

Functional Role for Cannabinoids in Respiratory Stability During Sleep

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Study Objectives: Serotonin, acting in the peripheral nervous system, can exacerbate sleep-related apnea, and systemically administered serotonin antagonists reduce sleep-disordered respiration in rats and bulldogs. Because cannabinoid receptor agonists are known to inhibit the excitatory effects of serotonin on nodose ganglion cells, we examined the effects of endogenous (oleamide) and exogenous (Δ^9 -tetrahydrocannabinol; Δ^9 THC) cannabimimetic agents on sleep-related apnea.

Design: Sleep architecture, respiratory pattern, and apnea expression in rats were assessed by polysomnography. A repeated measures, within-subjects, fully nested crossover design was used in which each animal was recorded on exactly 12 occasions.

Participants: Eleven adult male Sprague-Dawley rats were instrumented for chronic polysomnography.

Interventions: Animals were recorded following intraperitoneal injection of various doses of Δ^9 THC, oleamide, and serotonin, alone and in com-

bination.

Measurements and Results: Our data show that Δ^9 THC and oleamide each stabilized respiration during all sleep stages. With Δ^9 THC, apnea index decreased by 42% ($F=2.63$; $p=0.04$) and 58% ($F=2.68$; $p=0.04$) in NREM and REM sleep, respectively. Oleamide produced equivalent apnea suppression. This observation suggests an important role for endocannabinoids in maintaining autonomic stability during sleep. Oleamide and Δ^9 THC blocked serotonin-induced exacerbation of sleep apnea ($p<0.05$ for each), suggesting that inhibitory coupling between cannabinoids and serotonin receptors in the peripheral nervous system may act on apnea expression.

Conclusions: This study demonstrates potent suppression of sleep-related apnea by both exogenous and endogenous cannabinoids. These findings are of relevance to the pathogenesis and pharmacological treatment of sleep-related breathing disorders.

INTRODUCTION

CANNABIS PRODUCES WELL-RECOGNIZED BEHAVIORAL EFFECTS AND TWO TYPES OF G PROTEIN-COUPLED RECEPTORS (CB₁ AND CB₂) HAVE BEEN CHARACTERIZED WHICH BIND THE ACTIVE COMPONENTS OF CANNABIS.¹ Still, the functional roles of endogenous ligands for CB₁ and CB₂ receptors remain poorly defined.² Early observations suggest a potent neuromodulatory role for these endocannabinoids, including alterations in sleep/wake behaviors³⁻⁵ and cardiovascular control.^{6,7} Cannabinoid receptors interact with opiate, GABA, dopamine, glutamate, and serotonin systems,⁸ all of which influence level of arousal and autonomic regulation.

Of particular interest to us was the fact that activation of serotonin (5-HT) receptors within the peripheral nervous system can lead to significant autonomic perturbations, especially during sleep. For example, stimulating peripheral 5-HT subtype 3 (5-HT₃) receptors exacerbates apnea expression in sleeping rats,⁹ whereas blockade of 5-HT₃ receptors suppresses sleep apnea in rats^{10,11} and bulldogs.¹² By altering serotonin receptor function, endocannabinoids also may impact sleep/wake and autonomic behaviors. In this respect, the published findings are not fully consistent because the serotonin/cannabinoid interaction appears to be receptor subtype specific. For example, some investigators report that serotonin-related behaviors are enhanced by cannabi-

noids,¹³ while others find that serotonin-mediated responses are attenuated in a dose-dependent manner.^{14,15}

The availability of specific cannabinoid receptor agonists and endogenous fatty acid amide ligands allowed us to examine the role of cannabinoids in determining autonomic stability during sleep. We used the rat model of sleep related breathing disorder to monitor the impact of CB₁ receptor agonist Δ^9 -tetrahydrocannabinol (Δ^9 THC) and endogenous fatty acid amide cis-9-octadecenoamide (oleamide) on respiratory stability during sleep. Involvement of serotonin receptor systems in the cannabinoid effects was examined by testing the ability of Δ^9 THC and oleamide to block serotonin-induced exacerbation of sleep-related apnea.⁹

