Dopaminergic Regulation of Sleep and Cataplexy in a Murine Model of Narcolepsy

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Study Objectives: To determine if the dopaminergic system modulates cataplexy, sleep attacks and sleep-wake behavior in narcoleptic mice.

Design: Hypocretin/orexin knockout (i.e., narcoleptic) and wild-type mice were administered amphetamine and specific dopamine receptor modulators to determine their effects on sleep, cataplexy and sleep attacks.

Patients or Participants: Hypocretin knockout (n = 17) and wild-type mice (n = 21).

Interventions: Cataplexy, sleep attacks and sleep-wake behavior were identified using electroencephalogram, electromyogram and videography. These behaviors were monitored for 4 hours after an i.p. injection of saline, amphetamine and specific dopamine receptor modulators (D1- and D2-like receptor modulators).

Measurements and Results: Amphetamine (2mg/kg), which increases brain dopamine levels, decreased sleep attacks and cataplexy by 61% and 67%, suggesting that dopamine transmission modulates such behaviors. Dopamine receptor modulation also had powerful effects on sleep attacks and cataplexy. Activation (SKF 38393; 20mg/kg) and blockade (SCH 23390; 1mg/kg) of D1-like receptors decreased and increased sleep attacks by 77% and 88%, without affecting cataplexy. Pharmacological activation of D2-like receptors (quinpirole; 0.5mg/kg) increased cataplectic attacks by 172% and blockade of these receptors (eticlopride; 1mg/kg) potently suppressed them by 97%. Manipulation of D2-like receptors did not affect sleep attacks.

Conclusions: We show that the dopaminergic system plays a role in regulating both cataplexy and sleep attacks in narcoleptic mice. We found that cataplexy is modulated by a D2-like receptor mechanism, whereas dopamine modulates sleep attacks by a D1-like receptor mechanism. These results support a role for the dopamine system in regulating sleep attacks and cataplexy in a murine model of narcolepsy.

Keywords: Sleep, narcolepsy, cataplexy, hypocretin/orexin, dopamine

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being specific for either WT or KO mice and the reverse primer being common to both). All procedures and experimental protocols were approved by the University of Toronto’s animal care committee and were in accordance with the Canadian Council on Animal Care.

Surgery

Mice were anesthetized using isoflurane (1-2%) and implanted with electroencephalographic (EEG) and electromyographic (EMG) electrodes. EEG recordings were obtained using four stainless steel micro-screws (1mm anterior ± 1.5mm lateral to bregma; 3mm posterior ± 1.5mm lateral to bregma). EMG electrodes were made from multistranded stainless steel (AS131, Cooner Wire, Chatsworth, CA) wires, which were sutured onto both neck and left/right masseter muscles. All electrodes were attached to a micro-strip connector (CLP-105-02-L-D, Electrosonic, Toronto, ON), which was affixed onto the animal’s head with dental cement (Ketac-cem, 3M, London, ON). After surgery mice were given 5% dextrose in 0.9% saline, as well as ketoprofen (3mg/kg). Mice were individually housed in a sound-attenuated and ventilated chamber on a 12:12 light-dark cycle (110 Lux; lights on 7:00, lights off 19:00) for 10-12 days post surgery. Food and water were available ad libitum.

Drug Preparation

The following drugs were used to modify dopaminergic transmission: quinpirole (0.125 and 0.5mg/kg; a D2-like receptor agonist; Tocris, Ellisville, MO), eticlopride (0.25 and 1mg/kg; a D2-like receptor antagonist; Sigma Aldrich, Oakville, ON), SKF 38393 (5 and 20mg/kg; a D1-like receptor agonist; Tocris), SCH 23390 (0.25 and 1mg/kg; a D1-like receptor antagonist; Tocris) and amphetamine (2mg/kg; Sigma Aldrich). Drugs were made from frozen stock solutions before each i.p. injection. Dose ranges were chosen based on previous studies demonstrating behavioral effects in mice.

In this study we refer to D1-like or D2-like dopamine receptors. D1-like receptors include D1 and D5 receptors while D2-like receptors include D2, D3, and D4 receptors. The drugs used are selective for either D1-like or D2-like receptors. Ki values for quinpirole are 4.8, 24, 30 and 1900nM at D2, D3, D4 and D1 receptors, respectively. Ki values for eticlopride are 0.50 and 0.16nM at D2 and D3 receptors, respectively. Ki values SKF 38393 are 1, 0.5, 150, 5000 and 1000nM for D1, D5, D2, D3 and D4 receptors, respectively. Ki values for SCH 23390 are 0.2, 0.3, 1100, 800 and 3000nM at D1, D5, D2, D3 and D4 receptors, respectively.

