Evidence That REM Sleep Is Controlled by the Activation of Brain Stem Pedunculopontine Tegmental Kainate Receptor

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Datta, Subimal. Evidence that REM sleep is controlled by the activation of brain stem pedunculopontine tegmental kainate receptor. J Neurophysiol 87: 1790–1798, 2002; 10.1152/jn.00763.2001. Glutamate, the neurotransmitter, enhances rapid-eye-movement (REM) sleep when microinjected into the brain stem pedunculopontine tegmentum (PPT) of the cat and rat. Glutamate and its various receptors are normally present in the PPT cholinergic cell compartment. The aim of this study was to identify which specific receptor(s) in the cholinergic cell compartment of the PPT are involved in glutamate-induced-REM sleep. To identify these glutamate-induced REM-sleep-generating receptor(s) in the PPT cholinergic cell compartment, specific receptors were pharmacologically blocked differentially by localized pretreatment of specific glutamate receptor antagonists; glutamate was then microinjected into the PPT cholinergic cell compartment while quantifying the effects on REM sleep in freely moving chronically instrumented rats. The results demonstrate that when kainate receptors were blocked by pretreatment with a kainate-specific receptor antagonist, microinjection of glutamate was unable to induce REM sleep. Pharmacological blockade of specific N-methyl-D-aspartate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors was unable to block glutamate-microinjection-induced-REM sleep. These findings suggest, for the first time, that the activation of kainate receptors within the cholinergic cell compartment of the PPT is an essential portion of the mechanism for the generation of glutamate-induced REM sleep in the rat.

INTRODUCTION

Rapid-eye-movement (REM) sleep is a distinctive sleep stage that alternates with episodes of slow-wave sleep (SWS). Considerable progress has been made in identifying the neuroanatomical, neurochemical, and neurophysiological events underlying the generation of REM sleep, which is thought to involve activation of brain stem pedunculopontine tegmentum (PPT) cholinergic cells (for review, see Datta 1995; Gillin et al. 1993; Morrison et al. 1999; Steriade and McCarley 1990). Less is known, however, about the mechanisms of PPT cholinergic cell activation. Recent local microinjection studies in the cat and rat suggest that the neurotransmitter, glutamate, may be involved in the activation of PPT cholinergic cells that triggers and maintains REM sleep (Datta and Siwek 1997; Datta et al. 2001).

Two endogenous sources of glutamate have been identified to account for glutamatergic action on PPT cells. First, glutamate is known to be present in high concentrations in subpopulations of cholinergic and noncholinergic cells in the PPT (Clements and Grant 1990; Lai et al. 1993; Lavoie and Parent 1994; Liu et al. 1995). Second, neurons in the pontine reticular formation (PRF) project to the PPT (Steininger et al. 1992), and this PRF region contains numerous cells that are immunoreactive for glutamate (Lai et al. 1993). Thus endogenously released and exogenously applied glutamate may be acting on specific glutamate receptors to activate PPT cells; this in turn contributes to the induction of wakefulness and REM sleep.

Recent immunohistochemical studies have demonstrated the presence of N-methyl-D-aspartate (NMDA) (Morin et al. 1989; Petralia et al. 1994a,c; Watanabe et al. 1994), AMPA (Martin et al. 1993; Petralia and Wenthold 1992; Sato et al. 1993), and metabotropic glutamate receptors (Ohishi et al. 1993; Shigemoto et al. 1992) in PPT cells. The pharmacological identification of glutamate receptors involved in PPT-modulated REM sleep regulation will be an important step toward future experiments to elucidate the molecular mechanisms of REM sleep generation.

The present study was designed to identify specific glutamate receptors involved in glutamate-induced, PPT-modulated REM sleep. To identify this REM sleep-inducing specific glutamate receptor in freely moving rats, the optimal dose of glutamate was microinjected into the cholinergic cell compartment at the PPT sites pretreated with one of the three specific receptor antagonists (NMDA, kainate, and AMPA) or vehicle control while simultaneously recording polygraphic signs of wake-sleep.

METHODS

Subjects and housing

Experiments were performed on 26 male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing between 300 and 400 g. The rats were housed individually at 24°C with food and water provided ad libitum with lights on from 07:00 to 19:00 (light cycle) and off from 19:00 to 07:00 (dark cycle). The principles for the care and use of laboratory animals in research, as outlined by the National Institute of Health Publication No. 85-23 (1985) were strictly followed.

