Muscimol and Midazolam Do Not Potentiate Each Other’s Effects on Sleep EEG in the Rat

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Lancel, Marike, Johannes Faulhaber, Thomas Schiffelholz, Stefan Mathias, and Rudolf A. Deisz. Muscimol and midazolam do not potentiate each other’s effects on sleep EEG in the rat. J. Neurophysiol. 77: 1624–1629, 1997. The interaction of a γ-aminobutyric acid-A (GABA_A) receptor agonist and a benzodiazepine-type modulator of GABA_A receptors on sleep was investigated. Low doses of muscimol (0.3 mg/kg) and the benzodiazepine midazolam (1.5 mg/kg) were administered alone and in combination, in random order, to eight rats. All injections were given intraperitoneally at light onset. Electroencephalogram (EEG) and electromyogram were recorded during the first 6 h post injection. Compared with vehicle, muscimol hardly affected the time spent in non-rapid eye movement sleep (non-REMS) and REMS, but significantly enhanced EEG activity in the frequency range between 2 and 6 Hz during non-REMS. Midazolam significantly increased the time spent in non-REMS, reduced EEG activity at frequencies <12 Hz, and elevated EEG activity in most higher frequencies during this state. The combined administration of muscimol and midazolam affected non-REMS-specific EEG activity in an unexpected fashion: the effects were intermediate between those of muscimol and midazolam. These results indicate that muscimol and midazolam have dissimilar effects on EEG within non-REMS and demonstrate that midazolam does not augment but attenuates the muscimol-induced changes in sleep EEG. Our data are at variance with established mechanisms, according to which agonistic modulators would have similar effects and should potentiate the effects of GABA_A agonists. The present data suggest that application of agonists and agonistic modulators of GABA_A receptors causes differential net effects on sleep parameters.

INTRODUCTION

γ-Aminobutyric acid (GABA) is a major neurotransmitter in the mammalian brain (Bloom and Iversen 1971; Young and Chu 1990). The usual inhibitory action is to a large extent mediated by GABA_A receptors. GABA_A receptors form transmembrane ligand-gated anion channels and contain recognition sites for GABA and various modulatory binding sites for, among others, benzodiazepines (for review see Macdonald and Olsen 1994; Sieghart 1995). On activation by GABA or GABA analogues such as the selective GABA_A agonists muscimol and 4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol (THIP), the membrane permeability for anions increases, usually resulting in a slight, short-lasting hyperpolarization and associated decreased excitability of the receptive neuron. Benzodiazepines are agonistic modulators of GABA_A receptors. There is ample evidence derived primarily from in vitro experiments indicating that these compounds allosterically potentiate GABA-induced chloride flux by increasing the efficacy of GABA in opening the GABA_A-associated chloride channels (for review see Macdonald and Olsen 1994; Sieghart 1995). Furthermore, GABA_A agonists facilitate the binding of benzodiazepines (Barker et al. 1986; Richards et al. 1991). On the basis of these observations, it is generally assumed that benzodiazepines and GABA_A agonists have similar effects and that benzodiazepines potentiate the action of GABA_A agonists. According to this classical view, the role of GABA_A receptors in sleep regulation can be deduced from the hypnotic actions of benzodiazepines. It is well established that these compounds promote non-rapid eye movement sleep (non-REMS), decrease delta (0.5–4 Hz) activity, and increase spindling (≈11–16 Hz) in the electroencephalogram (EEG) during non-REMS and suppress REMS in mammals such as humans and rats (Borbély et al. 1985; Dijk et al. 1989; Gaillard et al. 1973; Johnson et al. 1976; Lancel et al. 1996; Mendelson and Martin 1990). However, it has recently been shown that muscimol and THIP increase time in non-REMS and, in contrast to the benzodiazepines, enhance non-REMS-specific delta activity and do not inhibit REMS in the rat (Lancel and Faulhaber 1996; Lancel et al. 1996). These apparently contradictory findings obscure the specific involvement of GABA_A receptors in the processes underlying sleep. To further elucidate the influence of GABA_A agonists and benzodiazepines on sleep, we assessed the sleep response to a low dose of muscimol and midazolam, alone and in combination, in rats.