Data Acquisition

Sleep-wake state and muscle activity were recorded by attaching a lightweight cable to the plug on the mouse’s head, which was connected to a Physiologic Amplifier system (Grass 15LT, Astro Med, Brossard, QC). The EEG was amplified 1000 times and band-pass filtered between 1 and 100 Hz. EMG signals were amplified 1000 times and band-pass filtered between 30 Hz and 1 kHz. All electrophysiological signals were digitized at 500Hz (Spike 2 Software, 1401 Interface, CED Inc.) and monitored and stored on a computer. Infra-red video recordings were captured and synchronized with the electrophysiological recordings to couple motoric behavior with EEG and EMG recordings.

Experimental Protocols

Mice were placed in a round plexi-glass cage (diameter: 20cm) and given 24 hours to habituate to this new environment. After this period, mice were connected to the recording apparatus and given another 48 hours to habituate at which point a habituation injection (i.e., saline) was given. In one group of mice, a single dose of amphetamine (n = 12) was given and sleep, cataplexy and sleep attacks recorded. In another group of mice (n = 26), serial dopamine drug injections were given, each separated by 48 hours; injections were given in random order. All injections (0.3ml i.p.) were given at the onset of the dark phase (i.e., 19:00h) and behavior monitored for the following 4 hours.

Data Analysis

Data was collected for 4 hours after injections and was scored, using EEG, EMG (neck and masseter) and video. Each of the 5 second epochs was scored as wake, non-rapid eye movement (NREM) sleep, rapid eye movement (REM) sleep, cataplexy, sleep attack or transition state (e.g., NREM-REM). Sleep attacks were classified as a gradual loss of neck muscle tone associated with NREM-like EEG characteristics and automatic behavior. In narcoleptic mice, automatic behavior is defined as chewing, which we confirmed by both videography and masseter EMG recordings. Cataplexy was classified as a sudden loss of skeletal muscle tone in both neck and masseter following at least 40 seconds of active waking and with a duration of at least 10 seconds.

Both videography and electrophysiological recordings were used to identify sleep-wake behavior, cataplexy and sleep attacks. To demonstrate the fidelity and reliable identification of the narcoleptic phenotype, two separate people identified and scored cataplexy and sleep attacks in a cohort of mice (n = 8). There was 88% and 82% agreement between scorers for cataplexy and sleep attacks, respectively.

The frequency and duration of cataplectic and sleep attacks, as well as the total time spent in cataplexy/sleep attacks was determined for each drug and compared to the saline treatment. To determine the total time spent in cataplexy, we summed the duration of each cataplectic attack across the 4-hour recording period. For narcoleptic and WT mice the time spent in each sleep-wake state for all drug treatments was determined and compared to the saline treatment.

Statistical Analyses

The statistical tests used for each analysis are included in the results section. Comparisons between frequency (i.e., number of bouts), duration and total time spent in cataplexy/sleep attacks were made using one-way repeated measures analysis of variance (RM ANOVA). Drug effects on sleep-wake state were made using a two-way RM ANOVA. Differences in sleep-wake behaviors between narcoleptic and WT mice were determined using 2-way ANOVA. All statistical analyses used SigmaStat (SPSS Inc.) and applied a critical 2-tailed alpha value of P < 0.05. Data are presented as means ± standard error (SEM).

RESULTS

Hypocretin Knockout Mice Exhibit Cataplexy and Sleep Attacks

We found that hypocretin KO mice have a behavioral phenotype that mimics human narcolepsy with the most striking
abnormalities being cataplexy and sleep attacks. During the 4-hour recording period (i.e., 19:00-23:00h, lights off) hypocretin KO mice had an average of 1.9 ± 0.7 (range: 1-5) episodes of cataplexy that lasted 50 ± 11s (range: 10-140s). Cataplectic attacks occurred during periods of alert wakefulness and were characterized by postural collapse and loss of skeletal muscle tone with a theta-dominant, waking-like EEG pattern (Figure 1A and C). Cataplectic episodes were terminated by re-entrance into wakefulness, with mice resuming normal motoric behaviors such as grooming or eating. During baseline conditions (i.e., saline treatment), 12% of narcoleptic mice (2 of 17 mice) did not present with cataplexy.

