Sleep and Core Temperature Measures Following Microinjection of Muscimol into the Medial Preoptic Area in Rats

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Although benzodiazepines are thought to exert their effects on sleep by altering GABAergic function, several studies using peripheral administration have pointed to differences in pharmacologic actions of these compounds and the GABA-A receptor agonist muscimol. Among the possible explanations for these differences are the notions that these agents may have very different rates of entry into the central nervous system (CNS), or that muscimol might be affecting a wide range of GABA receptors throughout the CNS. One way to test these concerns would be to administer both agents directly into a localized site. It has previously been observed that triazolam, as well as nonbenzodiazepine agents such as pentobarbital and ethanol, induce sleep in the rat when microinjected into the medial preoptic area (MPA). To this end, we have microinjected muscimol into the MPA of rats, using doses (0.1 g and 1.0 g) substantially higher than those reported to produce endocrine effects after microinjection there, and examined the consequent sleep and core temperature. Neither dose of muscimol significantly differed from vehicle on any measure. Insofar as the current work involved localized administration, the implication is that differences between benzodiazepines and muscimol in pharmacologic effects that have been detected after peripheral administration may not be due to differences in CNS penetrance. Other possibilities, including differing duration of activation of ion channels or differing actions on receptors of various subtype composition, are considered. (Sleep and Hypnosis 1999;1:158-162)

Key words: muscimol, GABA, sleep, REM sleep, power spectra, benzodiazepines

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 GABAA 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 gypnotic 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,

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like benzodiazepines, induces sleep when injected into the MPA would strengthen the gypothesis 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 Spraque-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.

In addition to sleep, measures of core temperature were performed by use of a MiniMitter transmitter (MiniMitter Co., Sunriver, Ore.) placed in the intraperitoneal space at the time of surgical implantation of EEG electrodes. On the day of a sleep recording, temperature was recorded for a 20 minute baseline period and then for two hours subsequently. Following the study, data were analyzed using a series of standard measures, listed in detail previously (2).

Following the study, each animal was anesthetized and euthanized, and accurate placement of injections was histologically confirmed (1). Figure 1 shows a representation of the injection sites.

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

Table 1: Effects of muscimol microinjections into the MPA on sleep variables

Sleep parameter	Vehicle	Muscimol 0.1 g	Muscimol 1.0 g	Significance
Sleep latency	19.0– 1.6	19.3– 1.2	18.3– 1.0	NS
Total sleep time	59.0– 1.9	59.0– 2.7	56.1– 2.0	NS
NREM sleep time	56.1– 1.7	55.6– 2.6	53.6– 1.6	NS
REM sleep time	2.9– 0.5	3.4– 0.7	2.7– 0.6	NS
Wake time after sleep onset	44.8– 2.6	44.7– 2.9	46.0– 1.6	NS
Movement time	0	0	1.2– 0.8	NS
REM latency	63.6– 6.0	64.1– 6.9	71.4– 8.4	NS

All values represent mean + SEM minutes. Abbreviations: NS= not statistically significant by ANOVA. Definitions of sleep variables are described previously

Table 2: Core temperature[1]

	1st Hr.	1st Hr-baseline	2nd Hr	Sleep onset	Peak temp.	Time of peak[2]
Vehicle	37.79–0.16	0.72–0.15	38.14–0.28	37.68–0.19	38.54–0.22	173.25+37.12
Muscimol ,1 g	37.96–0.10	0.74–0.12	38.15–0.22	37.93–0.12	38.68–0.18	153.75+38.49
Muscimol 1 g	38.16–0.19	1.26–0.28	38.69–0.25	38.05–0.20	39.06–0.24	154.71+29.35

[1]= No significant treatment effects for any variable by analysis of variance.
[2]= Duration (min.) from injection until peak temperature.
All values except Time of peak represent degrees Centigrade+SEM.

