Effects of Microinjections of Triazolam into Medial Preoptic Area on Sleep and Brain Temperature in Rats

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The effects of microinjection of triazolam into the medial preoptic area on sleep and brain temperature in rats. We have previously reported that microinjections of triazolam into the medial preoptic area (MPA) of the anterior hypothalamus have a potent hypnotic effect in the rat. It has not been clear whether this response is due to "direct" effects on sleep-regulating structures or is secondary to drug-induced alterations in temperature. Previous studies found no evidence of drug-induced changes in core temperature, but the possibility remained open that alterations in brain temperature (Tbr) occurred. For this reason we have now examined the effects of triazolam microinjections into the MPA on sleep and Tbr in rats. Each animal received both vehicle and triazolam in random sequence, in studies separated by three days. As in earlier reports, triazolam 0.25 µg produced a potent hypnotic effect by significantly decreasing sleep latency and wake time after sleep onset while increasing NREM sleep. In contrast, Tbr changes were minimal. Tbr during the first and second hours following microinjection, and at the time of sleep onset, did not differ between the triazolam and vehicle treatments. In summary, there was little evidence to suggest that the hypnotic effect of triazolam microinjections into the MPA is mediated by drug-induced alterations in Tbr. (Sleep and Hypnosis 1999;1:10-13)

Key words: triazolam, benzodiazepines, sleep, thermoregulation

INTRODUCTION

A variety of hypnotic compounds including triazolam (1), pentobarbital (2) and ethanol (3) have been shown to induce sleep when microinjected into the medial preoptic area (MPA) of the anterior hypnothalamus. The mechanism by which they produce this effect, however, is not clear. Lesions of a basal forebrain area including the preoptic area decrease sleep (4), while electrical stimulation of this area increases it (5). As an integrative structure, the preoptic area is also involved in a variety of other physiological processes. It contains cells sensitive to osmotic, and cardiovascular signals (6) as well as glucose and steroids (7). It is a thermoregulatory site in mammals, and contains a high concentration of strongly warm-sensitive and cold-sensitive neurons that are involved in autonomic and behavioral thermoeffector activities (8). This raises the possibility that the effect of microinjection of hypnotic compounds on sleep is secondary to drug-induced changes in temperature. In the past we have explore this possibility using rectal (1) and intraperitoneal temperature (9). After microinjection of both vehicle and triazolam into the medial preoptic area, there was an increase in core temperature, which did not differ between the two treatments. Thus, although the act of microinjecting materials into the MPA appears to produce a relatively consistent transient rise in temperature, there was no evidence to suggest that triazolam's hypnogenic property is secondary to drug-induced alterations in core temperature. It is possible, however, that its effects on temperature are more subtle, involving more localized changes. Under some circumstances brain temperature may vary from trunk temperature (10), and although in general they move in parallel, changes in preoptic temperature may precede alterations in abdominal temperature in association with sleep or eating in the rat (11). Indeed, rat brain and rectal temperature may even transiently move in opposite directions in response to some behaviors (12). Thus, we believe that an examination of the possibility that drug-induced changes in sleep are secondary to effects on temperature requires direct measures of brain temperature

(Tbr). The present report examines Tbr and sleep after microinjections of triazolam and vehicle into the medial preoptic area.

METHODS

In this study, nine male Sprague Dawley 250-300 gm rats (Harlan Sprague Dawley Inc., Madison, Wisc.) were given microinjections of triazolam 0.25 μg and vehicle, in random sequence in studies separated by at least 3 days. Techniques for anesthesia and surgical implantation of injection cannulae have been described in detail in previous publications (1). In summary, each rat was anesthetized with an injection of 70 mg/kg ketamine and 6 mg/kg xylazine IP, and positioned in a Kopf stereotaxic apparatus with the mouth bar set at -5.0 mm. Once the initial scalp incision was made and the skull exposed, holes were drilled at stereotaxically determined locations for the placement of bilateral 24-gauge stainless steel guide cannulae. These were lowered to 1 mm above the medial preoptic area so that the tips of the cannulae were at the stereotaxic coordinates (in mm) of A-P: -0.4; M-L: 0.5; D-V: -7.1, derived from Paxinos and Watson (13) and assessed in previous work (1). Blocking stylets of 31-gauge stainless steel were placed in the guide cannulae. Another hole was drilled directly 4.5 mm anterior to the guide cannulae on the right, and a thermistor was lowered to DV -5.1 mm.

