Activation of Pedunculopontine Tegmental PKA Prevents GABA_B Receptor Activation–Mediated Rapid Eye Movement Sleep Suppression in the Freely Moving Rat

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INTRODUCTION

Research in the past 10 yr, ranging from developmental maturation to genetic model of Alzheimer’s, has provided evidence that the cholinergic cells in the pedunculopontine tegmentum (PPT) and laterodorsal tegmentum (LDT) are critically involved in the physiological regulation of rapid eye movement (REM) sleep (Datta 1995; Deurveilher and Hennevin 2001; Garcia-Rill 1997; Kobayashi et al. 2004a,b; Zhang et al. 2005). The PPT, situated in the dorsolateral mesopontine tegmentum, contains a prominent group of cholinergic and some noncholinergic neurons, which project widely throughout the brain stem and forebrain (for reviews, see Datta 1995; Garcia-Rill 1991; Jia et al. 2003; Jones 2004; Lai et al. 1993). Single cell recordings from the PPT/LDT of behaving cats and rats have identified several different types of cells whose firing rates correlate with both wakefulness and REM sleep (Datta 1995; Datta and Siwek 2002; El-Mansari et al. 1989; Steriade et al. 1990; Thakkar et al. 1998). Some of the recent pharmacological studies in cats and rats have shown that the activation of PPT kainate type glutamate receptors induces REM sleep (Datta 2002; Datta and Siwek 2002; Datta et al. 2002). Other studies have shown that the activation of PPT GABA_B receptors inhibit PPT REM-ON cells activity and suppresses REM sleep (Datta et al. 2003; Ulloor et al. 2004). Despite tremendous progress in the identification of specific neurotransmitters and receptors involved in the regulation of the PPT cells neuronal activity and REM sleep, very few attempts have been made to study the possible intracellular signal transduction mechanisms of the PPT that may be involved in the regulation of REM sleep.

It is known that the GABA_B receptors couple to Gi/Go G proteins (Couve et al. 2000; Kerr and Ong 1995; Mody et al. 1994; Robbins et al. 2001; Sivilotti and Nistri 1991; Takahashi et al. 1998; Thompson 1994). It is also known that Gi/Go G proteins inhibit adenylyl cyclase (AC) and inhibition of AC prevents activation of the cAMP-protein kinase A (PKA) signal transduction pathway (Gilman 1987; Marinissen and Gutkind 2001). Because it has been shown that the activation of GABA_B receptors within the PPT suppresses activity of REM-ON cells and REM sleep in the freely moving rat (Ulloor et al. 2004), it is reasonable to suggest that the PPT GABA_B receptor activation–induced suppression of REM sleep may have been caused by the inhibition of AC that ultimately prevented activation of cAMP-PKA. In support of this suggestion, two recent studies have shown that the local application of A13.5-cyclic monophosphothioate triethylamine (SpCAMPS), and vehicle control were microinjected into the PPT in selected combinations to determine effects on sleep-waking states of chronically instrumented, freely moving rats. Microinjection of SpCAMPS (1.5 nmol) induced REM sleep within a short latency (12.1 ± 3.6 min) compared with vehicle control microinjection (60.0 ± 6.5 min). On the contrary, microinjection of baclofen (1.5 nmol) suppressed REM sleep by delaying its appearance for ~183 min; however, the suppression of REM sleep by baclofen was prevented by a subsequent microinjection of SpCAMPS. These results provide evidence that the activation of cAMP-PKA within the PPT can successfully block the GABA_B receptor activation–mediated REM sleep suppression effect. These findings suggest that the PPT GABA_B receptor activation–mediated REM sleep regulating mechanism involves inactivation of cAMP-PKA signaling in the freely moving rat.
receptor, or both cAMP-PKA and GABA_B receptor within the PPT. Our results show that the activation of PPT cAMP-PKA induces REM sleep. Although activation of PPT GABA_B receptors suppresses REM sleep, these results also show that the activation of cAMP-PKA could rescue REM sleep from the GABA_B receptor activation–induced suppression. The results of these experiments indicate that, in the PPT, GABA_B receptor activation–mediated REM sleep regulation involves inactivation of the cAMP-PKA system.