Hypocretin KO mice also exhibited sleep attacks (also called gradual arrests). Even though sleep attacks also occurred during active wakefulness, they differed from cataplexy because they were characterized by gradual loss of posture and muscle tone and because EEG activity patterns were NREM sleep-like in nature (Figure 1B and C). Another feature separating sleep attacks and cataplexy is automatic behavior – a common feature in human and murine narcolepsy. In KO mice automatic behavior was defined as chewing, which was visualized by repeated jaw movements and cyclic oscillations in masseter EMG activity. Automatic behavior was common during sleep attacks, but never observed during cataplexy. Sleep attacks were more frequent than cataplectic episodes, and on average mice exhibited 4.7 ± 1.0 (range: 2-9) episodes that lasted 30 ± 3s (range: 10-70s). During baseline conditions (i.e., saline treatment), 100% of narcoleptic mice experienced sleep attacks.

Narcoleptic mice also had abnormal sleep-wake architecture (Figure 1D). Compared to WT littermates, hypocretin KO mice had more REM sleep (KO: 5.3 ± 0.8% and WT: 1.7 ± 0.4%; 2-way ANOVA, P = 0.001) and more transitions into and out of sleep (P = 0.009), while they spent the same amount of time in wakefulness (P = 0.839) and NREM sleep (P = 0.499; Figure 1D).

Amphetamine Reduces Cataplexy and Sleep Attacks in Narcoleptic Mice

Because dopamine modulates sleep and because dopamine receptor binding is altered in human narcoleptics, we aimed to determine if amphetamine, which increases brain dopamine levels, affects sleep and cataplexy in narcoleptic mice. Amphetamine stimulated wakefulness and suppressed sleep in both hypocretin KO and WT mice (n = 6, 2-way RM ANOVAs; WT: P < 0.001, KO: P < 0.001; Figure 2A and B). It increased wakefulness by 41% above saline levels (P < 0.001) but decreased NREM and REM sleep by 52% and 69%, respectively (NREM: P < 0.001 and REM: P = 0.002 Figure 2A). Amphetamine administration potently suppressed time spent in sleep attacks by 61% (RM ANOVA; P = 0.032; Figure 2C). This reduction was due to a decrease in sleep attack frequency (44% below saline; RM ANOVA; P = 0.030; Figure 2D); duration of individual attacks was unaffected by amphetamine treatment (RM ANOVA; P = 0.289; Figure 2E). Amphetamine also suppressed cataplexy, decreasing the number of episodes by 67% of saline levels (RM ANOVA, P = 0.042; Figure 2G) without affecting the average duration of individual episodes (RM ANOVA, P = 0.149; Figure 2H).
Although amphetamine significantly increased waking and suppressed sleep in both hypocretin KO and WT mice, its effects were less robust in the narcoleptic phenotype during REM sleep (2-way ANOVA, P = 0.004). In WT mice (n = 6) amphetamine completely blocked REM sleep during the entire recording period; however, in KO mice it only suppressed REM sleep for 1.5 - 2 hours before it returned to saline values. The degree to which amphetamine suppressed NREM sleep and increased wakefulness was similar in both groups (2-way ANOVA, P > 0.05 for both states).

D1-Like Receptors Modulate Sleep Attacks but not Cataplexy

First, we aimed to determine if blockade of excitatory D1-like receptors would reduce wakefulness and promote sleep in narcoleptic mice. Compared to saline treatment, low doses of the D1-like receptor antagonist (SCH 23390; 0.25mg/kg) had no affect on sleep-wake amounts (n = 5; 2-way RM ANOVA, P > 0.05; Figure 3A) or sleep latency (RM ANOVA, P = 0.279). However, a higher dose of SCH 23390 (1mg/kg) decreased wakefulness by 25% (2-way RM ANOVA, P = 0.016) and increased NREM sleep by 122% (P = 0.024). Neither high nor low doses of SCH 23390 had significant affects on REM sleep amounts (2-way RM ANOVA, P = 0.963; Figure 3A). Blockade of D1-like receptors also promoted sleepiness because sleep latency decreased from 1735 ± 717s (i.e., saline treatment) to 172 ± 17s following treatment with a high dose of SCH 23390 (RM ANOVA, P = 0.033; data not shown).