METHODS

The experiments were approved by the local commission for animal welfare. Eight adult male Wistar rats (Charles River Laboratories, Sulzfeld, Germany) were used for these experiments. The rats were housed individually in a ventilated, sound-attenuated Faraday room under a 12-h light:dark schedule (lights on from 8.30 to 20.30 h, 50–120 lx) at an ambient temperature of 21.5–23°C. Food and water were available ad libitum. Under deep inhalation halothane (Hoechst, Frankfurt am Main, Germany) anesthesia, the rats were implanted with four stainless steel screws for epidural EEG recording (electrode positions: frontal cortex A3.9, L±2; occipital cortex P6.4, L±2 relative to bregma), and two stainless steel wires were inserted under the neck muscles for electromyogram (EMG) recording. The leads were connected to a socket, which was fixed on the skull by dental acrylic cement. At least 2 wk were allowed for recovery from surgery and 4 days to adapt to the recording conditions.

The rats were subjected to four randomized treatments, separated by ≥2 days. Each treatment consisted of two intraperitoneal injections, the first given at lights on and the second 10 min later, containing vehicle and vehicle (both pyrogen-free...
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**FIG. 1.** Typical 10-s electroencephalogram (EEG) record for wakefulness, non-rapid eye movement sleep (non-REMS), and REMS. During non-REMS, a spindle is marked by an arrowhead and delta waves by a bar.

RESULTS

Amount of time spent in each vigilance state

Muscimol alone and in combination with midazolam did not significantly affect the time spent in each vigilance state (Fig. 2, Table 1). For midazolam, ANOVA revealed a significant treatment effect on wakefulness \(F(1,7) = 9.8, P < 0.02\) and non-REMS \(F(1,7) = 14.7, P < 0.006\). Compared with vehicle, it significantly decreased wakefulness.

**FIG. 2.** Amount of the vigilance states during the 6-h recording period of muscimol, midazolam, and muscimol + midazolam. Values are means ± SE \((n = 8)\). Data are expressed as percentage of recording time (RT), in deviation from vehicle (% time treatment − % time vehicle). Differences from vehicle were tested with a 2-sided, paired \(t\)-test. Single asterisk: \(P < 0.05\). Double asterisk: \(P < 0.01\).
and increased non-REMS over the 6-h recording period (Fig. 2), mainly because of marked changes during interval 3–4 (Table 1). Analysis of the muscimol, midazolam, and muscimol + midazolam data (2-factor repeated-measures ANOVA) found no significant differences between these treatments for any of the vigilance states.

**Non-REMS and REMS latency and sleep episode frequency and duration**

Muscimol hardly affected sleep latency and the sleep episode parameters (Table 2). Midazolam significantly lengthened the non-REMS episodes, whereas muscimol + midazolam significantly reduced the number of REMS episodes.

Analysis of the muscimol, midazolam and muscimol + midazolam data (1-factor repeated-measures ANOVA) revealed a significant treatment effect on REMS episode frequency \(F(2,14) = 4.9, P < 0.05\). During muscimol + midazolam the number of REMS episodes was lower than during muscimol \((P < 0.06)\) or midazolam \((P < 0.007)\) alone.

**EEG power densities within non-REMS**

ANOVA of the EEG power densities within non-REMS during vehicle and muscimol revealed a significant treatment effect on the frequencies from 2 up to 6 Hz. Compared with vehicle, muscimol enhanced EEG activity within these frequency bands over the 6-h recording period (Fig. 3). Slight, nonsignificant enhancements were present during all 2-h intervals (Fig. 4). For midazolam, ANOVA yielded a significant treatment effect for the frequencies from 2 up to 11 Hz, because of persistent reductions (Figs. 3 and 4). In addition, the interaction between the factors treatment and time was significant for the frequencies \(\approx 13\) Hz, caused by enhancements (Fig. 3) that were limited to the first 2-h interval (Fig. 4). For muscimol + midazolam a significant treatment effect was observed for the frequencies from 6 up to 9 Hz. Over the 6-h recording period, EEG activity in these frequencies was reduced (Fig. 3), because of significant decreases during the first 2-h interval (Fig. 4). Furthermore, significant interaction effects were found for the frequencies \(\approx 13\) Hz, referring to enhancements (Fig. 3) that occurred during the first 2 hours (Fig. 4).