METHODS

Subjects and housing

Experiments were performed on 28 adult male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing between 250 and 350 g. The rats were housed individually at 24°C with food and water provided ad libitum with lights on from 7:00 AM to 7:00 PM (light cycle) and off from 7:00 PM to 7:00 AM (dark cycle). The principles for the care and use of laboratory animals in research, as outlined by the National Institutes of Health Publication no. 85–23 (1985), were strictly followed.

Drug and vehicle for microinjections

In this study, GABA_B receptors in the PPT were activated by a GABA_B receptor–specific agonist, baclofen hydrochloride (baclofen; molecular weight: 250.13), purchased from Sigma Chemicals (St. Louis, MO). The selection of baclofen was based on the selective agonistic effects on GABA_B receptors (Bowery 1993; Enna and Maggi 1979; Falch et al. 1986; Fromm et al. 1990; Hill and Bowery 1981; Hong and Henry 1991; Kerr and Ong 1995; Misgeld et al. 1995; Paredez and Agmo 1989; Ulloor et al. 2004). In addition to specificity, the above studies have shown that baclofen is readily soluble in water and accessible to extracellular receptors. Baclofen was dissolved in 0.9% saline to make 1.5 nmol/100 nl dose. The optimum dose of baclofen (1.5 nmol/100 nl) was predetermined from an earlier dose–response study (Ulloor et al. 2004). Another drug used in this study was an activator of cAMP-PKA activity, Sp-cAMPS triethylamine (SpCAMPS; molecular weight 446.46), also purchased from Sigma Chemicals. The selection of this drug was based on the selective activation effect on intracellular cAMP-PKA activity (Capece and Lydic 1997; Cook et al. 1995; Punch et al. 1997; Wang et al. 1991). In addition to the selectivity, SpCAMPS was suitable for this study because these studies have shown that it is water soluble, cell membrane permeable, has reversible effects, and has been successfully in microinjection studies in behaving animals (Capece and Lydic 1997; Cook et al. 1995; Punch et al. 1997; Wang et al. 1991, 1993). SpCAMPS was dissolved in 0.9% saline to make 1.5 nmol/100 nl dose. This 0.9% saline (100 nl) was also used for the vehicle control microinjection. Control saline and drug solutions were freshly prepared under sterile conditions before each use.

Surgical procedures and implantation of electrodes

Treatment of the animals and surgical procedures were in accordance with an approved institutional animal welfare protocol (AN-14084). Efforts were made to minimize the number of animals used and their suffering. Rats were anesthetized with pentobarbital sodium (40 mg/kg, ip), placed in the stereotaxic apparatus, and secured using blunt rodent ear bars (Paxinos and Watson 1997). With the use of sterile procedures, cortical EEG, dorsal neck muscle EMG, and hippocampal EEG (to record theta waves) recording electrodes were chronically implanted, as described elsewhere (Datta 2000). In addition, a stainless steel guide tube (26 gauge), with an equal-length stylett inside, was stereotaxically implanted 2 mm above the PPT (in relation to stereotaxic “0”): anterior, 1.0 mm; lateral, 1.8 mm; and horizontal, 3.0 mm) as described previously (Datta 2002; Datta et al. 2002).

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 microinjection into the PPT. 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. In this study, a double-microinjection protocol was adopted as described earlier (Datta 2002; 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 stylett was removed, and a control vehicle-filled (100 nl volume of 0.9% saline) or any one of the two drug-filled (1.5 mmol in 100 nl, SpCAMPS or Baclofen) injectors were introduced through the guide tube for the first injection. One minute after the insertion of the injector cannulae, 100 nl of control saline or any one of the two drugs was microinjected over a 60-s period (Pump II Pico Plus, Harvard Apparatus, Holliston, MA). The cannula was gently withdrawn 2 min after the injection, and the stylett was reintroduced inside the guide tube. For the second injection, control saline or SpCAMPS was injected into the same site using the same injector system as described for the first injection. During the microinjections, the animals were free to move around the cage with the cannulae in place. Because of the extended tubing, the injections could be made without restraining mobility of the animal. Immediately after completion of the microinjection procedure, polygraphic variables were recorded continuously for 6 h (between 10:00 AM and 4:00 PM), when rats would normally be sleeping (Datta 2000). Each of these rats received a total of two microinjections (100 nl each) into the PPT (left or right side) in a single experimental recording session. None of the rats were used for more than one microinjection recording session.