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Drugs and vehicle for microinjections

The drugs used included l-glutamate (molecular weight: 183.59), NMDA receptor antagonist, 1r(-)-2-amino-5-phosphonovaleric acid (AP5, molecular weight: 197.13), kainate receptor antagonist, 1,3-dipropyl-2-pyridyldithiocarbamate methosulfate (AMOA, molecular weight: 244.2), 1-glutamate, AP5, and GAMS were purchased from Research Biochemicals International (Natik, MA) and AMOA was purchased from Tocris Cookson (St. Louis, MO). These drugs were dissolved in 0.9% saline, and solutions were adjusted to pH 7.0. This 0.9% saline was also used for the control vehicle microinjection. All of these drugs are hydrophilic. Control saline and drug solutions were freshly prepared under sterile conditions before each use. The selection of antagonists was based on the selective antagonistic effects on specific types of glutamate receptors (Davies and Watkins 1982, 1985; Davis et al. 1992; Frandsen et al. 1990; Krosggaard-Larsen et al. 1991; Porter and Greenamyre 1994; Turski et al. 1985). In addition to the specificity, these drugs are also water soluble and accessible to the extracellular receptors (Davies and Watkins 1982, 1985; Davis et al. 1992; Frandsen et al. 1990; Krosggaard-Larsen et al. 1991; Porter and Greenamyre 1994).

Surgical procedures and implantation of electrodes

Treatment of the animals and surgical procedures were in accordance with an approved institutional animal welfare protocol (No. 97-183). Rats were anesthetized with pentobarbital (40 mg/kg ip), placed in the stereotaxic apparatus, and secured using blunt rodent ear bars (Paxinos and Watson 1986). With the use of sterile procedures, cortical electroencephalogram (EEG), dorsal neck muscle electromyogram (EMG), electrocorticogram (EOG), hippocampal EEG (to record theta wave), and pontine EEG (to record P-wave) recording electrodes were chronically implanted as described elsewhere (Datta and Hobson 2000). In addition, bilateral stainless steel guide tubes (26 gauge) with an equal length styllet inside were stereotaxically implanted 2 mm above the PPT (A: 1.0; L: 1.8; H: 3.0) as described previously (Datta et al. 1993). The behavioral states of wakefulness, three behavioral states were distinguished based on the visual scoring of polygraphic records as described earlier (Datta and Hobson 2000). The injection protocol was adopted as described earlier (Datta et al. 2001). The injection protocol was designed so that during the first experimental session all 26 rats received saline + saline as their first set of injections into one of the two PPT sites. During the second experimental session, all rats received one of the four sets of drug injections (saline + glutamate; AP5 + glutamate; GAMS + glutamate; AMOA + glutamate) in their contralateral PPT site as a second set of injections. During the third experimental session, all rats received another set of drug injections in the site of the first injections during the first experimental session. Each rat received a total of three sets of microinjections in three different experimental sessions. Experimental sessions were separated by at least 3 days. At the end of all experimental sessions and before perfusion, with the use of same injector used for glutamate and antagonist microinjections, 100 nl of black ink was microinjected 1 mm dorsal to each injection site for localizing injection sites. In Fig. 1A, the arrow points to the ink injection mark. Thus the true position of the glutamate injection is 1 mm below the ink mark.