METHODS

Polysomnography

Sprague-Dawley rats exhibit respiratory disturbance in the form of spontaneous apnea during all sleep stages, but with the greatest frequency during REM sleep (for a recent review relevant to this model system see Carley and Radulovacki).¹⁶ Adult animals (300–350 g), maintained throughout on a 12:12 hour light:dark cycle at controlled temperature (22±0.5 °C), were instrumented, under ketamine (80 mg/kg ip) and xylazine (5 mg/kg ip) anesthesia. A surgical incision of the scalp was made to allow bilateral implantation of stainless steel screws into the frontal and parietal bones of the skull for electroencephalogram (EEG) recording. Bilateral wire electrodes were placed into the nuchal muscles for electromyogram (EMG) recording. The EEG and EMG leads were soldered to a miniature connector and fixed to the skull with cranioplastic cement. After at least seven days of post-surgical recovery, animals were polygraphically recorded with respiration monitored by single chamber plethysmography. All waveforms were low-pass filtered (-3 dB at 30 Hz), digitized

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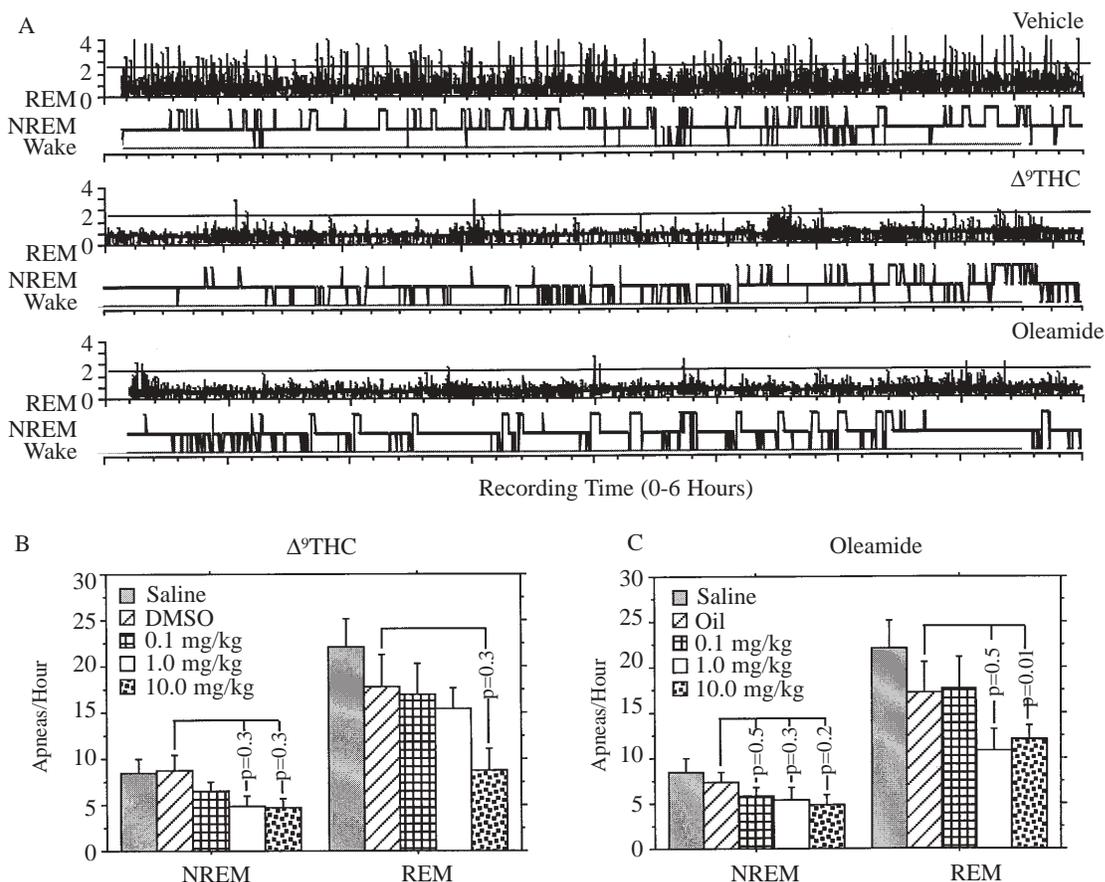


Figure 1—(A) Relationship between breath duration and sleep/wake states in typical 6-hour recordings made after injection of vehicle (upper panel), 10.0 mg/kg of Δ^9 THC (middle panel) or 10.0 mg/kg of oleamide (lower panel). Within each panel, the lower tracing depicts transitions among Wake, NREM sleep, and REM sleep as a step function. The upper tracing presents the duration in seconds (0–4) of each of the ~30,000 breaths during the recording. Whenever breath duration exceeded 2.5 seconds (above the horizontal threshold line) an apnea was scored. Note the dramatic reduction in the number and frequency of apneas elicited by Δ^9 THC and by oleamide. The paucity of apneas after oleamide or Δ^9 THC injection is apparent during both NREM and REM sleep. **(B)** Group mean data illustrating the dose-dependent suppression of spontaneous apnea by Δ^9 THC during NREM (left column) and REM (right column) sleep. Ordinate represents frequency of apnea during NREM sleep (apneas per hour of NREM sleep; left) or the frequency of apnea during REM sleep (apneas per hour of REM sleep; right). Injections are coded according to the inset legend. With respect to vehicle (DMSO), dose dependent reductions in apnea expression were observed as labeled. **(C)** Group mean data illustrating impact of oleamide on apnea expression presented in the format of panel B. Again, dose dependent apnea suppression (with respect to vehicle, peanut oil) was observed as labeled.