The injection protocol was designed so that 28 rats were divided equally into four groups (n = 7 rats/group). The first group received saline + saline injections (control group) into the PPT. The second group received baclofen + saline injections (GABA_B receptor activation group) into the PPT. The third group received baclofen + SpCAMPS injections (GABA_B receptor + PKA activation group) into the PPT. The fourth group received SpCAMPS + saline injections (PKA activation group) into the PPT. At the end of experimental session and before perfusion, with the use of same injector used for saline or drugs, 100 nl of black ink was microinjected 1 mm dorsal to each injection site for localizing the injection sites as described earlier (Datta 2002; Datta et al. 2001a).

Determination of behavioral states and data analysis

For the purpose of determining possible effects on sleep and wakefulness, polygraphic data were captured on-line in a computer using “Gamma” software (Grass Product Group, Astro-Med, West Warwick, RI). From this captured data, three behavioral states were distinguished and scored visually using “Rodent Sleep Stager” software (Grass Product Group, Astro-Med) as described earlier (Datta 2002; Datta et al. 2004). The behavioral states of wakefulness (W), slow-wave sleep (SWS), and REM sleep were scored in successive 10-s epochs. The polygraphic measures provided the following dependent variables, which were quantified for each recording session: 1) percentage of recording time spent in W, SWS, and REM sleep; 2) latency to onset of the first episode of REM sleep after the onset of recordings; 3) total number of REM sleep episodes; and 4) mean duration of REM sleep episodes. The effects of the four different
treatments (saline + saline; baclofen + saline; baclofen + SpCAMPS; SpCAMPS + saline) on the percentages of W, SWS, and REM sleep were statistically analyzed using a two-way ANOVA with time as a repeated-measure variable (6 levels corresponding to 1 h-each after injections) and treatment as a between-group variable (4 levels corresponding to the 4 different treatment groups). After a two-way ANOVA, post hoc Scheffe F tests were done to determine the individual levels of significant difference between the control (saline + saline) and the three different drug treatment protocols at six individual data points. Individual post hoc tests were also done to compare between three different drug treatment protocols. The latency, number, and duration of REM sleep episodes were analyzed using a one-factor ANOVA followed by post hoc Scheffe F tests. Statistical analyses (2-way ANOVA, 1-factor 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 sodium (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 2002; Datta et al. 2001a). This NADPH-diaphorase staining was done to identify the cholinergic cell compartment of the PPT (Bredt and Snyder 1992; Bredt et al. 1991; Datta 2002; Datta et al. 1997; Hope et al. 1991; Vincent et al. 1983).

**RESULTS**

Figure 1 shows the anatomical location of microinjection sites. Histological identification showed that all four combinations of microinjections (control saline + saline, baclofen + saline, baclofen + SpCAMPS, and SpCAMPS + saline) were within the PPT (Fig. 1). The microinjection sites were anatomically comparable between four different treatment groups; therefore, the analysis below quantifies the effects of baclofen + saline (n = 7 rats), baclofen + SpCAMPS (n = 7 rats) and SpCAMPS + saline (n = 7 rats) microinjected into the PPT on the W, SWS, and REM sleep states.

Figure 2 shows representative sleep-wake architectures for the 6-h postinjection recording sessions (10:00 AM to 4:00 PM) starting immediately after each of four different sets of microinjections. The figure shows that the latency between the end of microinjection and the first episode of REM sleep was much shorter after microinjections of SpCAMPS + saline compared with after saline + saline. In contrast, with microinjections of baclofen + saline, REM sleep latency was about 2 h longer than after saline + saline. However, after baclofen + SpCAMPS microinjections, REM sleep latency was shorter than after saline + saline and after baclofen + saline microinjections. Microinjections of baclofen + saline decreased latency of the first episode and increased duration of each SWS episodes compared with after saline + saline, SpCAMPS + saline, or baclofen + SpCAMPS. For about 2 h after microinjections of SpCAMPS + saline, baclofen + saline, and baclofen + SpCAMPS, the duration of W episodes were shorter compared with after saline microinjections. These results showed that these microinjections into the PPT did, in fact, change the sleep-wake architecture of the rat.