Intracerebral microinjections and experimental design

After the adaptation recording sessions, microinjection sessions began. During experimental sessions, animals were connected to the polygraphic recording system 15 min before the first injection. The microinjection system consisted of a 32-gauge stainless steel injector cannula with a 26-gauge collar that extended 2.0 mm beyond the implanted guide tube. The collar was connected to a 1.0-µl motor-driven Hamilton microsyringe with PE 20 tubing. After filling the injection system with drugs or control vehicle, a small air bubble was introduced into the PE tubing to monitor the movement of the fluid during the injection. In this study, a double-injection protocol was adopted as described earlier (Datta et al. 1993). Briefly, each microinjection session consisted of two injections in the same site with a 15-min interval between the first and second injections. While the animal was connected to the recording system, the styllet was removed and a control vehicle-filled (100 nl volume of 0.9% saline) or any one of the three types of glutamate receptor antagonist-filled (0.16 nmol in 100 nl, number of antagonist molecule is equal to the number of glutamate molecule) injector was introduced through the guide tube for the first injection. One minute after the insertion of the injector cannula, 100 nl of control saline or any one of the three types of glutamate receptor antagonist was microinjected over a 60-s period. The cannula was gently withdrawn 2 min after the injection, and the styllet was reintroduced inside the guide tube. For the second injection, control saline or glutamate (0.16 nmol in 100 nl) was injected into the same site using the same injector system as described for the first injection. During the time of microinjections, animals were free to move around the cage with the cannula in place. Due to the extended tubing, the injections could be made while the animals were moving around. Immediately after completion of the microinjection procedure, polygraphic variables were recorded continuously for a session of 6 h (between 10:00 and 16:00), when rats would normally be sleeping (Datta and Hobson 2000). The optimum dose of l-glutamate (0.16 nmol) was predetermined from our dose-response study (Datta et al. 2001). To block the specific receptor-mediated action of glutamate, an equal number of specific receptor antagonist molecules were microinjected in 100 nl of vehicle (Datta et al. 1993). Because the equimol dose of NMDA and AMPA receptor antagonists was not effective at blocking the REM sleep effect of glutamate, in three sets of microinjections, this dose was increased to three times (0.48 nmol) the glutamate dose. However, the kainate receptor antagonist at the equimol dose was effective at blocking the glutamate induced increase in REM sleep.

The injection protocol was designed so that during the first experimental session all 26 rats received saline + saline as their first set of injections into one of the two PPT sites. During the second experimental session, all rats received one of the four sets of drug injections (saline + glutamate; AP5 + glutamate; GAMS + glutamate; AMOA + glutamate) in their contralateral PPT site as a second set of injections. During the third experimental session, all rats received another set of drug injections in the site of the first injections during the first experimental session. Each rat received a total of three sets of microinjections in three different experimental sessions. Experimental sessions were separated by at least 3 days. At the end of all experimental sessions and before perfusion, with the use of same injector used for glutamate and antagonist microinjections, 100 nl of black ink was microinjected 1 mm dorsal to each injection site for localizing injection sites. In Fig. 1A, the arrow points to the ink injection mark. Thus the true position of the glutamate injection is 1 mm below the ink mark.

Determination of behavioral states and data analysis

For the purpose of determining possible effects on sleep and wakefulness, three behavioral states were distinguished based on the visual scoring of polygraphic records as described earlier (Datta and Hobson 2000). The behavioral states of wakefulness (W), SWS, and REM sleep were scored in successive 10-s epochs. The polygraphic measures provided the percentage of recording time spent in W, SWS, and REM sleep. The effects of the five different treatments (saline + saline; saline + glutamate; AP5 + glutamate; GAMS + glutamate; AMOA + glutamate) on the percentages of W, SWS, and REM sleep were statistically analyzed using a two-way ANOVA with time as a repeated measure within-subject variable (6 levels corresponding to 6 1-h epochs following injections) and treatment as a between-subject variable (5 levels corresponding to the 5 different treatments). Following a two-way ANOVA, post hoc Scheffe F tests were done to determine the individual levels of significant difference between control (saline + saline) and the four different drug treatment protocols at six individual data points. A second set of post hoc Scheffe F tests were done to determine the individual level of significant difference between glutamate without pretreatment of antagonist (saline + glutamate) and glutamate with pretreatment of glutamate receptor antagonists (AP5 + glutamate, GAMS + glutamate, or AMOA + glutamate) at six individual data points. Statistical analyses (2-way ANOVA and Scheffe F test) were performed with the use of StatView statistical software (Abacus Concepts, Berkeley, CA).
Histological localization of injection site

At the conclusion of the microinjection experiments, rats were deeply reanesthetized with pentobarbital (60 mg/kg ip) and perfused transcardially with heparinized cold phosphate buffer (0.1 M, pH 7.4) followed by 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were removed and processed for NADPH-diaphorase staining and histological localization of injection sites as described earlier (Datta et al. 2001).

RESULTS

A total of 78 sets of microinjections were made in 52 PPT injection sites. Histological identification showed that 34 sites were placed within NADPH-diaphorase-positive cell compartments and thus were considered to be within the cholinergic cell compartments of the PPT (Fig. 1). The remaining 18 microinjection sites were away from the NADPH-diaphorase-positive compartments of the PPT. Based on our earlier mapping studies (Datta et al. 2001), these 18 microinjection sites were considered to be negative sites for the glutamate-induced REM sleep effect. Microinjection of glutamate into these negative sites did not cause changes in wakefulness and SWS. Thus the results from these 18 negative sites were not included in the analyses of the data.

