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Enclosure:

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The Neurochemical and Anxiolytic-Like Effects of Centanafadine in Preclinical Assays in the Rat and Mouse

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Introduction

- Centanafadine is a norepinephrine (NE), dopamine (DA), and serotonin (5-HT) reuptake inhibitor (NDSRI) currently under review by the FDA as a novel drug to treat attention-deficit/hyperactivity disorder (ADHD)
- Overall, it has been shown to possess a favorable efficacy and safety profile in phase 3 trials in adults, children, and adolescents with ADHD[1-4]
- Emerging clinical and nonclinical data suggest that the pharmacological profile of centanafadine extends beyond core ADHD symptoms to associated features, including emotional dysregulation, executive dysfunction, and common psychiatric comorbidities such as anxiety[5-7]

Objective

- Characterize the neurochemical and behavioral effects of centanafadine using in vivo microdialysis and established preclinical models of anxiety-related behavior

Methods

Microdialysis

- Neurotransmitter levels were quantified in the rat prefrontal cortex (PFC) and ventral striatum (vSTR) following oral centanafadine (3, 10, and 30 mg/kg) or vehicle administration in male Sprague Dawley rats (n=7–8/group)
  - Comparisons versus vehicle used Williams' test ($P<0.05$)

Marble burying test

- In male C57BL/6J mice (8 weeks old; n=10/group), anxiety-related behavior was assessed using the marble burying test after 1 hour oral centanafadine (3, 10, 30, and 40 mg/kg), 30 min intraperitoneal chlordiazepoxide (15 mg/kg), or vehicle administration
- Endpoints included number of marbles buried and total distance traveled
  - Comparisons versus vehicle were analyzed using one-way ANOVA followed by Dunnett's test ($P<0.05$)

Elevated plus maze (EPM)

- In male wistar rats (n=10/group), anxiety related behavior was assessed using the EPM after 1 hour oral centanafadine (3, 10, and 30 mg/kg) or vehicle administration
- Endpoints included % time in open arms, % open arm entries, total arm entries
  - Comparisons versus vehicle were analyzed using Dunnett's test ($P<0.05$)

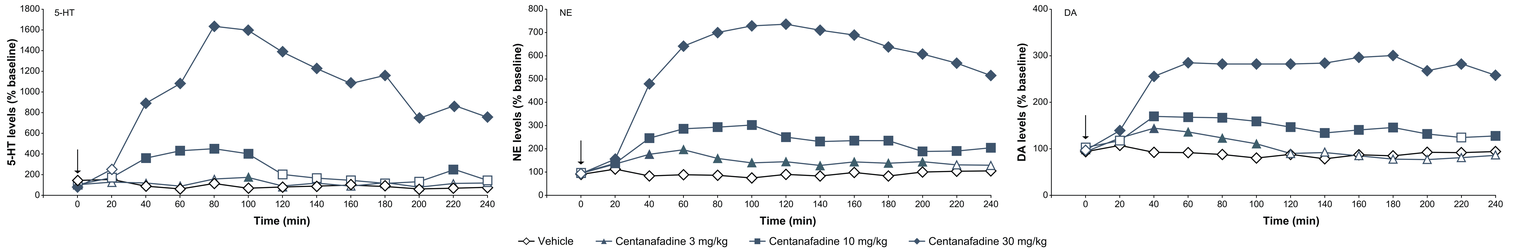
Contextual fear conditioning (CFC)

- In male wistar rats (n=8/group), anxietyrelated behavior was assessed using the CFC paradigm after 1 hour oral centanafadine (3, 10, and 30 mg/kg) or vehicle administration; a non-shock control group was also included (n=8)
  - Rats underwent conditioning on Day 1 and testing on Day 2
- Endpoints included freezing behavior and total distance traveled
- Differences between the non-shock and vehicle groups were analyzed using an unpaired t-test; differences versus vehicle were analyzed using Dunnett's test ($P<0.05$)

