INTRODUCTION

A number of compounds, including triazolam (1,2), pentobarbital (3), ethanol (4), and adenosine (5) have been shown to induce sleep when injected into the medial preoptic area of rats. The mechanism by which this occurs remains to be clearly elucidated, though the high affinity for triazolam for the benzodiazepine recognition site on the GABA-A-benzodiazepine receptor complex, and the interaction of pentobarbital and ethanol with its function, suggest that sleep may be initiated by increased GABAergic activity. In this view, the GABA recognition site and the benzodiazepine recognition site respond to binding of agonists by a complex interaction, the result of which is enhanced flux at the chloride ionophore (6,7). One difficulty with this approach has been two sets of studies, one of which indicated that microinjection of the GABA-A agonist muscimol into the anterior hypothalamus of cats increases wakefulness instead of sleep (8), while another reported that when given peripherally to rats, muscimol and the benzodiazepine hypnotic midazolam have differing effects on EEG power spectra during NREM sleep (9), and may have opposite effects on the amount of REM sleep (10). In order to explore the possible role of enhanced GABAergic activity in the MPA on sleep, we are now reporting on a study in which we have administered muscimol into the MPA in rats. A finding that muscimol,
like benzodiazepines, induces sleep when injected into the MPA would strengthen the hypothesis that a GABAergic mechanism mediates the hypnotic effects of benzodiazepines. If muscimol's effects should differ from those of benzodiazepines, this localized microinjection procedure would help eliminate two possible explanations, i.e., that differences in effects are secondary to differing effectiveness in entering the CNS, or to muscimol having affected GABA receptors throughout the CNS.

**METHODS**

The study was performed on nine 225-250 gm male Sprague-Dawley rats, which received muscimol 0.1 g, muscimol 1.0 g and vehicle in random sequence, in studies separated by at least four days each. A detailed account of anesthesia, surgical placement of injection cannulae, and implantation of stainless steel screws for EEG and stainless steel wires for nuchal EMG has been previously presented in a similar study (3) and a review (1). Following surgery, animals were given a one week recovery period while housed in individual cages in a 12:12 light:dark cycle in which lights come on at 8:00 AM, and the same lighting was maintained during the sleep recordings.

On the afternoon before a recording session, the animal was housed in the experimental chamber at 4:00 PM. Ambient temperature in the recording cages was maintained at 25-26 degrees C. At 10:00 AM on the morning of the recording, a 31 gauge stainless steel cannula was inserted through the guide cannula that had previously been surgically placed, extending 1 mm beyond it into the target area. Muscimol or vehicle, previously warmed to 37 degrees C, was administered over the course of one minute, and the cannula was allowed to remain in place for 30 seconds before withdrawal. The volumes given, and the infusion rate, were modeled after those of Myers (Myers, 1966) to minimize tissue damage and restrict drug diffusion to the area of interest.

Muscimol was prepared as 0.1 g and 1 g in 0.4 l of normal saline, based on doses previously used for microinjection of muscimol into the preoptic area in cats (8). A volume of 0.2 l was given on each side, using two syringes such that both sides were injected almost simultaneously.

A two hour sleep study was then performed, using a Grass Model 78 polygraph with a paper speed of 10 mm/sec, and calibrated such that a 50 V signal produced a 10 mm pen deflection. Recordings were interpreted in 30 second epochs by an investigator blind to the treatment given, and standard sleep measures, as described in detail previously (1) were tallied.

Statistical analysis was performed by a one-way analysis of variance (ANOVA) for repeated measures, in which treatment (muscimol at two doses, and vehicle) was the within-subjects factor. The statistical significance of differences was assessed with a Duncan's multiple range test.

Table 1: Core temperature

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1st Hr.</th>
<th>1st Hr-baseline</th>
<th>2nd Hr</th>
<th>Sleep onset</th>
<th>Peak temp.</th>
<th>Time of peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>37.79±0.16</td>
<td>0.72±0.15</td>
<td>38.14±0.28</td>
<td>37.68±0.19</td>
<td>38.54±0.22</td>
<td>173.25±37.12</td>
</tr>
<tr>
<td>Muscimol .1 g</td>
<td>37.96±0.10</td>
<td>0.74±0.12</td>
<td>38.15±0.22</td>
<td>37.93±0.12</td>
<td>38.69±0.18</td>
<td>153.75±38.49</td>
</tr>
<tr>
<td>Muscimol 1 g</td>
<td>38.16±0.19</td>
<td>1.26±0.28</td>
<td>38.69±0.25</td>
<td>38.05±0.20</td>
<td>39.06±0.24</td>
<td>154.71±29.35</td>
</tr>
</tbody>
</table>