Effects of different microinjections on sleep-wake architecture

Figure 2 illustrates representative sleep-wake architectures for the 6-h postinjection recording sessions (10 AM to 4 PM) starting immediately after each of five different sets of microinjections. The figure shows that the latency between the end of microinjections and the first episodes of REM sleep is much shorter after microinjections of saline + glutamate, AP5 + glutamate, and AMOA + glutamate compared with control saline + saline. In contrast, microinjections of GAMS + glutamate into the PPT increased REM sleep latency to about 3 h. REM sleep episodes are most frequent after microinjec-
tions of saline + glutamate, AP5 + glutamate, and AMOA + glutamate. For about 1 h after microinjections of saline + glutamate, AP5 + glutamate, and AMOA + glutamate, the duration of W episodes were much shorter than they were after the saline + saline microinjections. For about 2 h after microinjections of saline + glutamate, GAMS + glutamate, and AMOA + glutamate, the number of SWS episodes were fewer compared with after the control saline + saline microinjection. In contrast, for about 3 h after microinjection of AP5 + glutamate, the duration of SWS episodes was longer compared with SWS episode duration following saline + saline microinjections. These results demonstrate that microinjection of glutamate, alone or in combination with its specific receptor antagonist (AP5, GAMS, or AMOA), into the cholinergic compartment of the PPT changes the sleep-wake architecture of the rat.

Effects of glutamate and its receptor antagonists on REM sleep

Figure 3 shows representative polygraphic signs of REM sleep after control saline + saline (Fig. 3A) and saline + glutamate microinjections (Fig. 3B) into the cholinergic cell compartment of the PPT. Behavioral and polygraphic signs of REM sleep after glutamate application resembled those signs during REM sleep after control saline microinjections. During REM sleep, cortical EEG is activated, EMG records absence of muscle tone, EOG records REMs, hippocampal EEG records sinusoidal 5- to 7-Hz theta rhythms, and pontine EEG records frequent P waves.

The changes in the percentage of time spent in REM sleep after microinjection of glutamate with and without pretreatment of different glutamate receptor antagonists are summarized in Fig. 4. Two-way ANOVA indicated a significant main effect of treatment \( F(4,175) = 68.54, P < 0.0001 \), time \( F(5,175) = 9.48, P < 0.0001 \) and a significant treatment \( \times \) time interaction \( F(20,175) = 3.33, P < 0.001 \) on total percentage of time spent in REM sleep. The results of post hoc analysis (Scheffe \( F \) test) on total percentage of time spent in REM sleep are presented in Fig. 4. Post hoc analysis indicated that the total percentages of REM sleep at all six postinjection times (6 1-h epochs) after microinjection of saline + glutamate are significantly higher compared with after microinjection of control saline + saline. This evidence confirms our earlier observation that the microinjection of glutamate into the cholinergic cell compartment of the PPT increases REM sleep (Datta et al. 2001). In addition, post hoc analyses show that the total percentages of REM sleep after microinjection of GAMS + glutamate were significantly less in the first five postinjection times compared with after microinjection of control saline + saline. These results indicate that glutamate...
microinjection is unable to increase REM sleep when glutamate microinjection sites are pretreated with the kainate receptor antagonist. A second set of post hoc Scheffé F tests was done to determine the individual level of significant difference between glutamate without pretreatment of antagonist (saline + glutamate) and glutamate with pretreatment of glutamate receptor antagonists (AP5 + glutamate, GAMS + glutamate, or AMOA + glutamate) at six individual data points. This analysis indicated that after GAMS + glutamate microinjection, the percentages of REM sleep are significantly less in all six postinjection times (1–5 h, $P < 0.001$; and 6-hm $P < 0.01$) compared with after microinjection of saline + glutamate. However, after AP5 + glutamate microinjections, the percentages of REM sleep were less only during the second ($P < 0.01$) and third ($P < 0.05$) hour compared with after saline + glutamate microinjections. In contrast, the percentages of REM sleep after AMOA + glutamate microinjections were not significantly different compared with after saline + glutamate microinjections. To rule out the possibility that the 0.16 nmol doses of AP5 and AMOA may not be sufficient to block receptor-mediated action of the 0.16 nmol dose of glutamate, the doses of AP5 ($n = 3$ injections) and AMOA ($n = 3$ injections) were increased to 0.48 nmol (3 times the glutamate dose) for a limited number of trials. Again, the total percentages of REM sleep after a threefold higher dose (0.48 nmol) of AP5 and AMOA did not significantly change compared with after the lower doses (0.16 nmol) of AP5 and AMOA. These results indicate that only GAMS pretreatment effectively blocked the increase in REM sleep normally seen after glutamate microinjection.