**Effects on wakefulness**

The changes in the percentage of time spent in W after microinjections are summarized in Fig. 3. Two-way ANOVA indicated a significant main effect of treatment (df = 3, F = 9.32, P < 0.001), time (df = 5, F = 19.24, P < 0.001), and a significant treatment × time interaction (df = 15, F = 5.62, P < 0.001) on the total percentage of time spent in W. The results of post hoc analysis (Scheffe F tests) on total percentage of time spent in W are presented in Fig. 3. Post hoc analysis indicated that the microinjections of baclofen + saline and baclofen + SpCAMPS reduced total percentages of W significantly (F = 44.40 and 17.89; P < 0.001) compared with the microinjections of saline + saline (control group), only during the first hour. During the second hour, baclofen + saline and baclofen + SpCAMPS microinjected groups total percentages of W were less compared with saline control microinjections, but this reduction did not reach significance. Next, the total percentages of W in the baclofen + SpCAMPS and SpCAMPS + saline microinjection groups were compared (Scheffe F test) with the baclofen + saline microinjection group. This test revealed that the total percentage of W in the baclofen + SpCAMPS group was significantly higher only in the first hour.
The changes in the percentage of time spent in SWS after microinjections into the PPT are summarized in Fig. 4. A two-way ANOVA indicated a significant main effect of treatment (df = 3, F = 32.57, P < 0.001), time (df = 5, F = 14.19, P < 0.001), and a significant treatment × time interaction (df = 15, F = 3.61, P < 0.001) for total percentage of time spent in SWS. The results of post hoc analysis indicated that, after microinjections of baclofen + saline, the total percentages of SWS were significantly increased during the first (F = 41.81; P < 0.001), second (F = 5.0; P < 0.01), and third (F = 11.12; P < 0.001) hours of the postinjection period compared with after control saline + saline microinjections. Similar comparisons revealed that, after baclofen + SpCAMPS microinjections, total percentage of SWS increased significantly (F = 9.25; P < 0.001) only during the first hour compared with after saline + saline microinjections. The next set of post hoc tests (Scheffe F test) compared total percentages of SWS in the baclofen + SpCAMPS and SpCAMPS + saline microinjection groups with the baclofen + saline microinjection group. This test revealed that the total percentages of SWS in the baclofen + SpCAMPS and SpCAMPS + saline groups were significantly less during the first (F = 11.78; P < 0.001 and F = 53.15; P < 0.001), second (F = 3.26; P < 0.05 and F = 6.84; P < 0.01), and third (F = 18.89; P < 0.001 and F = 6.11; P < 0.001, respectively) hours of the postinjection period. A similar post hoc test between the baclofen + SpCAMPS and SpCAMPS + saline groups revealed that the total percentages of SWS in the SpCAMPS + saline group were significantly less in the first (F = 14.89; P < 0.001), second (F = 4.16; P < 0.05), and third hour of microinjections.
Effects on REM sleep