at a rate of 100 per second and stored to computer disk. Based on power spectrum analysis of EEG and EMG activity, each 10-second recording epoch was scored as Wake, non rapid eye movement (NREM) sleep, or REM sleep. These recording and data analysis methods are described in detail elsewhere (see for example Carley and Radulovacki.⁹

Respiratory Measurements

For recording, each rat was placed in a bias-flow-ventilated whole body plethysmograph (6"W X 10"L X 6"H; Buxco Electronics, Inc., Sharon, CT) within which the animal had free movement and access to food pellets and water. The bias flow of room air (2 l/min) was more than one order of magnitude greater than the alveolar ventilation of the rat, ensuring that no rebreathing occurred. Thermal fluctuations associated with tidal respiration induced changes in plethysmograph pressure proportional to tidal volume. Plethysmograph pressure was monitored using a Validyne DP45-14 differential pressure transducer (± 2 cm H₂O).

Prior to each experimental study, the plethysmograph was calibrated for tidal volume using the method described by Epstein et al.¹⁷ An adaptive threshold algorithm (Datawave Systems, Longmont, CO) was used to measure the duration and tidal amplitude of each breath in each recording. From these values, instantaneous respiratory rate (RR) and minute ventilation (MV) were computed on a breath by breath basis. For statistical analysis, normalized values were also computed for respiratory rate (NRR), tidal volume (NVT), and minute ventilation (NMV). For this purpose, the value for each breath of each recording was divided by the mean value observed during wakefulness in that animal following saline injection. These normalized values thus described the relative changes in respiratory pattern associated with transitions among behavioral states as well as those induced by drug or vehicle injections.

The frequency of spontaneous apneas (total breath duration > 2.5 seconds) was computed as an "apnea index" (apneas per hour) to provide a measure of extreme respiratory pattern disturbance in each sleep state. A separate apnea index was computed

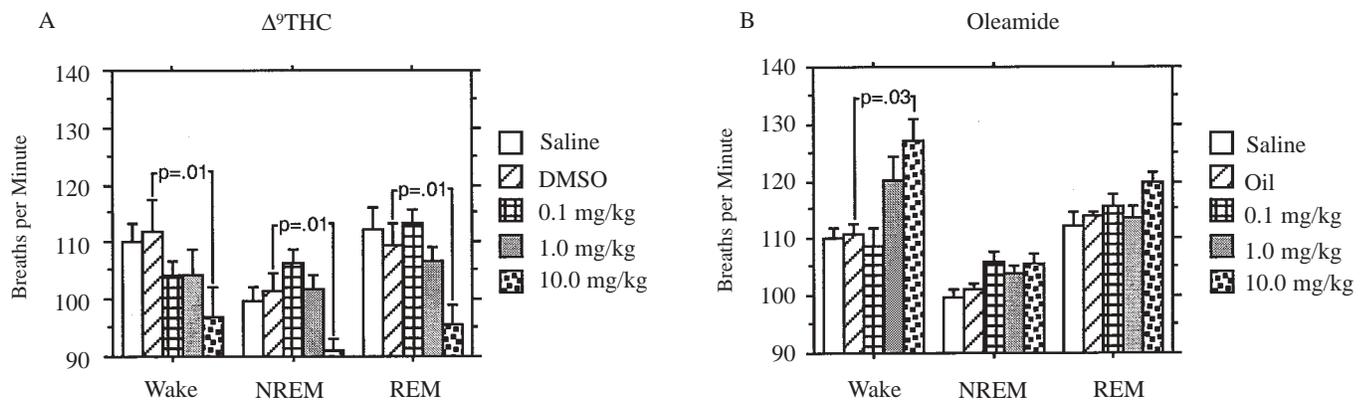


Figure 2—Effect of intraperitoneal $\Delta^9\text{THC}$ and oleamide on respiratory rate (RR). **(A)** Interaction plot depicting average RR during Wake (left column), NREM sleep (middle column) and REM sleep (right column) following $\Delta^9\text{THC}$ administration. Concentrations of $\Delta^9\text{THC}$ are coded by symbol according to the legend. RR was lowest during NREM sleep and was unaffected by time during the recording (see text for details). RR was significantly reduced in all sleep/wake states after administration of 10.0 mg/kg $\Delta^9\text{THC}$. **(B)** Group mean data ($N = 11$) for impact of oleamide on average RR throughout 6-hour recordings. RR tended to increase after administration of 10.0 mg/kg oleamide. Again, RR was lowest during NREM sleep and was unaffected by time during the recording (see text for details).