Results

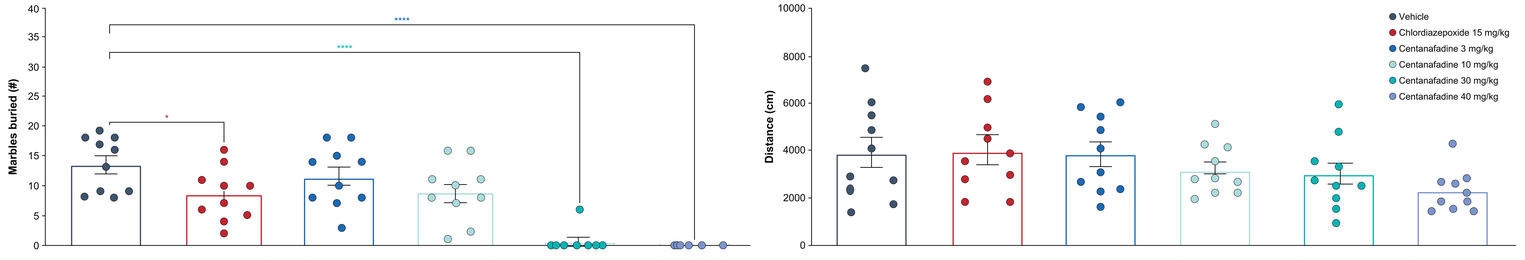
- Centanafadine dose-dependently increased the extracellular concentrations of NE, DA, and 5-HT in rats, with peak increases at 30 mg/kg in the PFC of 1638% for 5-HT, 739% for NE, and 302% for DA (**Figure 1**)
- At 3 and 10 mg/kg, peak increases in the PFC were 176% and 455% for 5-HT, 198% and 304% for NE, and 146% and 171% for DA, respectively
- Average increases over the 0–4h period in the PFC were 1184% for 5-HT, 604% for NE, and 281% for DA; over the same period in the vSTR, centanafadine increased 5-HT by 1118% and DA by 186%
- In mice, centanafadine significantly reduced the number of marbles buried following dosing at 30 and 40 mg/kg (both $P<0.0001$) compared with vehicle-treated animals (**Figure 2**)
- No significant differences in the total distance traveled were detected across the treatment groups
- In rats, centanafadine was associated with numerical increases in open arm exploration in the EPM at 30 mg/kg, including % time in the open arms, % open arm entries, and total arm entries compared with vehicle-treated animals (**Figure 3**)
- Vehicle-treated animals previously subjected to shocks showed a significantly increased ($P<0.0001$) percentage of the session duration exhibiting freezing behavior (**Figure 4**)
- Centanafadine significantly decreased the percentage of freezing behavior at 30 mg/kg compared with vehicle-treated group

Figure 1. Effect of centanafadine on 5-HT, NE, and DA in the PFC



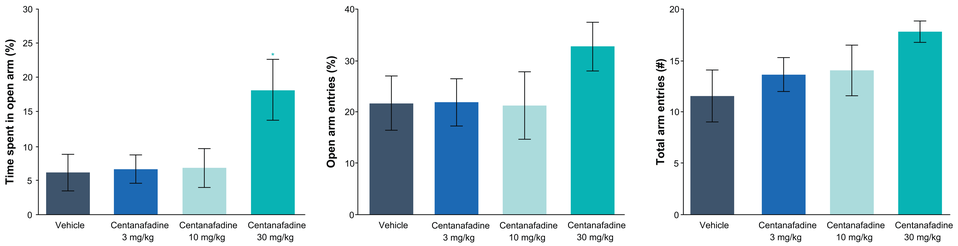
Data are means; n=7–8/group. For clarity, the SEMs are not shown on the figures. Centanafadine administration is shown by ↓. Significant differences from vehicle control are shown by the filled symbols.
5-HT, serotonin; DA, dopamine; NE, norepinephrine; PFC, prefrontal cortex.

Figure 2. Effects of centanafadine on anxiety-related behavior in the marble burying test



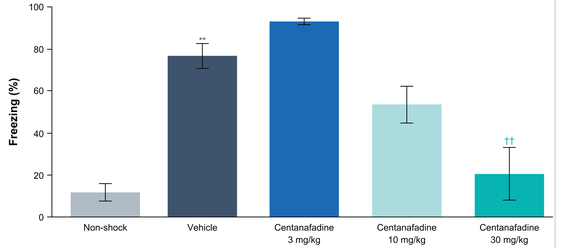
Data are presented as mean±SEM; n=10/group.
The differences between treatment groups were assessed by one-way ANOVA (*$P<0.05$, ****$P<0.0001$).

Figure 3. Effects of centanafadine on anxiety-related behavior in the EPM test



Data are presented as mean±SEM; the experiment was conducted with n=10/group, but due to some exclusions, the results are shown as n=9–10/group.
The differences between vehicle-treated and centanafadine-treated groups were assessed by Dunnett's test (*$P<0.05$).
EPM, elevated plus maze.

Figure 4. Effect of centanafadine on CFC



Data are presented as mean±SEM; n=8/group.
Differences between the non-shock and vehicle-treated groups were assessed by unpaired t-test (**$P<0.01$); differences between the vehicle-treated and centanafadine-treated groups were assessed by Dunnett's test (††$P<0.0001$).
CFC, contextual fear conditioning.

Conclusions

- Centanafadine increased NE, DA, and 5-HT in the rat PFC and 5-HT and DA in the vSTR, consistent with activity across all 3 monoamine systems in vivo
- The rank order of effects and the prominent serotonergic response in cortical and subcortical regions support centanafadine's profile as an NDSRI
- Centanafadine also demonstrated an anxiolytic-like profile across several well-established mouse and rat assays
- Together, these findings suggest that centanafadine's NDSRI activity may contribute to its anxiolytic-like effects and may provide potential benefit for ADHD-associated symptoms like emotional dysregulation, as well as comorbid anxiety symptoms

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At Otsuka, we hold a deep respect for the value of every mind. We will not rest until mental illnesses and brain diseases are approached with the same priority and urgency as our physical health and recognized as chronic diseases that warrant early, equitable, and accessible intervention for patients and caregivers everywhere.

Disclosures

RE, **MH**, **GSP** are all full-time employees of Otsuka Pharmaceutical Development & Commercialization, Inc. **DJH** received no financial support for authoring this work and the opinions expressed are exclusively his own and have not been influenced by any public, commercial, or not-for-profit organization. **TS** and **TK** are full-time employees of Otsuka Pharmaceutical Co., Ltd and own stock of Otsuka Holdings Co., Ltd.

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