Having documented the change in total percentage of REM sleep after microinjection of glutamatergic drugs, Fig. 5 illustrates effects on latency, number, and duration of REM sleep episodes after microinjection of glutamatergic drugs into the cholinergic cell compartment of the PPT. ANOVA indicated a significant change in the latency of the first episode of REM sleep between different treatments [$F(4,35) = 35.72, P < 0.0001$]. Post hoc analyses indicated that the latency to the first episode of REM sleep was significantly shorter after microinjection of saline + glutamate, AP5 + glutamate, and AMOA + glutamate compared with the control saline + saline. In contrast, compared with the control saline + saline microinjection, GAMS + glutamate microinjection significantly increased the REM sleep latency. The second set of post hoc analyses

![Figure 4](https://i.imgur.com/4G5G5G.png)

**FIG. 4.** Effects of pretreatment of specific glutamate receptor antagonists on PPT glutamate-induced REM sleep. Bar represents percentages (means and SE) of REM sleep during each of the 6-h periods after injection of saline + saline (Sal + Sal), saline + 0.16 nmol glutamate (Sal + Glut), 0.16 nmol AP5 + 0.16 nmol glutamate (AP5 + Glut), 0.16 nmol GAMS + 0.16 nmol glutamate (GAMS + Glut), and 0.16 nmol AMOA + 0.16 nmol glutamate (AMOA + Glut). Post hoc $t$-tests (Scheffe test): triangle represents the comparison with control saline + saline and asterisk represents the comparison with saline + glutamate. $\Delta$ or *: $P < 0.05$; $\Delta\Delta$ or **: $P < 0.01$; $\Delta\Delta\Delta$ or ***: $P < 0.001$.

![Figure 5](https://i.imgur.com/5G5G5G.png)

**FIG. 5.** Effects of pretreatment of specific glutamate receptor antagonists on the PPT-glutamate-induced latency (in minutes), number, and duration (in seconds) of REM sleep episodes. Top: REM sleep latencies (means ± SE) in min; middle: the number of REM sleep episodes per 6 h period (means ± SE); bottom: duration of REM sleep episodes (means ± SE) after microinjections. Data was collected after microinjections of: saline + saline (Sal + Sal), saline + 0.16 nmol glutamate (Sal + Glut), 0.16 nmol AP5 + 0.16 nmol glutamate (AP5 + Glut), 0.16 nmol GAMS + 0.16 nmol glutamate (GAMS + Glut), and 0.16 nmol AMOA + 0.16 nmol glutamate (AMOA + Glut). Post hoc $t$-tests (Scheffe test): triangle represents the comparison with control saline + saline and asterisk represents the comparison with saline + glutamate. $\Delta$: $P < 0.05$; $\Delta\Delta\Delta$ or ***: $P < 0.001$. [www.jn.org](http://www.jn.org)
indicated that the REM sleep latencies after microinjections of AP5 + glutamate, and AMOA + glutamate were not significantly different compared with REM sleep latency after microinjection of saline + glutamate. The REM sleep latency was increased significantly after microinjection of GAMS + glutamate compared with after saline + glutamate microinjection. In addition to the change in REM sleep latency, ANOVA indicated a significant change in the total number of REM sleep episodes between different treatments \([F(4,35) = 52.16, P < 0.0001]\). Post hoc analyses indicated that the total number of REM sleep episodes was significantly increased after microinjections of saline + glutamate, AP5 + glutamate, and AMOA + glutamate compared with the control saline + saline. However, the total number of REM sleep episodes was significantly smaller after microinjection of GAMS + glutamate compared with after both saline + saline and saline + glutamate microinjections. Post hoc analyses also indicated that the number of REM sleep episodes after microinjections of AP5 + glutamate, and AMOA + glutamate were not significantly different compared with after microinjection of saline + glutamate. ANOVA analysis indicated that the differences in mean duration of REM sleep episodes between different treatments are not statistically significant \([F(4,35) = 1.78, P = 0.15]\). These results demonstrate that the changes in the total percentages of time spent in REM sleep after microinjection of glutamatergic drugs into the PPT are due to the changes in the latency and number of REM sleep episodes.