for each sleep state (i.e., NREM apnea index = apneas per hour of NREM sleep; REM apnea index = apneas per hour of REM sleep). These two distinct apnea indexes allowed us to identify changes in apnea expression resulting from altered respiratory rhythm and motor output patterning in each sleep state. This method also allowed us to exclude changes in overall apnea expression resulting from simple changes in sleep architecture. The minimum apnea duration corresponded to two to three “missed” breaths.

Preparation of Injections

$\Delta^9\text{THC}$ (dronabinol, Roxane Laboratories, Columbus, Ohio) was dissolved in DMSO; oleamide (ICN Biomedicals, Aurora, Ohio) was suspended in peanut oil with sonication for 20 minutes; and serotonin was dissolved in saline. The study was a fully nested, repeated measures crossover design, such that each animal received each of 12 intraperitoneal injections (1 ml/kg) exactly one time: vehicle alone (saline, DMSO, or peanut oil); $\Delta^9\text{THC}$ alone (0.1, 1.0, or 10.0 mg/kg); oleamide alone (0.1, 1.0, or 10.0 mg/kg); serotonin alone (0.79 mg/kg); or combination injection (0.1 mg/kg $\Delta^9\text{THC}$ followed, after 15 minutes, by 0.79 mg/kg serotonin or 0.1 mg/kg oleamide followed by 0.79 mg/kg serotonin). Each injection was made 15 minutes prior to polygraphic recording for six hours (10:00–16:00). Successive recordings for an individual animal were separated by at least three days and the treatments were given in random order. Enzymatic degradation of oleamide by fatty acid amide hydrolase occurs within hours,¹⁸ but metabolism and excretion of $\Delta^9\text{THC}$ has a half-life of approximately 30 hours.¹⁹ For this reason, successive recordings for an individual animal were separated by at least three days and the treatments were given in random order. This recording interval was at least 2.5 times the half-life of $\Delta^9\text{THC}$, allowing for a return to baseline between recordings.

RESULTS

Figure 1A depicts the typical relationship between breath duration and sleep/wake states after injection of vehicle (upper panel), 10.0 mg/kg of $\Delta^9\text{THC}$ (middle panel) or 10.0 mg/kg of oleamide (lower panel). Within each panel, the lower tracing depicts transitions among Wake, NREM sleep, and REM sleep as a step function. The upper tracing presents the duration in seconds (0–4) for each of the ~30,000 breaths during the six hour recording. Whenever breath duration exceeded 2.5 seconds (above the horizontal threshold line), an apnea was scored. It can be observed during vehicle (control) recordings that respiratory rate was quite variable: apneas occurred intermittently throughout the recording and REM sleep often was associated with flurries of apnea. In contrast, injection of either $\Delta^9\text{THC}$ or oleamide produced a clear reduction in breath durations exceeding 1.5 seconds.

It is clear from the grouped data that extreme variations in respiratory pattern, as represented by apneas, were reduced by both test compounds. $\Delta^9\text{THC}$ strongly suppressed spontaneous apnea during all sleep stages (Fig. 1B). This effect was dose dependent during NREM ($F=2.63$, $p=0.04$) and REM ($F=2.68$; $p=0.04$) sleep, but was not time-dependent ($F=0.91$, $p=0.57$ for main effect of time during NREM; $F=0.71$, $p=0.82$ for main effect of time during REM) throughout the 6-hour recording period. Post hoc comparisons (controlled by Fisher’s protected least significance difference) demonstrated that $\Delta^9\text{THC}$ doses of 1.0 and 10.0 mg/kg reduced the frequency of apneas during NREM sleep ($p=0.03$ for each), whereas only the 10.0 mg/kg dose produced a significant decrease in apnea frequency during REM sleep ($p=0.03$).