During this procedure, four 0-80 stainless steel screws were also implanted through the skull to serve as dural EEG electrodes, while the stripped ends of two 0.01 in. Teflon-coated stainless steel wires were placed in the neck musculature for use as electromyographic (EMG) electrodes. The EEG and EMG electrodes were connected to an Amphenol socket, and the entire assemble, along with the cannulae and thermistor probe, was attached to the skull with dental acrylic. The edges of the wound were treated with an ointment containing bacitracin, polymyxin, and neomycin. After surgery, each rat, individually housed in plastic cage set on a 12:12-h light-dark cycle {lights were on from 8:00 a.m. until 8:00 p.m.), was allowed at least one week to recover prior to use is a study. The ambient temperature in the recording chambers was maintained at 26.25±0.14 °C.

The animals were placed in the recording chambers at 4:00 p.m. the day before the beginning of the experiment in order for them to adapt to the recording environment. At 10:00 a.m. the next morning, the blocking stylet was removed from each side, an injection cannula of 31-gauge stainless steel was inserted into each cannula so that the tip extended 1.0 mm past the tip of the guide cannula. Either triazolam (Upjohn Co., Kalamazoo, Mich.) or its vehicle was injected from a 1 microliter Hamilton syringe through a length of PE 20 tubing attached to the injection cannula. A volume of 0.2 μl was injected into each side over a period of a minute, with the inner cannula left in place for 30 seconds following injection. The volumes and infusion rate used were designed to minimize tissue damage and restrict diffusion of drug from injection site (14). A syringe was used on each side so that injections essentially occurred at the same time. Prior to injection, triazolam, dissolved in a 1:1 mixture of Emulphor polyoxyethylated vegetable oil and ethanol diluted 10-fold with artificial cerebrospinal fluid, was warmed to 37 °C. After injections were completed, blocking stylets were placed back into the guide cannulae, and the animal returned to its recording chamber.

Polygraphic sleep data, provided by the bifrontal EEG, fronto-occipital EEG, and EMG, was obtained by means of a Grass Model 78 polygraph. The paper speed was set at 10 mm/sec, with the vertical deflection of the pen calibrated so that 1.0 cm signified an electrical potential of 50 μV. Upon completion of the study, polygraphic recordings for each animal were visually scored in 30 seconds epochs by the investigator who was "blind" to the treatment conditions. For each epoch, the investigator determined whether the animal was awake, in NREM, or in REM sleep. Results were tallied in terms of the following parameters: sleep latency (time from drug injection until the first three consecutive epochs of sleep, REM latency (time from sleep onset until the first two consecutive epochs of REM sleep), NREM sleep time, REM sleep time, total sleep time, and intermittent wakefulness (waking time after initial sleep onset.) As previous work (15) indicated that drug-induced changes in sleep were largely confined to the first two hours after administration, therefore data were presented for this period.

The accuracy of the injection site was confirmed histologically using light microscopy to localize the tip of the injection cannula track. Each rat was injected with 400 mg/kg sodium pentobarbital intraperitoneally and perfused transcardially (1). The brain was then removed and fixed in a formalin solution. Using a freezing microtome, coronal brain sections (30 μm) were sliced, then mounted on slides, and stained in cresyl violet.

Measurement of Brain Temperature (Tbr)

Tbr was measured using the MiniMitter telemetry system (MiniMitter Co., Sunriver, Ore.). A temperature-sensitive probe was implanted in the rat at the time of the placement of the electrodes and cannulae, at the following coordinates: A-P: +4.0; M-L: +0.5; D-V: -5.1 mm. A transmitter mounted on the skull sends out a signal, encoding temperature, to a receiver situated underneath each of the testing cages. The signal was encoded at an IBM PC microcomputer.

For each animal, Tbr was recorded for a baseline period of at least 30 minutes prior to drug injection and for two hours following injection, at one minute intervals. Tbr data were accumulated for specific variables such as temperature

for the baseline period (prior to injection), mean temperature for the first and second hours after injection, difference in temperature between the mean of the first hour and baseline, and Tbr at sleep onset.

Statistical Analysis

Effects of triazolam and vehicle on the sleep and Tbr variables discussed above were statistically assessed using paired t-test for dependent samples. Analyses of variance were performed in which the sequence of drug or vehicle administration was the independent variable, while total sleep time and sleep latency were the dependent variables; sequence effect was found to be not significant. A two-way analysis of variance was also conducted in which the dependent was Tbr, while treatment (drug or vehicle) and time were the independent variables.