ANOVA run on the muscimol, midazolam, and muscimol + midazolam data found differential effects on EEG activity within non-REMS (see bars at bottom of Fig. 3 for results of ANOVA). Over the 6-h recording period, EEG power densities in the 1.5- to 10-Hz frequencies differed substantially between the treatments, being the highest during muscimol, intermediate during muscimol + midazolam, and the lowest during midazolam (Fig. 3). These differences were most prominent during the first 2 h (Fig. 4A), but were also evident during the subsequent 2-h intervals (Fig. 4, B and C). Moreover, the time course of EEG activity in several high-frequency bands differed significantly between the treatments. The midazolam-induced pronounced increase in high-frequency EEG activity during the first 2-h interval resulted in a sharper decline across the 6-h recording period than during the other conditions.

**DISCUSSION**

The relatively low dose of muscimol tended \((P < 0.1)\) to increase the amount of non-REMS (Fig. 2, Table 1) and

### TABLE 1. Vigilance states (% of recording time)

<table>
<thead>
<tr>
<th>State</th>
<th>2-h</th>
<th>VEH</th>
<th>MUSC</th>
<th>MIDA</th>
<th>MUSC + MIDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wake</td>
<td>1–2</td>
<td>47.8 ± 15.8</td>
<td>38.3 ± 9.9</td>
<td>41.3 ± 12.1</td>
<td>40.8 ± 8.9</td>
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<tr>
<td></td>
<td>3–4</td>
<td>33.6 ± 12.2</td>
<td>30.8 ± 14.6</td>
<td>22.0 ± 7.9*</td>
<td>29.7 ± 13.2</td>
</tr>
<tr>
<td></td>
<td>5–6</td>
<td>29.3 ± 8.7</td>
<td>29.6 ± 14.1</td>
<td>29.9 ± 8.4</td>
<td>29.1 ± 13.6</td>
</tr>
<tr>
<td>non-REMS</td>
<td>1–2</td>
<td>46.6 ± 12.9</td>
<td>56.0 ± 7.8</td>
<td>55.5 ± 9.8</td>
<td>55.7 ± 5.5</td>
</tr>
<tr>
<td></td>
<td>3–4</td>
<td>54.6 ± 11.4</td>
<td>56.6 ± 14.5</td>
<td>66.5 ± 7.8*</td>
<td>60.1 ± 12.0</td>
</tr>
<tr>
<td></td>
<td>5–6</td>
<td>56.0 ± 7.9</td>
<td>55.1 ± 11.1</td>
<td>54.5 ± 8.6</td>
<td>54.8 ± 10.7</td>
</tr>
<tr>
<td>REMS</td>
<td>1–2</td>
<td>5.6 ± 3.2</td>
<td>5.7 ± 4.1</td>
<td>3.3 ± 2.8</td>
<td>3.5 ± 3.6</td>
</tr>
<tr>
<td></td>
<td>3–4</td>
<td>11.8 ± 3.4</td>
<td>12.6 ± 4.2</td>
<td>11.6 ± 2.8</td>
<td>10.2 ± 4.7</td>
</tr>
<tr>
<td></td>
<td>5–6</td>
<td>14.7 ± 3.6</td>
<td>15.4 ± 3.3</td>
<td>15.6 ± 2.5</td>
<td>16.1 ± 5.2</td>
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</tbody>
</table>

Data are means ± SD from 8 subjects. VEH, vehicle; MUSC, muscimol; MIDA, midazolam; REMS, rapid eye movement sleep. * Significant differences from vehicle \((P < 0.05)\, 2-sided, paired \(t\)-test).