Oleamide, an endogenous fatty acid amide, mimicked the apnea suppression produced by the exogenous CB_1 receptor agonist $\Delta^9\text{THC}$ (Fig. 1C). With respect to the vehicle, oleamide suppressed apnea expression during NREM sleep at all three doses and during REM sleep at the two higher doses ($p<0.05$ for each, see Fig. 1C for details). At the highest dose, the degree of apnea

Table 1—Impact of Δ^9 THC and oleamide on normalized minute ventilation

Injection	State	Mean	SE	P vs. saline	P vs. vehicle
Saline	Wake	1.00	0.00		
	NREM	0.90	0.04		
	REM	0.99	0.05		
DMSO	Wake	1.04	0.03	°	
	NREM	0.87	0.04	°	
	REM	0.95	0.04	°	
Oil	Wake	1.03	0.04		
	NREM	0.95	0.05		
	REM	1.03	0.05		
Dro-0.1 mg/kg	Wake	1.03	0.06		°
	NREM	0.95	0.13		°
	REM	0.99	0.14		°
Dro-1.0 mg/kg	Wake	1.04	0.06		°
	NREM	0.96	0.14		°
	REM	0.97	0.13		°
Dro-10.0 mg/kg	Wake	0.87	0.05		0.04
	NREM	0.81	0.05		°
	REM	0.84	0.06		0.05
OI-0.1 mg/kg	Wake	1.00	0.04		°
	NREM	0.95	0.05		°
	REM	1.00	0.06		°
OI-1.0 mg/kg	Wake	1.08	0.06		°
	NREM	0.99	0.05		°
	REM	1.05	0.06		°
OI-10.0 mg/kg	Wake	1.17	0.05		0.01
	NREM	1.01	0.04		°
	REM	1.08	0.06		°

Dro=dronabinol OI=oleamide ° = $p > 0.05$

Normalized minute ventilation computed by dividing the minute ventilation for each breath by the mean minute ventilation observed during wakefulness following saline injection (see text for details).

suppression was equivalent for Δ^9 THC and oleamide. Oleamide's ability to suppress apnea persisted throughout the six hour recording interval ($F=1.24$, $p=0.23$ for main effect of time during NREM; $F=0.72$, $p=0.80$ for main effect of time during REM).

Δ^9 THC and oleamide each altered average respiratory rate (RR), but with opposite effects (Fig. 2). Δ^9 THC evoked a dose dependent reduction in RR during all sleep/wake states ($F=4.67$, $p=0.003$). Conversely, oleamide produced dose dependent elevation of RR, an effect which achieved statistical significance only during wakefulness ($p=0.03$ for 10.0 mg/kg vs. vehicle). As detailed in Table 1, equivalent effects of Δ^9 THC and oleamide were observed for respiratory minute ventilation. Average tidal volume was unaffected by any injection (Table 2). RR also displayed the expected decrease during NREM sleep under all conditions ($F=25.8$, $p<0.0001$ for effect of sleep; $F=1.49$, $p=0.17$ for interaction between sleep state and injection type); an effect that was evident throughout the recording interval ($F=0.98$, $p=0.52$ for interaction between sleep state and recording hour).

Oleamide produced a significant alteration of sleep/wake architecture (Figure 3). Throughout the 6 hour recording ($F=1.38$, $p=0.14$ for interaction of time and dose), administration of 0.1 or 1.0 mg/kg oleamide produced increased REM sleep with decreased wakefulness, whereas the 10.0 mg/kg dose resulted in increased NREM sleep with decreased REM sleep. These

hypnotic properties of oleamide confirm similar previous observations.^{4,18,20} The only change in sleep architecture produced by Δ^9 THC was a decrease in REM sleep expression at the highest dose tested (-66%, $p=0.0002$).

The reductions in apnea frequency produced by Δ^9 THC and oleamide are not a simple result of changes in sleep architecture elicited by these compounds. Several observations clarify this viewpoint. As can be seen in Figure 1B and 1C, Δ^9 THC reduced the number of apneas per hour of NREM sleep at doses of 1.0 mg/kg and 10.0 mg/kg, yet Δ^9 THC had no effect on the percentage of recording time spent in NREM at any dose. Δ^9 THC produced a reduction in apnea expression during REM sleep only at a dose of 10.0 mg/kg (Figure 1B), a dose that also produced a 66% reduction in REM sleep itself. However, the reduction in REM sleep time does not explain the reduction in REM-related apneas, because the frequency of apnea during REM sleep also decreased, as shown in Figure 1B.