All values represent mean ± SEM minutes. Abbreviations: NS= not statistically significant by ANOVA. Definitions of sleep variables are described previously.
Figure 1. Injection sites for muscimol as confirmed by histological analysis. Abbreviations used: AC = anterior commissure; CC = corpus callosum; CPU = caudate putamen (striatum); CTX = cerebral cortex; F = fornix; MPA = medial preoptic area; OX = optic chiasm; VP = ventral pallidum.
independent variable and the various sleep or temperature measures were the dependent variables. In those cases in which the ANOVA showed a significant treatment effect, a post-hoc Least Significant Difference (LSD) test was performed.

RESULTS

As seen in Table 1, microinjections of muscimol into the MPA had no significant on any sleep measure, including sleep latency, total sleep time, amounts of NREM or REM sleep, or waking time after initial sleep onset. Similarly, there were no significant effects on a wide range of measures of core temperature, including mean temperature in the first or second hours after injection, the difference between the first hour following injection and baseline, temperature at sleep onset, peak temperature, duration from injection until the time peak temperature was reached, or mean temperature for six hours after injection (Table 2).

DISCUSSION

In summary, muscimol, injected into the MPA in doses higher than those known to have endocrine effects when administered into the same site (13) was found to have no significant effects on sleep or a variety of measures of core temperature. This relative lack of effect of muscimol, when compared to the hypnotic actions of agents such as triazolam (2) or pentobarbital (3) administered into the MPA, is analogous to a similar discrepancy in effects of muscimol or THIP and benzodiazepines when given peripherally to rats or humans (11) The absence of effects on sleep has also been seen with systemic injections of GABA transaminase inhibitors, which increase CNS GABA concentrations (14). Muscimol and the benzodiazepine hypnotic midazolam have differing effects on power spectra during NREM sleep (9), and may have opposite effects on the amount of REM sleep (10). Explanations which have been offered to explain differential effects of peripherally administered muscimol and benzodiazepines include the possibilities that muscimol or THIP given peripherally may produce nonspecific actions at GABA receptors throughout the entire brain, that, in contrast to GABA, they may be poor substrates for uptake into neurons or glial cells (11), or that systemically administered muscimol may have poor penetration into the CNS. Only 0.02% of tritiated muscimol enters the CNS as the original compound (16), and some investigators have indicated concern about lack of effects due to difficulty entering the CNS or prereceptor metabolism (15, 16). The microinjection study reported here would tend to eliminate these as possibilities, since the administration was very localized. There are also data to suggest that the effects of muscimol on chloride channel function differ from those of GABA, the former activating channels for a longer duration (17). It seems possible that this might help explain a difference in hypnotic properties, although it remains unclear why an agent which activates channels for a longer time would have a smaller pharmacologic effect. Another possibility that could be considered is that muscimol activated all GABA-A receptors in the area of administration, while agents such as benzodiazepines may have effects only on receptors of specific compositions (13).

Previous studies of administration of muscimol into the preoptic area have had mixed results. A recent report indicates that microdialysis of smaller amounts of muscimol into the preoptic area of rats resulted in decreased firing of waking-related neurons (18). Lin et al. (8) reported that microinjection into the preoptic area/anterior hypothalamus of doses similar to those in the present study enhanced wakefulness when injected into the preoptic area of cats. Whether differences in results of the latter study are related to species differences or injection into a larger area including other parts of the anterior hypothalamus will need to be established in further work.

Finally, it should be mentioned that in areas other than sleep there have been instances in which muscimol effects differ from those of benzodiazepines. There has been a report, for instance, that muscimol's effects on voltage-dependent Ca+ currents and Cl- currents differ from those of diazepam in isolated frog sensory neurons (19). Muscimol has been reported to increase seizure occurrence, while diazepam and clonazepam have the opposite effect, in a pharmacologically-induced model of chronic petit mal epilepsy in the rat (20). An analogous set of opposite effects of the GABA-A agonist THIP and diazepam has been reported in spontaneous petit mal-like epilepsy in rats (21). Muscimol does not appear to potentiate, and indeed may even block, anticonflict effects of marginal doses of diazepam in the rat (22). These types of findings emphasize the continuing need to clarify the relationship between the binding of benzodiazepines to their recognition site and the possible GABAergic effector mechanisms.

REFERENCES