RESULTS

Sleep

In confirmation of previous studies (1,9), triazolam injections into the MPA had a potent hypnotic effect. Triazolam significantly increased total sleep time during the first two hours, by significantly decreasing sleep latency and increasing NREM sleep (Table 1). It also significantly decreased the amount of waking time after sleep onset. Total REM sleep and REM latency were not affected.

Table 1. Sleep Data After Injections (in minutes, mean ± SEM).

	Vehicle Injected	Triazolam Injected	Significance
Sleep Latency *	23.44 ± 1.70	12.94 ± 1.14	p< 0.001
Total Sleep Time **	59.83 ± 1.73	78.00 ± 2.25	p< 0.001
NREM ***	55.39 ± 1.64	72.89 ± 1.66	p< 0.001
REM	4.44 ± 1.64	4.72 ± 1.66	NS
Wake Time ****	40.11 ± 2.26	30.89 ± 3.08	p< 0.027
REM Latency	51.44 ± 5.24	64.83 ± 9.49	NS

*df= 8, t= 5.12, p< 0.001; ** df 8, t= -8.12, p< 0.00004;
*** df 8, t= -7.85, p< 0.00005; **** df 8, t= 2.70, p< 0.027

Tbr

Prior to injection, the baseline Tbr did not significantly differ between the triazolam and vehicle treatments. In contrast to its potent effect of sleep, triazolam microinjection induced minimal alterations in Tbr (Table 2). there were no significant drug effects on mean temperature in the first or second hours after injection, although there was a non-significant trend for the triazolam condition values to be lower for the first two hours combined (df 1,6; F= 4.55; p< 0.08). Tbr at the time of sleep onset rose slightly above

Table 2. Brain Temperature Variables (in °C, mean ± SEM).

	Vehicle Injected	Triazolam Injected	Significance
First Hour	38.400 ± 0.197	38.181 ± 0.237	NS
Second Hour	38.774 ± 0.283	38.438 ± 0.202	NS
First Two Hours Combined	38.589 ± 0.240	38.311 ± 0.208	NS
Sleep Onset	38.203 ± 0.311	38.395 ± 0.318	NS
First Hour Minus Baseline *	0.830 ± 0.141	0.439 ± 0.072	p< 0.021

* df= 8, t= -2.8670, p< 0.0209

baseline (37.544 ± 0.177 °C) to 38.298 ± 0.217 °C for both treatments combined (df 17; t= -3.34; p<0.01), and did not significantly differ between the vehicle (38.203±0.311 °C) and triazolam (38.395±0.318 °C) conditions. The only significant drug effect was that the rise in Tbr was smaller in the triazolam group: the mean Tbr in the first hour minus the pre-injection baseline, was approximately 0.4 °C lower in the drug compared to the vehicle condition (p<0.02). Thus, although the specific treatment did not alter Tbr, the act of microinjecting materials into the medial preoptic area appears to produce a transient rise in Tbr.

DISCUSSION

It is well established that Tbr drops relative to waking in NREM sleep (16), and preoptic temperature declines during behaviorally-measured sleep (11). Insofar as parentally administered benzodiazepines decrease core (17) and brain (18) temperature in rodents, one hypothesis might be that sleep enhancement by benzodiazepines results from drug-induced alterations in temperature. Indeed. such a hypothesis is strengthened by the observation that the preoptic area contains thermosensitive neurons, the sensitivity of which may change in NREM sleep relative to wakefulness (19), and that increasing the ambient temperature restores sleep in cats with preoptic/hypothalamic lesions (20). It has been hypothesized that NREM sleep is controlled by thermoregulatory systems in the preoptic area/anterior hypothalamus (21). What has not been certain is whether these mechanisms of normal physiology might mediate pharmacologically-induced changes in sleep. We have previously shown that microinjections of triazolam and vehicle into the medial preoptic area produce similar transient increase in core temperature, though the triazolam enhanced sleep while vehicle did not (1,9). It seemed possible, however, that more localized temperature alterations might occur which would not reflected in core temperature. The present study found no significant change in Tbr associated with sleep induction by triazolam microinjection. This indicates that its effects on sleep are not mediated by drug-induced alterations in Tbr, and suggests that the mechanisms of pharmacologic sleep induction and physiologic sleep initiation may differ in this regard.

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