Sleep attacks were affected by D1-like receptor blockade. Compared to saline treatment, low doses of SCH 23390 (0.25mg/kg) had no affect on sleep attacks (RM ANOVA, P = 0.101; Figure 3C); however, higher doses (1mg/kg) increased the total time spent in sleep attacks (RM ANOVA, P = 0.009; Figure 3C) by increasing the number of sleep attacks by 88% (RM ANOVA, P = 0.022; Figure 3D); this drug dose had no affect on the duration of individual attacks (RM ANOVA; P = 0.265; Figure 3E). Blockade of D1-like receptors had no significant affect on cataplexy (RM ANOVA, P = 0.870; Figure 3F-H).

Next, we wanted to determine if D1-like receptor activation would increase wakefulness and decrease sleep in narcoleptic mice. Compared to saline treatment, both low (5mg/kg) and high doses (20mg/kg) of SKF 38393 increased wakefulness by 25% (n = 6; 2-way RM ANOVA, P < 0.001) and 23% (P < 0.001), while decreasing NREM sleep by 88% (P < 0.001) and 76% (P < 0.001; Figure 4A). Neither high nor low drug doses had significant affects on REM sleep even though REM amounts decreased by 92% (P = 0.157) and 98% (P = 0.127). Activation of D1-like receptors also promoted arousal because sleep latency increased from 1768 ± 785s (i.e., saline) to 10789 ± 932s (RM ANOVA, P < 0.001) and 11374 ± 1056s (P < 0.001) following treatment of 5mg/kg and 20mg/kg of SKF 38393 (data not shown).

Both high and low doses of SKF 38393 potently suppressed sleep attacks in narcoleptic mice (RM ANOVA, P = 0.004; Figure 4C). Compared to saline treatment, high and low doses of SKF 38393 decreased the number of sleep attacks by 77% (P = 0.004; Figure 4D) and 58% (RM ANOVA, P = 0.022), but neither dose had an affect on duration of attacks (P = 0.560; Figure 4E). High and low doses of SKF 38393 completely abolished sleep attacks in 83% and 33% of narcoleptic mice. Neither high nor low doses of SKF 38393 had significant affects on cataplexy (RM ANOVA, P = 0.549; Figure 4F-H).

Lastly, we aimed to determine if D1-like receptor manipulation similarly affects sleep-wake behavior in normal (i.e., WT) and narcoleptic mice. In WT mice, SCH 23390 administration (1.0mg/kg) decreased wakefulness by 13% (n = 4; 2-way RM ANOVA, P = 0.003) and increased NREM sleep by 73% (P = 0.002; Figure 3B); whereas receptor activation by SKF 38393 treatment (20mg/kg) increased wakefulness by 23% (n = 5;
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without affecting cataplexy duration (RM ANOVA, P = 0.107; Figure 5H). Even though this dose increased the total time spent in cataplexy by 174%, this effect was not statistically significant (RM ANOVA, P = 0.193; Figure 5F). Partial activation of D2-like receptors with modest quinpirole doses (0.125mg/kg) had no measurable affects on either the duration (RM ANOVA, P = 0.058) or frequency (RM ANOVA, P = 0.931) of attacks.

Blockade of D2-like receptors with low doses (0.25mg/kg) of eticlopride decreased wakefulness by 18% (n = 7; 2-way RM ANOVA; SCH 23390: P = 0.029; SKF 38393: P = 0.341). High doses of eticlopride (1mg/kg) had no detectable affects on sleep-wake behavior in narcoleptic mice (2-way RM ANOVA, P = 0.366; Figure 6A). Even though high and low doses did not affect sleep attacks (RM ANOVA, P = 0.357; Figure 5C-E), they had robust suppressive affects on cataplexy. Compared to saline treatment, high doses of eticlopride potently reduced the total time spent in cataplexy by 97% (RM ANOVA, P = 0.024; Figure 6D); this decrease was attributable to the 88%

Figure 3—Inactivation of D1-like receptors increases sleep attacks. A: In narcoleptic mice, SCH 23390 (D1-like antagonist; 1mg/kg) increased NREM sleep and decreased wakefulness. B: In wild-type mice, SCH 23390 also increased NREM sleep and decreased wakefulness. C-E: SCH 23390 (1mg/kg) increased both the total time spent in sleep attacks (C) and sleep attack frequency (D), but had no affect on attack duration (E). F-H: SCH 23390 treatment had no affect on time spent in cataplexy (F) or on cataplexy frequency (G) or duration (H). *denotes P < 0.05 when compared to saline.

