Trace Amine-Associated Receptor 1 Agonists as Narcolepsy Therapeutics

Sarah W. Black, Michael D. Schwartz, Tsui-Ming Chen, Marius C. Hoener, and Thomas S. Kilduff

ABSTRACT

BACKGROUND: Narcolepsy, a disorder of rapid eye movement (REM) sleep, is characterized by excessive daytime sleepiness and cataplexy, a loss of muscle tone triggered by emotional stimulation. Current narcolepsy pharmacotherapeutics include controlled substances with abuse potential or drugs with undesirable side effects. As partial agonists at trace amine-associated receptor 1 (TAAR1) promote wakefulness in mice and rats, we evaluated whether TAAR1 agonism had beneficial effects in two mouse models of narcolepsy.

METHODS: In the first experiment, male homozygous B6-Taar1tm1(NLSLacZ)Blt (Taar1 knockout) and wild-type mice were surgically implanted to record electroencephalogram, electromyogram, locomotor activity, and body temperature, and the efficacy of the TAAR1 agonist, RO5256390, on sleep/wake and physiological parameters was determined. In the second experiment, the effects of the TAAR1 full agonist RO5256390 and partial agonist RO5263397 on sleep/wake, locomotor activity, body temperature, and cataplexy were assessed in two mouse narcolepsy models.

RESULTS: RO5256390 profoundly reduced rapid eye movement sleep in wild-type mice; these effects were eliminated in Taar1 knockout mice. The TAAR1 partial agonist RO5263397 also promoted wakefulness and suppressed nonrapid eye movement sleep. Both compounds reduced body temperature in the two narcolepsy models at the highest doses tested. Both TAAR1 compounds also mitigated cataplexy, the pathognomonic symptom of this disorder, in the narcolepsy models. The therapeutic benefit was mediated through a reduction in number of cataplexy episodes and time spent in cataplexy.

CONCLUSIONS: These results suggest TAAR1 agonism as a new therapeutic pathway for treatment of this orphan disease. The common underlying mechanism may be the suppression of rapid eye movement sleep.

Keywords: Cataplexy, Hypocretin, Mouse models, Orexin, Sleep, Trace amines

http://dx.doi.org/10.1016/j.biopsych.2016.10.012

The sleep disorder narcolepsy affects 1 in 2000 individuals in the United States and is characterized by excessive daytime sleepiness (EDS); abnormalities of rapid eye movement (REM) sleep; and cataplexy, the sudden loss of muscle tone triggered by emotional stimulation (1). Specific degeneration of neurons that contain hypocretin (Hcrt; also known as orexin) is the likely cause of narcolepsy (2,3). Cerebrospinal fluid Hcrt1 levels are reduced in narcoleptic patients relative to control subjects (4–6), presumably from the loss of Hcrt neurons (2,3). Numerous animal models of narcolepsy exist that are characterized by cataplexy, sleep/wake fragmentation, and increased REM sleep propensity (7–10); some models exhibit both construct and face validity (11,12).

Current treatments for narcolepsy involve symptomatic management of EDS and cataplexy. Amphetamines and other stimulants with abuse potential treat EDS through presynaptic stimulation of dopaminergic transmission (1,13). Modafinil has become the first-line treatment for sleepiness because it has fewer side effects than amphetamine-like stimulants (14). The wake-promoting mechanism of action of modafinil may be through inhibition of the dopamine (DA) transporter (15), but it could also be through enhancement of trace amine activity (16). Antidepressants, both tricyclic antidepressants and selective monoaminergic reuptake inhibitors, alleviate cataplexy to the extent that they, or their metabolites, inhibit noradrenaline uptake (17,18). The only approved drug that treats both cataplexy and EDS is gamma-hydroxybutyric acid (19,20).

Trace amine-associated receptor 1 (TAAR1) (21,22) is a negative modulator of monoaminergic neurotransmission (23,24) and may present a novel therapeutic pathway for the treatment of narcolepsy. Whereas Taar1 knockout (KO) mice appear similar to wild-type (WT) littermates in most neurological and behavioral analyses (23,24), when challenged with d-amphetamine, Taar1 KO mice show enhanced hyperlocomotion and exaggerated striatal release of DA, noradrenaline, and 5-hydroxytryptamine (23,24). Conversely, Taar1-overexpressing mice show little response to amphetamine (25). The spontaneous firing rate of dopaminergic ventral tegmental area (VTA) and serotonergic neurons in...
the dorsal raphe nucleus is greatly increased in the absence of TAAR1 (23); TAAR1 tonically activates G protein-coupled inwardly rectifying potassium channels to reduce basal firing activity of these neurons (26,27). TAAR1 may also regulate glutamatergic activity in the cerebral cortex (27,28). The endogenous ligands for TAAR1 are trace amines such as beta-phenylethylamine, p-tyramine, octopamine and tryptamine, molecules that are closely related to the classic biogenic amines (29,30), and the thyronamines thyronamine and 3-iodothyronamine (31), which are structurally related to thyroid hormones triiodothyronine and thyroxine. Although thyroid hormones have been hypothesized to be regulated by Hct, circulating levels are associated with changes in sleep/wake in both narcoleptic patients and control subjects (32). Abnormal levels of trace amines have been associated with neuropathological disorders (29,30,33–35).