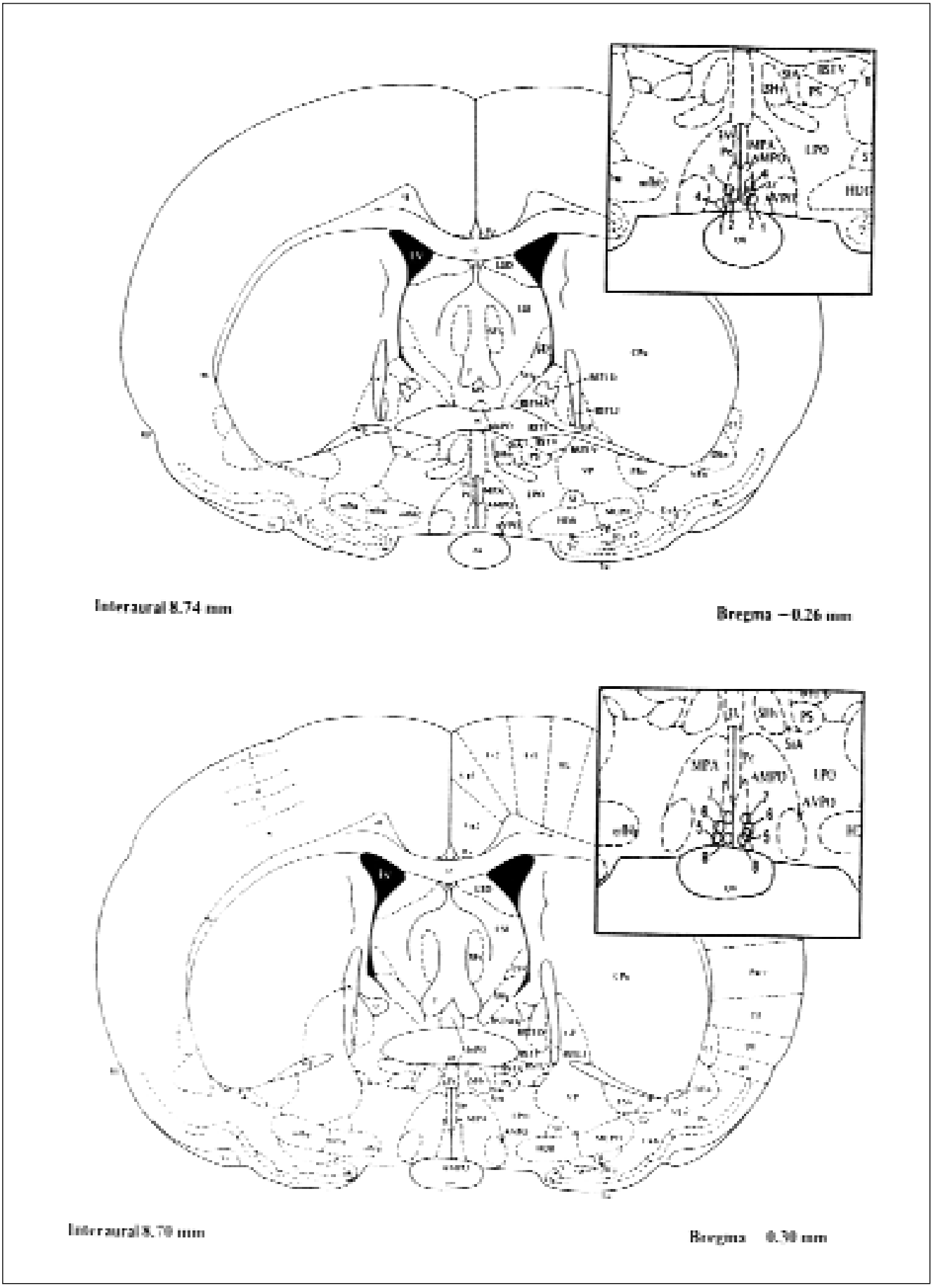

Figure 1. Injection sites for muscimol as confirmed by histological analysis. Abbreviations used: AC= anterior commissure; CC= corpus callosum; CPU= caudate putamen (striatum); CTX= cerebral cortex; F= fornix; MPA = medial preoptic area; OX = optic chiasm; VP= ventral pallidum.

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 SignificantDifference (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 efects when administeded 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) administeded 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 administeded 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 penetrance 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 mentined that in areas other than sleep there have been instances i 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 efects 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.

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