### TABLE 2. Sleep latency and sleep episodes

<table>
<thead>
<tr>
<th></th>
<th>VEH</th>
<th>MUSC</th>
<th>MIDA</th>
<th>MUSC + MIDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-REMS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency, min</td>
<td>34.6 ± 19.9</td>
<td>24.6 ± 11.3</td>
<td>19.5 ± 9.1</td>
<td>17.1 ± 7.0</td>
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<tr>
<td>Episode duration, min</td>
<td>2.76 ± 0.50</td>
<td>2.82 ± 0.68</td>
<td>3.20 ± 0.59*</td>
<td>3.29 ± 0.93</td>
</tr>
<tr>
<td>Episode frequency</td>
<td>70.9 ± 10.3</td>
<td>74.5 ± 11.1</td>
<td>68.6 ± 9.9</td>
<td>66.9 ± 14.3</td>
</tr>
<tr>
<td>REMS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency, min</td>
<td>73.4 ± 39.9</td>
<td>71.7 ± 40.4</td>
<td>84.9 ± 28.6</td>
<td>92.2 ± 50.1</td>
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<tr>
<td>Episode duration, min</td>
<td>1.32 ± 0.28</td>
<td>1.35 ± 0.20</td>
<td>1.30 ± 0.20</td>
<td>1.49 ± 0.39</td>
</tr>
<tr>
<td>Episode frequency</td>
<td>29.1 ± 6.6</td>
<td>30.0 ± 8.0</td>
<td>28.4 ± 7.1</td>
<td>23.0 ± 6.8†</td>
</tr>
</tbody>
</table>

Data are means ± SD from 8 subjects. non-REMS and REMS latency were arbitrarily defined as the 20th epoch of non-REMS and the 3rd epoch of REMS. For abbreviations see Table 1. Differences from vehicle were tested with a 2-sided, paired \(t\)-test \(* P < 0.01; † P < 0.05\).
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activity and initially enhanced EEG activity in the higher-frequency bands, especially spindling, within non-REMS (Figs. 3 and 4). The decay in the effects of midazolam over the 6-h recording period corresponds with the bioavailability known from plasma measurements after intravenous administration of midazolam in the rat (Mandema et al. 1991). A higher dose of midazolam has previously been shown to have more pronounced effects on non-REMS and to suppress REMS (Lancel et al. 1994, 1996). The present findings confirm earlier results demonstrating that the GABA_A agonists muscimol and THIP and the benzodiazepine midazolam induce different changes in the EEG within non-REMS (Lancel and Faulhaber 1996; Lancel et al. 1994, 1996).

However, combined administration of muscimol and midazolam augmented neither the increase in non-REMS evoked by each substance alone nor the suppression of REMS induced by midazolam (Fig. 2, Tables 1 and 2).

These observations confirm earlier results from studies on muscimol combined with the benzodiazepines flurazepam and triazolam (Mendelson and Martin 1990; Mendelson and Monti 1993) and indicate that at least the GABA_A agonist muscimol and benzodiazepines do not potentiate each other's effects on sleep architecture. A possible exception concerns the distribution of REMS. Only after combined administration of muscimol and midazolam was the number of REMS episodes significantly reduced (Table 2), and episode duration tended ($P < 0.06$) to be lengthened. Intriguingly, the inhibition of REMS induced by a higher dose of midazolam and the promotion of REMS evoked by a higher dose of muscimol are associated with a decreased number and an increased duration, respectively, of the REMS episodes (Lancel et al. 1996). Conceivably, midazolam and muscimol facilitate each other’s influence on REMS generation and maintenance, resulting in no change in the total amount of REMS. However, the present data show for the first time that muscimol and midazolam do not potentiate, but even reduce each other’s effects on non-REMS-specific EEG activity (Figs. 3 and 4). This finding indicates that selective

**FIG. 3.** EEG power densities within non-REMS over the 6-h recording period of muscimol, midazolam, and muscimol + midazolam. Curves connect values (mean ± SE; $n = 8$). For plotting purposes, the standardized data were expressed as percentage of the corresponding vehicle value. Dots below graph denote frequency bands and serve as visual aids. Lines through the dots indicate significant results of analysis of variance (ANOVA) ($P < 0.05$) run on log-transformed 2-h interval values of muscimol, midazolam, and muscimol + midazolam. treat, Treatment; time, 2-h interval.