In similar fashion, the number of apneas per hour of NREM sleep was reduced by all doses of oleamide; yet only at the highest (10.0 mg/kg) dose was expression of NREM sleep itself affected. Furthermore, it is counter-intuitive to postulate that an increase in NREM sleep expression could account for the observed decrease in NREM apneas. During REM sleep, apnea expression was decreased by the 1.0 mg/kg and 10.0 mg/kg doses of oleamide. Yet, one of these doses (1.0 mg/kg) increased REM

Table 2—Impact of Δ^9 THC and oleamide on normalized tidal volume

Injection	State	Mean	SE	P vs. saline	P vs. vehicle
Saline	Wake	1.00	0.00		
	NREM	0.94	0.06		
	REM	1.03	0.07		
DMSO	Wake	1.02	0.06	°	
	NREM	0.87	0.05	°	
	REM	1.03	0.07	°	
Oil	Wake	1.03	0.05		
	NREM	0.94	0.05		
	REM	1.08	0.07		
Dro-0.1 mg/kg	Wake	1.09	0.07		°
	NREM	0.90	0.16		°
	REM	1.06	0.12		°
Dro-1.0 mg/kg	Wake	1.09	0.08		°
	NREM	0.95	0.15		°
	REM	1.08	0.16		°
Dro-10.0 mg/kg	Wake	0.99	0.07		0.04
	NREM	0.89	0.08		°
	REM	1.06	0.09		0.05
OI-0.1 mg/kg	Wake	1.03	0.05		°
	NREM	0.91	0.07		°
	REM	1.02	0.06		°
OI-1.0 mg/kg	Wake	0.98	0.08		°
	NREM	0.97	0.06		°
	REM	1.09	0.08		°
OI-10.0 mg/kg	Wake	1.04	0.07		0.01
	NREM	0.97	0.06		°
	REM	1.08	0.07		°

Dro=dronabinol OI=oleamide ° =p>0.05

Normalized tidal volume computed by dividing tidal volume for each breath by the mean tidal volume observed during wakefulness following saline injection (see text for details).

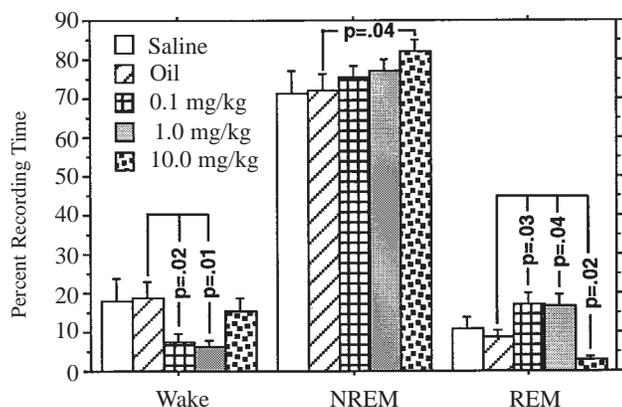


Figure 3—Group mean data for the impact of oleamide on sleep architecture. Ordinate displays the percentage of each 6-hour recording scored as Wake (left column), NREM sleep (middle column), or REM sleep (right column). Injection type is coded according to the inset legend. Significant changes in sleep architecture with respect to vehicle were observed as labeled (see text for details).

sleep expression while the other (10.0 mg/kg) decreased REM sleep expression. Again, there is no straightforward way in which these changes in REM sleep expression could account for the

changes in apnea expression during REM sleep.

As previously demonstrated, serotonin produced a significant increase in the frequency of apnea during REM sleep⁹ (Figure 4). This peripheral effect of serotonin was completely blocked by pretreatment with 0.1 mg/kg of either Δ^9 THC or oleamide. At this dose, neither Δ^9 THC nor oleamide had any effect on apnea expression during REM sleep when administered alone (Fig. 1).

DISCUSSION

Transient but physiologically significant perturbations of autonomic homeostasis can be observed during all levels of sleep, but with the greatest frequency and intensity during REM sleep. A particularly dramatic example is transient cessation of respiration, or apnea. When apnea occurs with sufficient frequency during sleep, significant behavioral and clinical morbidity can result.^{19,21} Our data show that the CB₁ receptor agonist Δ^9 THC stabilizes respiratory pattern during all sleep stages by producing a dose-dependent reduction in apnea expression.