The changes in the percentage of time spent in REM sleep after microinjections into the PPT are summarized in Fig. 5. A two-way ANOVA indicated a significant main effect of treatment (df = 3, F = 75.94, P < 0.001), time (df = 5, F = 44.92, P < 0.001), and treatment × time interaction (df = 15, F = 11.81, 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. 5. Microinjections of baclofen + saline into the PPT suppressed REM sleep completely for the first 3 h of the postinjection period. During the fourth hour after injection, REM sleep began to reappear, but the total percentages of REM sleep were significantly less both during the fourth (F = 21.05, P < 0.001) and fifth (F = 3.82, P < 0.05) hours compared with their saline + saline control values. On the contrary, in the first postinjection hour, the total percentages of REM sleep were significantly higher in the baclofen + SpCAMPS (F = 17.72, P < 0.001) and SpCAMPS + saline (F = 26.5, P < 0.001) groups compared with after microinjections of the control saline + saline group. Similar comparisons after baclofen + saline microinjection revealed that the total percentages of REM sleep after microinjections of both baclofen + SpCAMPS and SpCAMPS + saline were significantly higher during postinjection hours 1–5. The total percentages of REM sleep were not significantly different between the baclofen + SpCAMPS and SpCAMPS + saline microinjection groups. These results indicate that the microinjection of baclofen into the PPT suppress REM sleep, and this baclofen-induced REM sleep–suppressing effect could be eliminated by the subsequent application of SpCAMPS.

Having documented the changes in REM sleep after microinjections of baclofen + saline, baclofen + SpCAMPS, and SpCAMPS + saline into the PPT, Fig. 6 shows the effects on latency, number, and duration of REM sleep episodes after these microinjections. One-factor ANOVA indicated a significant change in the latency of the first episode of REM sleep between different treatments (df = 3, F = 1795.17, P < 0.001). Post hoc (Scheffe F test) analyses indicated that the latency to the first episode of REM sleep was significantly higher (F = 73.34, P < 0.001) after microinjections of baclofen + saline compared with after microinjections of control saline + saline. In contrast, latency of the first episode of REM sleep was significantly reduced after microinjections of baclofen + SpCAMPS (F = 69.69, P < 0.001) and SpCAMPS + saline (F = 110.53, P < 0.001) compared with after control saline + saline microinjection. The second set of post hoc analyses indicated that the REM sleep latencies after microinjections of baclofen + SpCAMPS and SpCAMPS + saline were significantly less compared with REM sleep latency after baclofen + saline microinjection. In addition to changes in REM sleep latency, one-factor ANOVA indicated a significant change in the total number of REM sleep episodes between different treatments (df = 3, F = 59.42, P < 0.001). Post hoc analyses indicated that the total number of REM sleep episodes was significantly (F = 31.07, P < 0.001) decreased after baclofen + saline microinjection compared with the control saline + saline microinjection. Post hoc analyses also indicated that the total number of REM sleep episodes after microinjections of baclofen + SpCAMPS and SpCAMPS + saline were not significantly different compared with after microinjection of saline + saline. Compared with after microinjection of baclofen + saline, the total number of REM sleep episodes were significantly higher after baclofen + SpCAMPS (F = 33.91, P < 0.001) and after SpCAMPS + saline (F = 50.0, P < 0.001) microinjections. Similar one-factor ANOVA and post hoc analyses did not reveal any significant difference in the mean duration of REM sleep episodes between different treatment groups. These results showed that the reduction in the total percentage of time spent in REM sleep after microinjection of baclofen + saline was caused by an increase in the latency and decrease in the number of REM sleep episodes. These results also showed that the increased REM sleep after baclofen + SpCAMPS and SpCAMPS + saline are only caused by a decrease in the REM sleep latency.

**Discussion**

In this study, I examined the role of cAMP-PKA signal transduction pathway in the PPT for the regulation of GABA<sub>B</sub> receptor activation–induced REM sleep suppression. The principal findings of this study are that 1) microinjection of the GABA<sub>B</sub> receptor selective agonist, baclofen, into the PPT suppressed REM sleep, 2) microinjection of the cAMP-PKA activator, SpCAMPS, into the PPT increased REM sleep, and 3) microinjection of SpCAMPS into the PPT blocked the baclofen-induced REM sleep suppression effect. These results showed that the activation of PPT cAMP-PKA increases REM sleep and also blocks PPT GABA<sub>B</sub> receptor activation–induced REM sleep suppression. The findings are discussed in relation to ongoing efforts to understand the PPT neurotransmitter receptor activation–dependent intracellular signaling mechanisms for the regulation of REM sleep.
GABAB receptor selective agonist suppresses REM sleep. In this study, I showed that the local application of GABA activates GABAB receptors and suppresses REM sleep through inhibition of AC (Bandyopadhya et al. 2006). Thus it is reasonable to suggest that the cAMP-PKA intracellular signaling system in the PPT is critically involved in the regulation of PPT cell activity and REM sleep. Besides these studies, a number of other studies in behaving cats and rats have looked at the involvement of signal transduction pathways in the pontine reticular formation (PRF), another site known to be involved in the regulation of REM sleep (Capece and Lydic 1997; Marks and Birabil 2000; Shuman et al. 1995). These studies have indicated that the signal transduction pathway activated by muscarinic cholinergic receptors involves a pertussis toxin–sensitive G protein, AC, cAMP, and PKA. In the rat, this signal transduction pathway in the PRF is also shown to be involved in spontaneous REM sleep (Marks and Birabil 2000). Thus it is reasonable to conclude that, depending on the anatomical site and involved neurotransmitter receptors, activation of the intracellular cAMP-PKA signaling system is excited and/or inhibited for the regulation of REM sleep.