**Effects of glutamate and its receptor antagonists on wakefulness and SWS**

After pretreatment of control saline or pretreatment of one of the glutamate receptor antagonists, glutamate microinjection yielded different changes in the percentage of time spent in W and SWS. These results are summarized in Table 1. Two-way ANOVA indicated a significant main effect of treatment \([F(4,175) = 7.41, P < 0.001]\), time \([F(5,175) = 51.37, P < 0.0001]\), and a significant treatment \(\times\) time interaction \([F(20,175) = 2.83, P < 0.01]\) on total percentage of time spent in W. Post hoc analyses indicated that the microinjection of saline + glutamate and AMOA + glutamate reduced total percentages of W significantly \((P < 0.05)\) compared with the microinjection of control saline + saline, only during the first hour. Compared to the control saline + saline, microinjection of AP5 + glutamate reduced W significantly \((P < 0.05)\) in the first, second, and sixth hours of recordings. In contrast, microinjection of GAMS + glutamate increased W significantly \((P < 0.05)\) during the fourth and fifth hours of recordings. Post hoc analyses also indicated that the total percentages of W after AP5 + glutamate and AMOA + glutamate are not significantly different when compared with the total percentages of W after saline + glutamate. The total percentages of W after GAMS + glutamate during the first, fourth, and fifth hours of recordings were significantly higher \((P < 0.05)\) compared with the total percentages of W after saline + glutamate microinjections (Table 1).

Two-way ANOVA indicated a significant main effect of treatment \([F(4,175) = 7.0, P < 0.001]\), time \([F(5,175) = 34.0, P < 0.0001]\), and a significant treatment \(\times\) time interaction \([F(20,175) = 1.84, P < 0.05]\) on total percentages of time spent in SWS. Post hoc analyses indicated that after microinjection of saline + glutamate, the total percentages of SWS were significantly reduced \((P < 0.05)\) during hours 2, 3, and 6 of the postinjection period compared with after control saline + saline microinjections (Table 1). However, the total percentages of SWS after AP5 + glutamate, GAMS + glutamate, and AMOA + glutamate are not significantly different compared with after saline + saline microinjection. Post hoc analyses also show that compared with the microinjection of saline + glutamate, AP5 + glutamate microinjection increases SWS significantly (see Table 1 for the level of significance) during the first, second, and sixth hours of recordings. Microinjections of GAMS + glutamate increase SWS significantly \((P < 0.05)\) only during the third hour compared with the microinjection of saline + glutamate. The total percentages of SWS after AMOA + glutamate microinjections are not significantly different compared with after saline + glutamate microinjections (Table 1).

### TABLE 1. Effects of pretreatment of glutamate receptor antagonists on the PPT glutamate-induced changes in wakefulness and slow-wave sleep