slightly but significantly enhanced delta activity within this state (Figs. 3 and 4). These findings are in accordance with earlier results demonstrating that muscimol dose-dependently increases the amount of non-REMS and REMS and elevates non-REMS-specific delta activity in rats (Lancel et al. 1996). Midazolam promoted non-REMS (Fig. 2, Table 1) and persistently reduced low-frequency ($\leq 11$ Hz) EEG

**FIG. 4.** EEG power density within non-REMS during interval 1–2 h (A), 3–4 h (B), and 5–6 h (C) after the administration of muscimol, midazolam, and muscimol + midazolam. Data are means ($n = 8$). For plotting purposes, the standardized data were expressed as percentage of the corresponding vehicle value. Dots below graphs: frequency bands. Squares and lines through the dots refer to significant differences between the stated treatments ($P < 0.05$, 2-sided, paired $t$-test run on log-transformed values). veh, Vehicle; muse, muscimol; mida, midazolam.
GABA_A agonists and benzodiazepine-agonistic modulators of GABA_A receptors have differential and reciprocal effects on neural electric activity in vivo. This postulate is substantiated by observations in epilepsy research: whereas GABA analogues such as muscimol and THIP trigger/aggravate absence (petit mal) epilepsy in normal/genetic epileptic rats (Vergnes et al. 1984), it is well established that benzodiazepines suppress spontaneous and attenuate THIP-induced absence epilepsy (Marescaux et al. 1985).

The differential effects of GABA_A agonists and benzodiazepines are at odds with the classical view stating that agonists and agonistic modulators of GABA_A receptors have similar effects. Although other investigators have hypothesized that the hypnotic and antabiase actions of benzodiazepines are chiefly mediated by non-GABAergic processes (Marescaux et al. 1985; Mendelson and Martin 1990; Mendelson and Monti 1993), this explanation is not very plausible in view of the fact that zolpidem and zopiclone, both nonbenzodiazepines with a high affinity for the GABA_A receptor-associated benzodiazepine binding site, have benzodiazepine-like sleep effects (Aeschbach et al. 1994; Brunner et al. 1991; Depoortere et al. 1995). In addition, zolpidem has been shown to suppress absence epilepsy too (Depoortere et al. 1995). One may also argue that the opposite effects may be due to the activation of different GABA_A receptor subtypes. GABA_A receptors are composed of combinations of distinct polypeptides, divided at least into five classes (α, β, γ, δ, and ρ), with one to six variants each. The precise configuration determines the physiological and pharmacological properties of a GABA_A receptor. Whereas muscimol and THIP probably activate all GABA_A receptors, the ability to respond to benzodiazepines requires the coexpression of the γ2 or γ3 subunits with an α- and β-subunit (for review see Lüddens and Korpi 1995). If the opposing effects of benzodiazepines are due to a selective activation of specific GABA_A receptor subtypes, one would expect that the massive unselective activation of all GABA_A receptors by GABA analogues dominates the response and masks possible differences. But this is observed neither for the hypnotic (present data; Mendelson and Martin 1990; Mendelson and Monti 1993) nor for the antiabsence action (Marescaux et al. 1985). Alternatively, GABA and GABA analogues may have dissimilar effects on GABA_A receptor functioning in the in vivo situation. One of the prominent differences between GABA and its analogs muscimol and THIP is that the latter are poor substrates for uptake mechanisms. Compared with the distinct spatiotemporal pattern of released GABA, these drugs produce more tonic hyperpolarizations, which probably affect neural electric activity in a different manner. If so, the in vivo effects of muscimol and THIP would not cast light on the role of GABA_A receptors in sleep, but may reflect a pathophysiological state of elevated GABA levels, e.g., during impaired reuptake. Therefore the role of GABA_A receptors in sleep regulation may be reflected more accurately in the sleep effects of benzodiazepines, indicating that GABA_A receptors are positively involved in the production of non-REMS and spindles, but not in the generation of delta waves and the production of REMS.