The results of this study also showed that the local activation of intracellular cAMP-PKA blocked PPT GABA receptor activation–induced REM sleep suppression. It is known that the activation of GABA receptors is capable of inhibiting AC (Couve et al. 2000; Kerr and Ong 1995; Mody et al. 1994; Robbins et al. 2001; Sivilotti and Nistri 1991; Takahashi et al. 1998; Thompson 1994), and inhibition of AC prevents activation of the cAMP-PKA signal transduction pathway (Gilman 1987; Marinissen and Gutkind 2001). Thus it is logical to suggest that the PPT GABA receptor activation–mediated REM sleep suppression effect may have been caused by the inhibition of cAMP-PKA signal transduction pathway. Earlier studies have shown that the inhibition of both AC and cAMP-PKA in the PPT suppresses REM sleep (Bandyopadhya et al. 2006; Datta and Prutzman 2005).

The results of this study are particularly valuable given recent evidence from studies in both academic and industrial settings indicating that novel compounds designed to modify intracellular signaling molecules have promising therapeutic potential for the treatment of neurodegenerative disorders, endogenous depression, and other psychiatric disorders (Coyle et al. 2003; Moon-Edley and Graybiel 1983; Reese et al. 1995; Scarnati and Fiorio 1997; Scarnati et al. 1988; Steininger et al. 1992). The possibility that endogenous GABAergic neurotransmission in the PPT may modulate PPT cell activity is also supported by the presence of different types of GABA receptors and GABAergic fibers in the PPT (Bowery et al. 1987; Chu et al. 1990; Kosaka et al. 1987; Mugnaini and Oertel 1985). Collectively, the results of this study and results of earlier studies discussed above provide conclusive evidence to support that the endogenous GABA released in the PPT regulates physiological REM sleep and wakefulness by activating GABA receptors.

This study showed that the activation of PPT cAMP-PKA by local application of SpCAMPS, an activator of cAMP-PKA activation, increases REM sleep. This result suggests that the activation of PPT cAMP-PKA is involved in the generation of REM sleep. Because earlier studies have shown that the inhibition of AC (Datta and Prutzman 2005) and cAMP-PKA (Bandyopadhya et al. 2006) in the PPT suppresses REM sleep, it is reasonable to suggest that the cAMP-PKA intracellular signaling system in the PPT is critically involved in the regulation of PPT cell activity and REM sleep. Besides these studies, a number of other studies in behaving cats and rats have looked at the involvement of signal transduction pathways in the pontine reticular formation (PRF), another site known to be involved in the regulation of REM sleep (Capece and Lydic 1997; Marks and Birabil 2000; Shuman et al. 1995). These studies have indicated that the signal transduction pathway activated by muscarinic cholinergic receptors involves a pertussis toxin–sensitive G protein, AC, cAMP, and PKA. In the rat, this signal transduction pathway in the PRF is also shown to be involved in spontaneous REM sleep (Marks and Birabil 2000). Thus it is reasonable to conclude that, depending on the anatomical site and involved neurotransmitter receptors, activation of the intracellular cAMP-PKA signaling system is excited and/or inhibited for the regulation of REM sleep.