<table>
<thead>
<tr>
<th>Substance Injected</th>
<th>n</th>
<th>1 h (min)</th>
<th>2 h (min)</th>
<th>3 h (min)</th>
<th>4 h (min)</th>
<th>5 h (min)</th>
<th>6 h (min)</th>
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<tbody>
<tr>
<td>Wakefulness</td>
<td></td>
<td></td>
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<tr>
<td>Saline + saline</td>
<td>12</td>
<td>61.9 ± 2.8</td>
<td>37.5 ± 2.2</td>
<td>28.9 ± 1.8</td>
<td>29.6 ± 2.9</td>
<td>27.2 ± 2.5</td>
<td>28.8 ± 2.4</td>
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<tr>
<td>Saline + glut</td>
<td>7</td>
<td>47.8 ± 3.7</td>
<td>33.3 ± 3.9</td>
<td>27.9 ± 4.6</td>
<td>29.5 ± 2.3</td>
<td>23.3 ± 3.9</td>
<td>29.1 ± 2.3</td>
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<tr>
<td>AP-5 + glut</td>
<td>6</td>
<td>34.7 ± 5.2</td>
<td>17.0 ± 2.9</td>
<td>23.3 ± 5.1</td>
<td>17.8 ± 2.5</td>
<td>27.1 ± 8.3</td>
<td>24.0 ± 3.1</td>
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<tr>
<td>GAMS + glut</td>
<td>8</td>
<td>66.3 ± 4.3*</td>
<td>43.2 ± 9.0</td>
<td>27.4 ± 3.6</td>
<td>48.6 ± 2.6*</td>
<td>47.2 ± 3.8*</td>
<td>25.0 ± 2.8</td>
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<td>7</td>
<td>49.1 ± 4.2</td>
<td>33.7 ± 4.9</td>
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<td>37.7 ± 2.7</td>
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<td>61.7 ± 2.7</td>
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<tr>
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<td>30.5 ± 3.0</td>
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<tr>
<td>AP-5 + glut</td>
<td>6</td>
<td>49.3 ± 7.9*</td>
<td>66.7 ± 2.5**</td>
<td>61.8 ± 4.6</td>
<td>65.8 ± 2.4</td>
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<tr>
<td>AMOA + glut</td>
<td>7</td>
<td>33.8 ± 5.1</td>
<td>45.3 ± 6.9</td>
<td>52.5 ± 5.8</td>
<td>49.9 ± 2.3</td>
<td>56.6 ± 1.1</td>
<td>52.1 ± 3.0</td>
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Effects of pretreatment of glutamate receptor antagonists on the pedunculopontine tegmentum (PPT)-glutamate-induced changes in wakefulness and slow-wave sleep. Percentages (means ± SE) of wakefulness and slow-wave sleep during each of the 6-h sessions after injection of saline + saline, saline + 0.16 nmol glutamate (saline + glut), 0.16 nmol AP5 + 0.16 nmol glutamate (AP5 + glut), 0.16 nmol GAMS + 0.16 nmol glutamate (GAMS + glut), and 0.16 nmol AMOA + 0.16 nmol glutamate (AMOA + glut). \(n\) = number of microinjection sessions. Post hoc \(t\)-tests (Scheffe test): triangle represents the comparison with control saline + saline and asterisk represents the comparison with saline + glutamate. \(\times\) or *: \(P < 0.05\); **: \(P < 0.01\).
The principal findings of this study are that microinjection of the excitatory amino acid L-glutamate into the cholinergic cell compartment of the PPT increased the total amount of REM sleep, microinjection of specific kainate receptor antagonist GAMS into the cholinergic cell compartment of the PPT blocked glutamate-induced REM sleep, and microinjections of NMDA and AMPA receptor antagonists AP5 and AMOA were unable to block glutamate-induced increases in REM sleep. These results show for the first time that PPT glutamate-induced REM sleep is mediated through the kainate receptor.

Role of the PPT in REM sleep generation

The notion that PPT neurons are involved in the generation of REM sleep comes from extracellular single-cell recording studies (Datta 1995; El-Mansari et al. 1989; Saito et al. 1977; Steriade et al. 1990). These studies recorded the activity of a group of PPT cholinergic neurons that increased their firing rates during REM sleep. Although these neuronal correlating studies have suggested that excitation of PPT cells may be causal for the generation of REM sleep, these single-cell recording studies were not definitive proof of a causal relationship between the activation of PPT cholinergic cells and REM sleep generation. Those earlier studies have raised the important question of how PPT cholinergic cells are activated to trigger REM sleep. This was probably one of the most important questions about the mechanism of REM sleep generation. More direct evidence implicating the excitation of PPT cholinergic cells in the generation of REM sleep comes from recent local microinjection studies (Datta and Siwek 1997; Datta et al. 2001). These microinjection studies have shown that microinjection of glutamate into the cholinergic cell compartment of the PPT of the freely moving cat and rat increases REM sleep (Datta and Siwek 1997; Datta et al. 2001). The results of this present study demonstrate that a single microinjection of 0.16 nmol of glutamate into the cholinergic cell compartment of the PPT increased REM sleep and confirms earlier glutamate microinjection studies (Datta and Siwek 1997; Datta et al. 2001). These microinjection studies provide direct evidence that activation of PPT cholinergic cells is causal for the generation of REM sleep. Furthermore, these results indicate that the excitatory neurotransmitter, glutamate, is involved in the regulation of PPT cell activity that contributes to the generation of REM sleep.

