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RESEARCH ARTICLE

Effect of fasedienol (PH94B) pherine nasal spray and steroidal hormones on electrogram responses and autonomic nervous system activity in healthy adult volunteers

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Abstract

Objective: Fasedienol (PH94B) is a pherine compound formulated as a nasal spray that is hypothesized to regulate olfactory-amygdala circuits of fear and anxiety. Fasedienol's effect on the local electrogram of nasal chemosensory neurons (EGNR) and autonomic nervous system (ANS) responses versus steroidal hormones and controls in healthy adults is reported.

Methods: Eight males and 8 females randomly received aerosolized control (propylene glycol) and study drugs (fasedienol, 17β-estradiol, progesterone, cortisol, and testosterone, 0.4 μg each in propylene glycol) onto the nasal septum mucosal lining at 30-min intervals over 2 sessions. EGNR was continuously monitored; autonomic parameters were recorded before and after administration.

Results: Fasedienol significantly increased EGNR amplitude (males: 5.0 vs. 0.6 mV, $p < 0.001$; females:5.7 vs. 0.6 mV, $p < 0.001$), and rapidly reduced respiratory rate ($p < 0.05$), heart rate ($p < 0.01$), and electrodermal activity ($p < 0.05$) versus control. EGNR and ANS responses after steroidal hormone administration were similar to control. 81% reported feeling less tense/more relaxed after receiving fasedienol, but not after receiving either control or steroidal hormones.

Conclusions: Intranasal fasedienol, but not control or steroidal hormones, activated EGNR and rapidly reduced ANS responses, consistent with sympatholytic effects. Combined with subjective reports, results suggest fasedienol may provide acute relief in anxiety conditions.

KEYWORDS
anti-anxiety, autonomic nervous system effects, chemosensory receptor neurons, intranasal administration, pherines, social anxiety disorder, steroidal hormones

1 | INTRODUCTION

Social anxiety disorder (SAD) is common, with a lifetime prevalence of 12.1% for adults in the United States (National Institute of Mental Health, 2021). Social anxiety disorder compromises social, academic, and occupational functioning, and is often comorbid with and a predictor for other serious psychiatric disorders, including other anxiety, major depressive, substance use, and avoidant personality disorders (Koyuncu et al., 2019; Leichsenring & Leweke, 2017), as well as suicidal ideation and suicide. Behavioral therapies are considered first-line treatments in SAD but are not uniformly effective (Leichsenring & Leweke, 2017). Pharmacotherapy options—

including antidepressants, benzodiazepines, and beta-blockers—require long-term use, can have troubling side effects and safety concerns, and are not suitable or effective in many patients (Blanco et al., 2013; Rodebaugh et al., 2004; Williams et al., 2017). Additionally, in September 2022, the US Food and Drug Administration issued a drug safety communication requiring the *Boxed Warning* for all benzodiazepines be updated to describe the risks of abuse, misuse, addiction, physical dependence, and withdrawal reactions consistently across all medicines in the class. Currently, no treatment is US Food and Drug Administration-approved for the acute treatment of anxiety associated with SAD.

Hyperactivation of the amygdala occurs in many anxiety disorders, including SAD (Shin & Liberzon, 2010). Aberrant neurosteroid homeostasis has been documented in anxiety disorders (Garakani et al., 2020). Classical neurosteroids are synthesized in the brain and are positive allosteric modulators of GABA-A receptors that control the excitability of the central nervous system (CNS) (Almeida et al., 2021; Dubrovsky, 2005). Pherines, such as fasedienol (PH94B; 3β-androsta-4,16-dien-3-ol), are synthetic neuroactive steroids that regulate and modify neural activities via nongenomic mechanisms, binding to membrane receptors (Monti et al., 2022; Ruprecht, 2003), and their proposed mechanism of action is fundamentally differentiated from that of classical neurosteroids (Monti & Liebowitz, 2022). When administered onto the nasal mucosal surface in microgram quantities, pherines produce a robust, dose-dependent depolarization of the local electrogram of nasal receptors (EGNR) from receptor cells of the nasal chemosensory mucosa, along with behavioral and autonomic nervous system (ANS) responses (Monti & Liebowitz, 2022).