2-way RM ANOVA, P = 0.002) and decreased NREM sleep by 87% (P = 0.005; Figure 4B). Neither intervention had significant affects on REM sleep amounts (SCH 23390: P = 0.853; SKF 38393: P = 0.706). The impact of D1-like receptor manipulation on sleep-wake behavior was identical in WT and KO mice (2-way ANOVA; SCH 23390: P = 0.295; SKF 38393: P = 0.341).

D2-Like Receptors Modulate Cataplexy but not Sleep Attacks

Here, we aimed to determine if D2-like receptors influence sleep, sleep attacks and cataplexy in narcoleptic mice. To do this, we administered quinpirole (0.125 and 0.5mg/kg) to activate D2-like receptors and eticlopride (0.25 and 1mg/kg) to inactivate them. Neither high nor low doses of quinpirole affected amounts of sleep or wakefulness (n = 7; 2-way RM ANOVA, P = 0.262; Figure 5A); these interventions also had no affect on sleep attacks (RM ANOVA, P = 0.357; Figure 5C-E). However, quinpirole had potent affects on cataplexy. The highest dose (0.5mg/kg) increased the number of cataplexy episodes by 172% above baseline levels (RM ANOVA, P = 0.030; Figure 5G), without affecting cataplexy duration (RM ANOVA, P = 0.107; Figure 5H). Even though this dose increased the total time spent in cataplexy by 174%, this effect was not statistically significant (RM ANOVA, P = 0.193; Figure 5F). Partial activation of D2-like receptors with modest quinpirole doses (0.125mg/kg) had no measurable affects on either the duration (RM ANOVA, P = 0.058) or frequency (RM ANOVA, P = 0.931) of attacks.

Blockade of D2-like receptors with low doses (0.25mg/kg) of eticlopride decreased wakefulness by 18% (n = 7; 2-way RM ANOVA, P = 0.026; Figure 6A), increased NREM sleep by 90% (P = 0.039), but had no affect on REM sleep (P = 0.922). High doses of eticlopride (1mg/kg) had no detectable affects on sleep-wake behavior in narcoleptic mice (2-way RM ANOVA, P = 0.366; Figure 6A). Even though high and low doses did not affect sleep attacks (RM ANOVA, P = 0.758; Figure 6C-E), they had robust suppressive affects on cataplexy. Compared to saline treatment, high doses of eticlopride potently reduced the total time spent in cataplexy by 97% (RM ANOVA, P = 0.024; Figure 6D); this decrease was attributable to the 88%
We demonstrate that a dopamine mechanism modulates cataplexy and sleep attacks in a murine model of narcolepsy. Specifically, we show that amphetamine suppresses both cataplexy and sleep attacks, suggesting that dopamine transmission modulates these behaviors. We then show that pharmacological activation of D2-like receptors triggers cataplectic attacks and blockade of these receptors potently suppresses them. Manipulation of D2-like receptors does not influence sleep attacks. We also show that activation and blockade of D1-like receptors decreases and increases sleep attacks, respectively; however, manipulation of D1-like receptors does not affect cataplexy. Our results suggest that dopamine transmission modulates cataplexy and sleep attacks by different receptor mechanisms.

**DISCUSSION**

We demonstrate that a dopamine mechanism modulates cataplexy and sleep attacks in a murine model of narcolepsy. Specifically, we show that amphetamine suppresses both cataplexy and sleep attacks, suggesting that dopamine transmission modulates these behaviors. We then show that pharmacological activation of D2-like receptors triggers cataplectic attacks and blockade of these receptors potently suppresses them. Manipulation of D2-like receptors does not influence sleep attacks. We also show that activation and blockade of D1-like receptors decreases and increases sleep attacks, respectively; however, manipulation of D1-like receptors does not affect cataplexy. Our results suggest that dopamine transmission modulates cataplexy and sleep attacks by different receptor mechanisms.