One may question the relevance of this finding, or findings from other animal model systems, to the mechanisms and management of human sleep apnea syndromes. It is our view that both central and obstructive apnea reflect, at least in part, dysregulation of central neural motor output patterning to the respi-

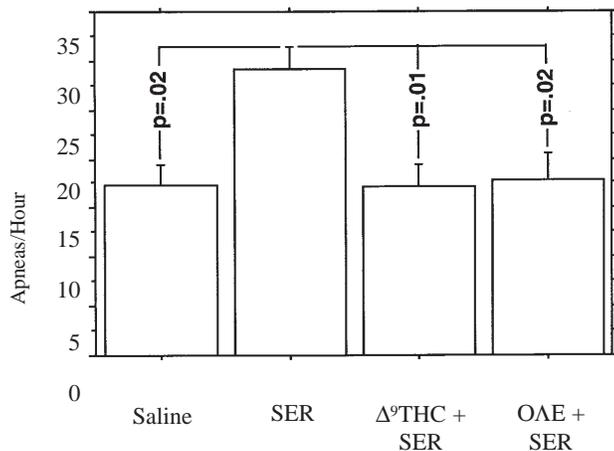


Figure 4—Group mean data for 6-hour recordings demonstrating the frequency of apnea during REM sleep (apneas per hour of REM sleep) following intraperitoneal injection of vehicle (SALINE), serotonin (0.79 mg/kg, SER) alone, or serotonin following pretreatment by Δ^9 THC (0.1 mg/kg, SER+ Δ^9 THC) or oleamide (0.1 mg/kg, SER+OLE). Serotonin produced a significant increase in REM sleep-apnea; an effect which was completely blocked by pretreatment with either Δ^9 THC or oleamide.

ratory system. In humans or English bulldogs with upper airways predisposed to collapse by anatomical, mechanical, or muscular factors, this dysregulation may be manifest primarily by obstructive apneas. In humans or rats with mechanically stable upper airways, dysregulation of respiratory motor output patterning may be expressed primarily by central apneas or hypopneas.

Indirect support for our view comes from several lines of investigation. Most patients with sleep apnea syndrome exhibit a combination of central, mixed, and obstructive apneas in a single sleep period, leading to the suggestion that any factor which destabilizes respiratory drive during sleep promotes apnea genesis. Önal and Lopata²² demonstrated that patients with sleep apnea exhibited obstructive apneas when breathing through their own upper airways, but central apneas when breathing through a tracheostomy. These authors concluded that obstructive apnea reflects unstable central respiratory drive in individuals with upper airways predisposed to collapse by anatomical or neuromuscular defects. Furthermore, in some cases, continuous positive airway pressure converts obstructive apneas to central apneas, again supporting the conclusion that unstable central respiratory motor patterning contributes to the pathogenesis of obstructive sleep apnea syndrome.

If apnea reflects unstable respiratory motor patterning, interventions stabilizing respiratory drive during sleep may reduce or eliminate apnea. Indeed, inspired carbon-dioxide, used to elevate respiratory drive, reduced the expression of both central^{23,24} and obstructive^{23,25} apnea in man. Conversely, supplemental inspired oxygen that raises mean arterial oxygen saturation is often associated with longer or more frequent apneas in man.²⁵ The above human findings suggest that central and obstructive apnea during sleep share common central neural pathogenic mechanisms.

In testing the validity of the normal rat model of sleep disordered breathing we have demonstrated that central apneas in rats are expressed in similar patterns and are influenced by interventions in a fashion similar to human central and obstructive apnea.

In patients, both central and obstructive apnea are most severe in REM sleep.²⁶ In the rat, central apnea is 2 to 10 times more frequent during REM than non-REM sleep.⁹⁻¹¹ In both man²³⁻²⁵ and rat,²⁷ inspired hypercapnia decreases, whereas hyperoxia increases the severity of apnea. In addition, administration of the serotonin antagonist ondansetron resulted in reduced frequencies of central apnea in rats¹¹ and of obstructive apneas in English bulldogs.¹² The impact of this drug on human sleep apnea has not yet been tested.

The above evidence demonstrates similar patterns of expression and responses to intervention for central and obstructive apnea in man, rat, and bulldog. Thus, the significant cannabinoid-induced suppression of apnea in all sleep stages documented by the present investigation in rats is expected to be of relevance to the mechanisms and management of human sleep-related apnea. Still, limitations of this model system must be considered before extrapolating the present results to human sleep disordered respiration.

First, the impact of 2.5 second respiratory pauses on gas exchange in the rat has not been directly demonstrated. However, mathematical modeling suggests that during a brief apnea, alveolar and arterial carbon dioxide partial pressure will rise exponentially toward the mixed venous partial pressure with a time constant which scales according to the ratio of mlv/Qc , where mlv is the mean lung volume and Qc is the cardiac output.²⁸ Normal values of these parameters yield approximate time constants of 9.9 seconds for an adult man²⁸ and 1.7 seconds for a 500 gram rat.^{29,30} Assuming equivalent arterio-venous differences in carbon-dioxide partial pressure in man and rat, a 1.7 second apnea in rat should produce equivalent arterial hypercarbia to a 9.9 second apnea in man. On this basis, we believe that by analogy to man expression of 2.5 second apneas does represent “sleep disordered breathing” in the rat.