Several small molecule TAAR1 ligands have been described. The TAAR1 selective antagonist EPPTB (26) increased the firing frequency of DA neurons in vitro, suggesting that TAAR1 either exhibits constitutive activity or is tonically activated by endogenous agonists. Conversely, the full agonist RO5166017 inhibited the firing of dopaminergic VTA and serotoninergic dorsal raphe nucleus neurons but did not affect locus coeruleus neurons, an area devoid of Taar1 expression (27). We have described the effects of partial TAAR1 agonists in rodent and primate neurobehavioral paradigms (36–38) that suggested therapeutic potential for TAAR1 agonism in psychosis, mood disorders, and substance abuse. These studies also revealed dose-dependent, wake-promoting and REM sleep-inhibiting properties of TAAR1 partial agonists. However, unlike psychostimulants, the wakefulness produced by TAAR1 partial agonists was not accompanied by hyperlocomotion and increased body temperature (Tb) (36,37). Consequently, we hypothesized that TAAR1 agonism might be a novel therapeutic pathway for treatment of narcolepsy. We report here the efficacy of TAAR1 agonists to promote wakefulness and alleviate cataplexy in two models of murine narcolepsy.

METHODS AND MATERIALS

All experimental procedures were approved by the Institutional Animal Care and Use Committee at SRI International and were conducted in accordance with the principles set forth in the Guide for Care and Use of Laboratory Animals. Detailed methods are described in Supplemental Methods.

Protocol 1: Efficacy of TAAR1 Full and Partial Agonists in WT and Taar1 KO mice

Animals. To evaluate wake-promoting efficacy of ROS5256390, adult male homozygous B6-Taar1tm1(NLSlacZ)dit (Taar1 KO) mice (23) (n = 11) and their WT littermates (n = 13) were used. As a comparison to ROS5256390, data are presented from a second cohort of Taar1 KO mice (n = 8) and a pooled group of 5 WT littermates and 7 WT B6-Tg(Taar1)27 (Taar1-overexpressing) mice (n = 12) (25) treated with ROS5363397, as described previously (38). Both strains were maintained on a C57BL/6 background. Mice were obtained from F. Hoffmann-LaRoche, Ltd. (Basel, Switzerland) or were bred at SRI International using founders obtained from Roche.

Surgical Procedures and Data Recording. Mice were instrumented for tethered recording of electroencephalogram (EEG), electromyogram (EMG), telemetered monitoring of locomotor activity (LMA), and core Tb as described previously (38–40). EEG/EMG data were continuously recorded using iox2 version 2.8.0.11 (emka TECHNOLOGIES, Paris, France) and analyzed as described (38–40).

Drugs. ROS5256390 and RO5263397 (synthesized at Roche) and caffeine (Caf) (Sigma-Aldrich, St. Louis, MO) were prepared fresh as solutions or suspensions with 1 hour sonication and serial dilutions using 0.3% Tween-80 in water as the vehicle (Veh). Doses (37) were delivered at 10 mL/kg final volume and were administered per os [p.o.].

Experimental Design. One group of mice (n = 11 Taar1 KO mice and 13 WT littermates) received ROS5256390 (1 mg/kg, 3 mg/kg, and 10 mg/kg, p.o., half-life = 3.7 hours) (37), Caf (10 mg/kg, positive control), or Veh. As described previously (38), the other group (n = 8 Taar1 KO mice and 12 WT littermates) received RO5263397 (0.1 mg/kg, 0.3 mg/kg, and 1 mg/kg, p.o., half-life = 6 hours), Caf (10 mg/kg, positive control), or Veh. All mice were dosed at zeitgeber time 6 (ZT 0 = 8 AM) in balanced order with at least 3 days between treatments.

Data Analysis and Statistics. EEG and EMG data were visually scored offline in 10-second epochs as wakefulness, non-REM sleep, and REM sleep using ecgAUTO (emka TECHNOLOGIES) by expert scorers blinded to drug treatment and genotype. Time spent in each state and LMA and Tb were assessed for 3 hours after dosing. Drug efficacy was evaluated using two-way mixed analysis of variance (ANOVA) comparing genotype and drug treatment followed by Bonferroni t tests, where appropriate.

Protocol 2: TAAR1 Full and Partial Agonists in Mouse Narcolepsy Models

Animals. Male, hemizygous transgenic C57BL/6-Tg(orexin/ataxin-3)/Sakurai mice (Atax) mice (11) and transgenic C57BL/6-Tg(orexin/tTA; TetO diphtheria toxin A fragment)/Yamanaka (DTA) mice (12) bred at SRI International were used. DTA mice were studied after dietary doxycycline withdrawal [Dox(−)] for 28 days, which produces degeneration of Hcrt cells and severe cataplexy (12).

Surgical Procedures and Data Recording. Mice were surgically implanted with transmitters for EEG, EMG, Tb, and locomotor activity recording as described (41,42). Physiological and video-recorded behavioral data were simultaneously acquired with Dataquest A.R.T. version 4.2 (Data Sciences International, St. Paul, MN).

Drugs. Almorexant (Alm), (2R)-2-[(1S)-6,7-dimethoxy-1-[2-(4-trifluoromethyl-phenyl)-ethyl]-3,4-dihydro-1H-isouquinolin-2-yl]-N-methyl-2-phenyl-acetamide (43), was synthesized at SRI International (>99% purity as determined by nuclear magnetic
دریافت فوری متن کامل مقاله

امکان دانلود نسخه تمام متن مقالات انگلیسی
امکان دانلود نسخه ترجمه شده مقالات
پذیرش سفارش ترجمه تخصصی
امکان جستجو در آرشیو جامعی از صدها موضوع و هزاران مقاله
امکان دانلود رایگان ۲ صفحه اول هر مقاله
امکان پرداخت اینترنتی با کلیه کارت های عضو شتاب
دانلود فوری مقاله پس از پرداخت آنلاین
پشتیبانی کامل خرید با بهره مندی از سیستم هوشمند رهگیری سفارشات