**Amphetamine Alleviates Cataplexy and Sleep Attacks**

We found that amphetamine suppresses cataplexy, sleep attacks and sleep. One mechanism by which amphetamine may...
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This study shows that D2-like receptors play a significant role in regulating murine cataplexy. Dopamine receptor modulation of cataplexy shows specificity for the D2-like receptor because neither D1-like receptor activation nor blockade affects cataplexy even though D1 drugs have pronounced effects on sleep and sleep attacks. Multiple lines of evidence recognize the importance of a D2-like receptor mechanism in regulating cataplexy. For example, there is a positive correlation between striatal D2 receptor binding and the frequency of cataplectic attacks in human narcoleptics. A D2-like receptor mechanism has also been linked to canine narcolepsy; Nishino et al. demonstrate that activation of D2-like receptors increases cataplexy and blockade of these receptors decreases it in narcoleptic dogs.

Amphetamine-induced changes in dopamine levels may also contribute to the suppression of cataplexy in narcoleptic mice. This assertion is supported by the fact that both systemic amphetamine administration and direct manipulation of dopamine cell fields modulate cataplexy in narcoleptic dogs. However, because amphetamine also increases levels of both serotonin and noradrenaline it is possible that amphetamine-induced changes in cataplexy are mediated by multiple monoaminergic systems. Indeed, clomipramine, a tricyclic antidepressant that affects dopaminergic, noradrenergic and serotonergic transmission, reduces cataplexy without affecting sleep attacks in hypocretin KO mice.

**A D2-Like Receptor Mechanism Modulates Cataplexy**

This study shows that D2-like receptors play a significant role in regulating murine cataplexy. Dopamine receptor modulation of cataplexy shows specificity for the D2-like receptor because neither D1-like receptor activation nor blockade affects cataplexy even though D1 drugs have pronounced effects on sleep and sleep attacks. Multiple lines of evidence recognize the importance of a D2-like receptor mechanism in regulating cataplexy. For example, there is a positive correlation between striatal D2 receptor binding and the frequency of cataplectic attacks in human narcoleptics. A D2-like receptor mechanism has also been linked to canine narcolepsy; Nishino et al. demonstrate that activation of D2-like receptors increases cataplexy and blockade of these receptors decreases it in narcoleptic dogs.

![Figure 5](image-url)

**Figure 5**—Activation of D2-like receptors increases cataplexy. A: Quinpirole (D2-like receptor agonist) had no affect on sleep-wake behavior in narcoleptic mice. B: Quinpirole also had no affect on sleep-wake behavior in wild-type mice. C-E: Quinpirole had no affect on the total time spent in sleep attacks (C) or on the frequency (D) or duration of attacks (E). F-H: Quinpirole did not significantly increase the total time spent in cataplexy (F), but at 0.5mg/kg it increased cataplexy frequency (G) without affecting duration (H). *denotes P < 0.05 when compared to saline.
triggers sleepiness by reducing the latency from wakefulness to NREM sleep, it also increases NREM sleep amounts and sleep attacks. These findings suggest that a dopaminergic drive acting on D1-like receptors stimulates wakefulness. Wake-active dopamine cells in the ventral periaqueductal gray could be one source of this excitatory drive because lesioning these dopamine cells triggers increased sleep in rats.

It is unlikely that dopamine-mediated suppression of sleep attacks and sleepiness acts on the hypocretin system because 1) dopamine inhibits rather than excites wake-promoting hypocretin neurons and 2) KO mice do not synthesize hypocretin. This assertion is also supported by the fact that modafinil’s wake-promoting effects are stronger in hypocretin KO than WT mice.

There is evidence that D2-like drugs modulate sleep-wake behavior. We confirmed that blockade of D2-like receptors can influence sleep-wake amounts. However, we did not observe sleep-wake effects with injection of a D2-like receptor agonist. Previous studies have shown that quinpirole does not impact sleep-wake regulation in a linear dose-depen-