Fasedienol, which is formulated as a nasal spray and is being investigated for the acute treatment of SAD, is proposed to bind peripheral receptors in nasal chemosensory mucosa that in turn activate subsets of olfactory bulb neurons to modulate olfactory-amygdala neural circuits (Liebowitz et al., 2014; Monti & Liebowitz, 2022). When stimulated, these neural circuits regulate GABA release in the amygdala, attenuating sympathetic ANS activity and pathways underlying fear and anxiety (Monti & Liebowitz, 2022; Zhang et al., 2021). Unlike benzodiazepines, which directly activate GABA receptors in the brain, fasedienol indirectly modulates GABA activity from the periphery by activating nasal chemosensory neurons that stimulate a subset of olfactory bulb neurons projecting onto GABAergic neurons in the limbic amygdala, leading to a rapid anxiolytic effect, without benzodiazepine-like abuse potential (Monti et al., 2022). In a preclinical investigation, nasal instillation of radiolabeled fasedienol (ie, [^{14}C]-fasedienol) in rats resulted in minimal radioactivity distribution to the CNS 15 min after fasedienol administration, and no quantifiable CNS radioactivity was present 1 h after dosing (Monti et al., 2022). In clinical studies, neither single-dose (≤19.2 μg), nor single and repeat dosing of fasedienol (1.6 μg/dose) onto nasal chemosensory mucosa resulted in quantifiable fasedienol plasma concentrations. Without requiring systemic absorption or CNS penetration, and with its rapid elimination, fasedienol has shown robust efficacy in the acute treatment of SAD in phase 2 and phase 3 clinical studies (Liebowitz et al., 2014, 2016, 2023) and is in ongoing phase 3 development for the acute treatment of anxiety in adults with SAD. To differentiate the unique mechanism of action of fasedienol from other steroidal hormones, EGNR and ANS effects (respiratory rate, heart rate, and electrodermal activity) were compared following intranasal administration of fasedienol, steroidal hormones, and control in healthy adult volunteers.

2 | METHODS

2.1 | Study design

This single-blind randomized study was conducted in 16 healthy volunteers (8 males, 8 females) aged 20–60 years in accordance with Good Clinical Practice Guidelines and ethical principles derived from the Declaration of Helsinki; all subjects provided informed consent. The study was conducted at the University of Utah Neuropsychiatric Institute following protocol approval by the University of Utah Institutional Review Board. Volunteers with nasal septum perforation or past/current drug or alcohol use were excluded. Volunteers participated in 2 study sessions, 2–3 days apart, during which they randomly received control (propylene glycol) and 0.4 μg each of fasedienol, 17β-estradiol, progesterone, cortisol, and testosterone dissolved in polypropylene glycol, tested 30 min apart and delivered as a 1-sec aerosolized pulse via a Multifunctional Miniprobe.

2.2 | Measurements

The local EGNR was measured via a nonpolarizable silver chloride recording electrode positioned on the surface of the nasal chemosensory mucosa of the medial and dorsal nasal septum and was continuously monitored. Noninvasive recording electrode leads on the skin surface enabled continuous monitoring of autonomic reflex activity. Respiratory rate was recorded with a strain gauge placed around the lower thorax; heart rate was measured from the electrocardiogram (standard bipolar I); and electrodermal activity was recorded as skin conductance from the palmar surface of the third and fourth finger. Autonomic parameters were recorded in 5-min epochs before and 15-min epochs after intranasal drug administration. Recordings were amplified, digitized, continuously monitored, and stored for further offline processing and statistical analysis. The amplitude and frequency (as appropriate) of the various digitized signals was measured offline using ACQKnowledge software (Biopac Systems, Inc.). At the conclusion of recording for each drug tested, participants were asked by the investigator to provide feedback on their experience and about any changes in mood that occurred.

2.3 | Statistical analyses

Electrogram amplitude and ANS effects associated with administration of study drugs were compared using a paired *t*-test. No adjustments for multiplicity were made.