FIG. 6. Effects on latency, total number, and mean duration of REM sleep episodes observed after microinjection of baclofen and SpCAMPS into PPT. Data presented as mean + SE. Note that compared with control saline + saline, microinjections of baclofen + saline increased latency for 1st episode of REM sleep and decreased number of REM sleep episodes. On the contrary, microinjections of baclofen + SpCAMPS and SpCAMPS + saline decreased latency for 1st episode of REM sleep. Post hoc tests (Scheffe test): *comparison with control saline + saline and *comparison with Baclofen + saline. *** or ΔΔΔp < 0.001.
Depression, and other major psychiatric and neurological disorders are closely related to those of normal REM sleep (Garcia-Rill 1997; Garcia-Rill et al. 2003; Rye 1997), there may be a convergence of mechanisms at the level of the brain stem. Therefore the results of these experiments will be important in developing rational schematics of pharmacological therapies for these persistent and debilitating disorders.

I chose to study chronically implanted freely moving rats because this preparation provides the most physiological approach to a longitudinal analysis of the behavioral and electrographic events related to the wake-sleep cycle (Datta and Siwek 2002; Datta et al. 2002; Ulloor et al. 2004). After the adaptation period of each experimental condition, the rats showed regular values of wake and sleep stages. Thus the microinjections of a pharmacologically active selective signal transduction-altering activator that affected electrophysiological signs of wake-sleep patterns could be evaluated accurately (Capece and Lydic 1997; Marks and Birabil 2000). Moreover, understanding the role of the intracellular signal transduction pathway in a behaviorally active living organism is a vital step toward creating ways to converge functional biochemistry and integrative physiology. However, a major limitation of the microinjection method relates to the diversity in the neurochemical and functional nature of the neuronal population affected by the drug application. In this study, a GABA_B receptor selective antagonist, baclofen, and a selective activator of cAMP-PKA, SpCAMPS, were microinjected into the PPT. I injected these drugs into the part of the PPT (pars compacta) where most cells are known to be cholinergic (Mesulam et al. 1983; Rye et al. 1987) and express REM-ON type of activity (Datta and Siwek 2002). Nonetheless, I acknowledge that if there are noncholinergic and REM-OFF cells located within the targeted site, they will also be affected by the application of these drugs. Another limitation of the microinjection method is the extent of diffusion of injected materials. In recent years, several laboratories, including our own, have improved this technique tremendously in many different ways. Recent microinjection studies have shown that a major advantage of the microinjection technique is its ability to stimulate a relatively circumscribed neuronal pool (Bandyopadhyya et al. 2006; Capece and Lydic 1997; Datta et al. 2001a,b, 2003; Marks and Birabil 2000). This view is supported by biophysical studies of drug diffusion (Nicholson 1985), and distribution kinetics (Herz and Teschemacher 1971; Martin 1991). Diffusion data obtained from radiolabeled drug studies have shown that 30 min after brain microinjections of 500-nl volumes, 72% of the drug remained within a radius of 0.75 mm (Yakhsh and Rudy 1978). In this study, the target area of the 100-nl drug solutions injected into the PPT (approximate size: length: 1.0; height: 1.0; and width: 0.8 mm), were well within the diffusion limit of this small volume. Moreover, in our recent microinjection mapping study in the PPT, it was shown that 100 nl of glutamate with drug concentrations similar to those used in this proposal were ineffective when injections were 0.5 mm away from the center of the PPT site (Datta 2002; Datta et al. 2001a,b). Additionally, using a similar microinjection technique, I showed that ibotenic microinjection-induced cell loss in the brain stem of rats was limited to a maximum radius of 0.35 mm. Other sleep-wake–related structures that are close to the PPT microinjection sites are the LDT and subcoeruleus nucleus. These two structures are ~1 mm away from the PPT microinjection sites. Thus it is highly improbable that microinjected drugs used in this study (100 nl volume and 1.5 nmol dose) diffused into the LDT and subcoeruleus nucleus.