Figure 6—Inactivation of D2-like receptors decreases cataplexy. A: In narcoleptic mice, 0.25mg/kg of eticlopride (D2-like antagonist) increased NREM sleep and decreased wakefulness, but at 1mg/kg it had no effect on sleep-wake behavior. B: Eticlopride had no effect on sleep-wake behavior in wild-type mice. C-E: Eticlopride had no effect on the total time spent in sleep attacks (C) or on the frequency (D) or duration of attacks (E). F-G: Eticlopride (1mg/kg) decreased both the total time spent in cataplexy (F) and its frequency (G) without affecting the duration of cataplectic episodes (H). *denotes P < 0.05 when compared to saline.
dent fashion; instead, only low (0.015mM) or high (1mM or greater) doses modulate sleep-wake behavior. Because we used mid-range doses (0.125 and 0.5mM), we did not expect changes in sleep-wake behaviors. Indeed, we used this approach to determine whether D2 drugs could affect cataplexy independent of sleep-wake regulation. We found that D2 drugs can modulate cataplexy with negligible effects on sleep and sleep attacks, while D1 drugs modulate sleep and sleep attacks without affecting cataplexy. This is an important observation because it illustrates that sleep attacks and cataplexy are controlled by distinct mechanisms.

Physiological Significance

Narcolepsy and REM sleep share some physiological and behavioral similarities, the most salient example being the loss of skeletal muscle tone (i.e., cataplexy vs. REM atonia). This commonality led to the hypothesis that narcolepsy is a REM sleep disorder and that a faulty REM mechanism underlies narcolepsy/cataplexy. However, we show that dopamine drugs can manipulate REM sleep and cataplexy independently. Okura et al. also show that D2 antagonists reduce cataplexy without affecting REM sleep in narcoleptic dogs. Although REM sleep atonia and cataplexy may be caused by the same mechanism (i.e., at the motoneuron level), our data suggest that REM sleep and cataplexy are triggered by distinct mechanisms. Two other pieces of experimental data support this claim. First, Thankachan et al. demonstrate that putative REM-generating cells only discharge during REM sleep but never during cataplexy in narcoleptic mice; and second, Nishino et al. show in narcoleptic dogs that REM sleep follows an ultradian rhythm whereas cataplexy does not. Based on these observations, we assert that REM sleep and cataplexy are triggered by separate physiological mechanisms.

Clinical Implications

The dopamine system has an established role in regulating both sleep and motor control, and loss of normal dopamine function contributes to common sleep disorders. For example, changes in dopamine receptor function are associated with the sleep attacks that occur in both narcolepsy and Parkinson’s disease (PD). A reduction in striatal dopamine transporter expression has been observed in REM sleep behavior disorder (RBD) – a sleep disorder that is characterized by excessive motor activity during REM sleep. It is noteworthy that ~65% of RBD patients eventually develop PD; this is important because both disorders are characterized by abnormal sleep and motor function, and dopamine system dysfunction contributes to each.

Drugs targeting the dopamine system effectively treat some sleep and motor disorders. For example, D2 drugs (e.g. pramipexole) are prescribed for treating the motor symptoms associated with restless leg syndrome and RBD. Modafinil and amphetamine, both of which influence dopamine neurotransmission, are used to combat excessive sleepiness in narcolepsy, sleep apnea syndrome and PD. Our current study in narcoleptic mice and a previous one in narcoleptic dogs both show that D2 receptor activators and blockers increase and decrease cataplexy, respectively. We also show that D1 receptor stimulation reduces sleep attacks in hypocretin KO mice. These findings support the use of dopaminergic drugs in the treatment of narcolepsy/cataplexy and other sleep disorders.

Another link between the dopamine system and narcolepsy is gamma-hydroxybutyrate (GHB) – an effective treatment for both cataplexy and sleep fragmentation. The exact physiological mechanism(s) by which GHB acts is unknown, however, it is well documented that it not only affects GABA_A receptor function, but it also impacts dopamine transmission. Although acute GHB treatment inhibits dopamine release chronic treatment upregulates both D1-like and D2-like receptor mRNA expression levels. Because oral doses of GHB cause almost immediate sleep initiation, whereas it takes several weeks to alleviate cataplexy, we suggest that GHB acts to trigger sleep by inhibiting dopamine release and hence the wake-promoting dopamine drive, and functions to suppress cataplexy by changing dopamine receptor expression. Both these explanations are consistent with our data showing that a D1 receptor mechanism suppresses sleep attacks and that a D2 receptor mechanism can regulate cataplexy. Dissecting the neural mechanisms and substrates by which dopamine modulates sleep and cataplexy is important given the potential clinical relevance of these findings.

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DISCLOSURE STATEMENT

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