Although the alterations in EEG within non-REMS induced by benzodiazepines and the GABA_A agonists muscimol and THIP are not readily explained, we briefly speculate on possible mechanisms. As the relay station for information toward the cerebral cortex, the thalamus plays a decisive role in sleep-wake behavior. Electrical activity of thalamic neurons depends on the membrane potential. At a depolarized membrane potential, typical for wakefulness and REMS, thalamic neurons fire tonically; and at a more negative membrane potential, as observed during non-REMS (Hirsch et al. 1983), they fire in a burst-pause pattern, associated with depressed responsiveness (for review see Steriade et al. 1993). Near resting membrane potential, thalamic cells display spindle oscillations. They are triggered by the GABAergic reticular thalamic nucleus, which imposes rhythmic (in the frequency of spindles) inhibitory postsynaptic potentials (IPSPs) onto relay neurons. Cessation of the IPSPs enables activation of low-threshold Ca^{2+} spikes with a series of high-frequency action potentials atop, which in turn induce excitatory postsynaptic potentials (EPSPs) in the cortex (Nuñez et al. 1992; Steriade et al. 1991). At a more negative membrane potential, thalamic neurons generate delta oscillations (0.5-4 Hz) (McCormick and Pape 1990; Nuñez et al. 1992; Steriade et al. 1991), resulting from the interplay between two voltage-gated currents: the hyperpolarization-activated inward cation current (McCormick and Pape 1990) and the hyperpolarization-deinactivated low-threshold Ca^{2+} current, which underlies the low-threshold Ca^{2+} spikes (Jahnsen and Llinàs 1984). Electrophysiological experiments revealed that spontaneous and evoked spindle oscillations in thalamic relay neurons are abolished by a GABA_A antagonist, but not by GABA_B antagonists (von Krosigk et al. 1993), which indicates that GABA_A receptors are critically involved in the generation of spindles. Thus benzodiazepines may increase spindling simply by potentiating GABA_A-mediated IPSPs from thalamic reticular nucleus or local circuit neurons. The benzodiazepine-induced decrease in delta activity may be partially accounted for by the electrophysiological finding that spindles block delta oscillations (Nuñez et al. 1992). However, the present (Fig. 4) and previous data (Aeschbach et al. 1994; Borbély et al. 1985) demonstrate that the attenuation of low-frequency EEG activity can outlast the increase in spindling, which suggests that multiple and in part separate processes are involved. Possibly, the enhancement of short-lasting IPSPs simply decreases the duration of EPSPs, resulting in a lower incidence of summation of EPSPs, thereby causing a less effective synchronization of thalamic and/or cortical neurons. If the GABA analogues muscimol and THIP produce longer-lasting hyperpolarizations, as speculated above, they are likely to dramatically remove inactivation of the hyperpolarization-deinactivated low-threshold Ca^{2+} current (Jahnsen and Llinàs 1984) and thereby contribute to the generation of delta oscillations in thalamic cells. Because polymorphic delta waves also occur in the cortex of athalamic animals (for review see Steriade et al. 1990), the cortex is an alternative site of action. Given these speculations, it is improbable that midazolam can potentiate the EEG effects of muscimol or vice versa. It is more likely that the spindle oscillations facilitated by midazolam interrupt long-lasting hyperpolarizations required for delta oscillations, whereas the postulated tonic hyperpolarization evoked by muscimol disfavors the midazolam-potentiated generation of spindles.
In conclusion, the present data demonstrate that muscimol and midazolam have dissimilar and reciprocal effects on the EEG activity within non-REMS. It is highly likely that, because of poor uptake, GABA_A agonists such as muscimol and THIP produce more tonic effects than physiological GABA concentrations. If this is correct, the role of GABA_A receptors in sleep regulation is reflected more accurately in the hypnic actions of benzodiazepines than in those induced by GABA analogues. Moreover, our findings substantiate the notion that, in contrast to the classical view, agonists and benzodiazepine-agonistic modulators of GABA_A receptors differentially affect neural electric activity in vivo.

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