The results of this study also confirm a well-established fact that the activation of PPT is positively involved in the regulation of REM sleep (for reviews, see Datta 1995; McCarley 2004). Contrasting this view, a recent publication claimed that the total amount of REM sleep increases in the rat after PPT lesion (Lu et al. 2006). This study also claimed that lesioning the subcoeruleus alpha [labeled as sublaterodorsal nucleus (SLD)] suppressed REM sleep (Lu et al. 2006). However, numerous previous PPT lesion studies in both cats (Shouse and Siegel 1992; Webster and Jones 1988) and rats (Deurveilh and Hennevin 2001) have shown that the elimination PPT cholinergic cells suppressed physiological REM sleep. Moreover, one study has shown that rats with inbred cholinergic hyperfunction have excess REM sleep (Shirmomani et al. 1988). More recently, it has also been shown that, in the animal model of Alzheimer’s, impaired REM sleep is caused by the reduction of cholinergic cells in the PPT (Zhang et al. 2005). Collectively, these lesion studies provided much stronger and definitive evidence to indicate that the intact PPT cholinergic cells are critical for the generation of REM sleep.

Assuming PPT cholinergic cells are necessary for the generation of REM sleep, a careful analysis of previous studies reveals a potential oversight in the study of Lu et al. (2006). Stimulation studies have shown that the electrical and chemical stimulation of the PPT increases active wakefulness in rats and cats (Datta and Siwek 1997; Datta et al. 2001b; Garcia-Rill 1991; Garcia-Rill et al. 1987). On the other hand, using an excitotoxin lesion technique, studies have shown that the elimination of PPT cells suppresses motivational behaviors (Bechara and van der Kooy 1989, 1992; Olmstead and Franklin 1994); and consequently PPT lesioned rats spend more time in quiet wakefulness by reducing active wakefulness. Cortical EEG waves amplitudes and frequencies are very similar during quiet wakefulness and REM sleep (Datta and Hobson 2000). During quiet wakefulness, EMG sign of muscle tone in the rat is also very low, especially in PPT-lesioned rats. Thus it is possible that while identifying sleep-wake states, Lu et al. (2006) might have misidentified some quiet wake episodes as REM sleep episodes. It is also known that the lesion of nucleus subcoeruleus alpha suppresses REM sleep muscle atonia (Henricks et al. 1982; Sakai 1980; Sanford et al. 2001; Shouse and Siegel 1992). Despite a reduction in REM sleep muscle atonia in these lesioned animals, the total amount of REM sleep is relatively unchanged. Similar to REM sleep muscle atonia, it has also been shown that the lesion of P-wave (one of the cardinal features of REM sleep) generator suppresses P-wave activity in the rat and cat during REM sleep (Datta and Hobson 1995; Mavanji et al. 2004). In these P-wave generator–lesioned rats and cats, the total amount of wakefulness, SWS, and REM sleep remains relatively unchanged. Therefore in these REM sleep sign generator–lesioned animals, identification of REM sleep is relatively more challenging than the identification of sleep-wake states in normal animals. It is likely that the SLD-lesioned rats of Lu et al. (2006) also exhibited some REM sleep episodes without muscle atonia. Therefore they might have failed to identify some of those dissociated REM sleep episodes. This could explain why the study of Lu et al. (2006)
presents paradoxical results, such as reduced REM sleep accompanying an SLD lesion. Indeed, lesion of subcoeruleus nucleus (part of SLD) does not change total amount of REM sleep or wakefulness (Mavani et al. 2004; Sanford et al. 1994).

In conclusion, this study showed that the inactivation of cAMP-PKA in the PPT is a critical step for the GABAB receptor activation–mediated regulation of REM sleep. The results also suggest that the activation of cAMP-PKA in the PPT is an important step to induce REM sleep. These data provide a novel perspective on the regulatory aspect of PPT cell activity in the regulation of REM sleep. These novel findings are critical for our complete understanding of the basic mechanisms in REM sleep regulation.

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References


Shouse MN, Siegel JM. Pontine regulation of REM sleep components in cats: integrity of the pedunculopontine tegmentum (PPT) is important for phasic events but unnecessary for atonia during REM sleep. Brain Res 571: 50–63, 1992.