The increase in REM sleep induced by glutamate microinjection leads to the question of which receptor type is activated in the cholinergic cell compartment of the PPT. It is known that glutamatergic actions in the neuronal system are mediated through six different types of receptors. Using immuno-histochemical techniques, a number of recent studies have demonstrated the presence of NMDA, kainate, AMPA, and metabotropic receptors within the cholinergic cell compartment of the PPT (Martin et al. 1993; Morin et al. 1989; Ohishi et al. 1993; Petralia and Wenthold 1992; Petralia et al. 1994a–c; Sato et al. 1993; Shigemoto et al. 1992; Watanabe et al. 1994). In the present study, pretreatment of GAMS, a specific antagonist for the kainate receptor (Davies and Watkins 1985; Frandsen et al. 1990; Krosggaard-Larsen et al. 1991; Turski et al. 1985), effectively blocked glutamate-induced REM sleep. This effect of GAMS pretreatment indicates that, within the PPT, glutamate-induced REM sleep is mediated by kainate receptors. Only one other study has suggested that the brain stem kainate receptors may be involved in REM sleep (Onoe and Sakai 1995). In that study, a very high concentration (50–100 μM) of kainic acid was continuously infused into the locus coeruleus alpha of the cat to increase the total amount of REM sleep (Onoe and Sakai 1995). Because the kainic acid concentration was very high and continuously infused for a long period of time, it was difficult to localize the actual site of the kainic acid-induced REM sleep response in the cat. It is also unfortunate that we cannot confirm this kainate-receptor-mediated response by direct application of kainic acid because, in behaving rats, microinjection of kainic acid into the brain stem produces seizure activity, circling behavior, and ultimately cell body lesions (Obrenovitch and Urenjak 1997; Sperk et al. 1985). It is also known that kainic acid not only activates kainate receptor but also activates AMPA receptor (Jayaraman 1998; Patneau and Mayer 1991; Sommer and Seeburg 1992; Zona et al. 2000). For these reasons, use of kainic acid for the identification of glutamate-induced REM sleep is not practical. Glutamate-induced REM sleep remained unchanged when specific NMDA and AMPA receptors were blocked with pretreatment of their specific receptor antagonists, AP5 and AMOA (Davies and Watkins 1982; Davis et al. 1992; Krosggaard-Larsen et al. 1991; Porter and Greenamyre 1994), an observation that further strengthens the case for kainate receptor involvement in glutamate-induced REM sleep. The present study also demonstrates that blocking the NMDA receptor with specific antagonist increases SWS by suppressing wakefulness. Also it has been shown that the activation of NMDA receptors in the PPT activates locomotion (Garcia-Rill et al. 1990). The results of the present study together with earlier studies suggest that different types of glutamate receptors within the cholinergic cell compartment of the PPT are involved in different physiological functions.

Limitations and future studies

Central microinjection is a powerful means of affecting brain physiology and behavior because it allows us to explore the regulatory role on sleep/waking behavior of suspected intrinsic neurotransmitters in a restricted brain region. However, in evaluating the outcome of the present study, it is important to consider that the central microinjection method does not permit knowledge of the absolute concentration of ligand (glutamate and its antagonists) at the location of the receptors mediating the behavioral response. Drugs are typically injected at high concentrations in small volumes, and following injection, concentrations probably decline rapidly as drugs diffuse away from the injection site. It is, however, reasonable to assume that agents with similar chemical properties will have similar diffusion rates when injected with the same volume and concentration. Therefore it is reasonable to believe that the REM sleep effect observed after kainate-receptor-blockage was a receptor- and site-specific response.

Another unresolved question that can be investigated in future studies, is whether metabotropic receptors are also involved in the glutamate-induced REM sleep generation process. Our preliminary unpublished results show that metabotropic receptor antagonists like (RS)-1-aminoisobutan-1,5-dicarboxylic acid/UPF 523, (2S)-alpha-ethylglutamic acid, and...
is not surprising that the metabotropic receptors may not be effective at blocking glutamate-induced REM sleep. Because the latency of glutamate-induced REM sleep is relatively short, it is not surprising that the metabotropic receptors may not be involved in the glutamate-induced REM sleep. Normally, metabotropic receptor mediated responses are longer-latency responses (Michaelis 1998).

In conclusion, the present study shows for the first time that REM sleep induced by glutamate injection into the cholinergic cell compartment of the rat PPT specifically involves the activity of kainate receptors. The data provide a novel perspective on the regulatory aspect of PPT cell activity in the generation of REM sleep. The results also suggest that the different types of glutamate receptors within the PPT may be involved in different types of physiological functions. The results encourage future studies to explore the role of glutamate receptors in the regulation of natural REM sleep.

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