CB₁ receptors have been demonstrated in peripheral neurons, albeit at lower densities than in the central nervous system.^{20,31} The function of these receptors, however, has not been well demonstrated. The ability of Δ^9 THC to completely block exogenous serotonin-induced apnea exacerbation, at a dose that exerted no independent effect on any behavior measured, argues that CB₁ receptors in the peripheral nervous system exert a significant influence on serotonin receptor signaling. We argue that the impact of cannabinoids on the increased apnea frequency elicited by exogenous serotonin is exerted in the peripheral nervous system because systemically administered serotonin does not effectively cross the blood-brain-barrier. Because 0.1 mg/kg of oleamide or Δ^9 THC was insufficient to alter apnea expression but did eliminate serotonin-induced elevation of apnea frequency, it is most probable that the blockade resulted from interference with the effects of exogenous serotonin by the cannabinoids. Moreover, because peripherally administered serotonin does not effectively enter the brain, the interaction between cannabinoids and serotonin relevant to apnea expression most likely occurred in the peripheral nervous system.

These speculations are supported by the observation that CB₁ receptor agonists inhibit excitatory responses to serotonin receptor agonists in nodose ganglion cells.¹⁴ The possibility that endogenous serotonin may act at receptors in the peripheral nervous system to promote autonomic perturbations such as apnea⁹ is consistent with our finding that sufficient doses of Δ^9 THC

alone can suppress spontaneous apnea (Fig. 1B). This may have resulted from interference with endogenous serotonin at relevant target tissues such as the nodose ganglia only occurring at higher cannabinoid doses.

Such mechanistic interpretations must remain speculative, however. The present data do not definitively demonstrate the relevant site(s) of action for either serotonin or the cannabinoids. We cannot rule the possibility that the pertinent site of action for oleamide or Δ^9 THC resides in the central nervous system. Also, certain areas of the brain, such as the area postrema and the circumventricular organs, have incomplete blood-brain-barriers. Exogenous serotonin may thus act in these areas as well as the peripheral nervous system. In fact, the area postrema is believed to act as a "trigger zone" for emesis. Specific respiratory functions for have not been identified, but cannot be ruled out.

The endocannabinoid oleamide completely mimicked the ability of Δ^9 THC to suppress spontaneous and serotonin-induced apnea (Figs. 2 and 3). Although not conclusive, this parallelism suggests that the actions of oleamide and Δ^9 THC are convergent at some point in the signaling cascade. It is unlikely that the demonstrated respiratory effects of oleamide are directly mediated via cannabinoid receptors, because oleamide does not appear to bind with high affinity to CB₁ or CB₂ receptors.^{21,32} It is likely that oleamide and Δ^9 THC, as well as anandamide, an endogenous CB₁ agonist, have common as well as distinct pathways of action. In accordance, the increased stability of respiratory pattern produced by Δ^9 THC and oleamide does not appear to be a nonspecific by-product of respiratory stimulation, because the two compounds exerted opposite effects on average respiratory rate and minute ventilation (Fig. 2A).

It may at first seem surprising that Δ^9 THC and oleamide produce opposite effects on respiratory rate. Although the present data do not demonstrate the mechanisms underlying these paradoxical effects, we may offer a speculation: the increased respiratory rate produced by oleamide results from enhanced signaling at 5-HT₂ receptors. Lindsay and Feldman demonstrated stimulation of respiration by 5-HT, effects that were mimicked by a selective 5-HT₂ agonist and blocked by a selective 5-HT₂ antagonist.³³ In addition to putative interference with 5-HT₃ mediated signaling, oleamide markedly potentiates the action of 5-HT at 5-HT₂ receptors.³⁴⁻³⁶ Similar effects of Δ^9 THC have not been described. Thus, enhancement of 5-HT₂-mediated signaling may contribute to the respiratory stimulation produced by oleamide but not Δ^9 THC. Again, the present data provide no direct evidence to support or refute this speculation.

Although our data do not provide direct evidence for a functional role of peripheral endocannabinoid systems in regulating autonomic stability, they are consistent with this possibility conclusion. Further, they provide a rationale for exploring the use of cannabimimetic drugs in the treatment of sleep-related breathing disorders. In this regard, endocannabinoids that can promote deep sleep, such as oleamide, may have advantages over agents that have no effect on, or interfere with sleep.

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