3 | RESULTS

Fasedienol significantly increased EGNR amplitude versus control, in males (5.0 vs. 0.6 mV, $p < 0.001$) (Figure 1a) and females (5.7 vs. 0.6 mV, $p < 0.001$) (Figure 1a) and compared with each

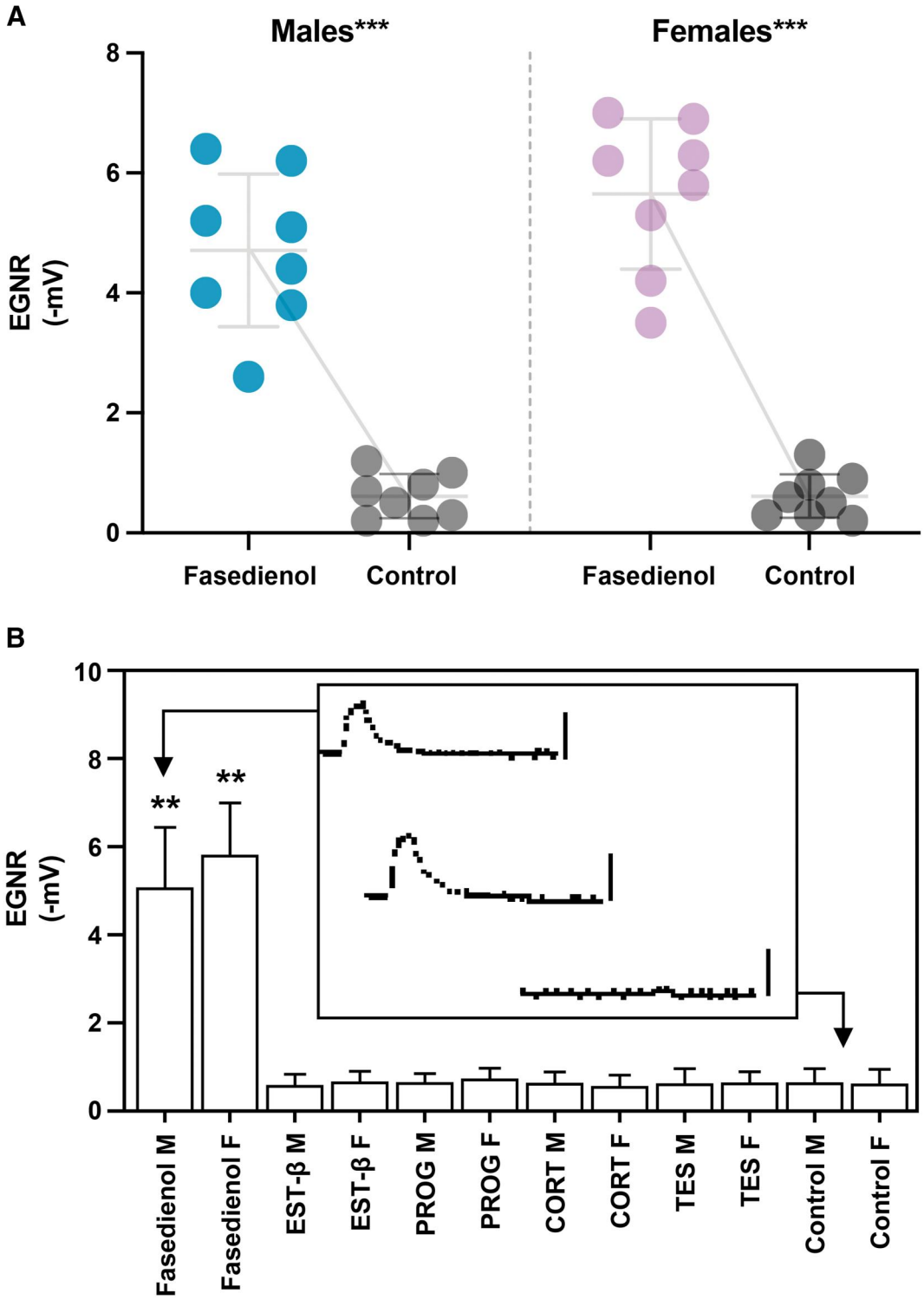

FIGURE 1 EGNR amplitude. Panel A shows the EGNR response to intranasal fasedienol administration in healthy male and female volunteers was significantly increased compared with control. Panel B shows representative electrogram traces from a male and female volunteer compared with a male and female control. EGNR responses following steroidal hormone administration were similar to that observed after administration of control. ***$p < 0.001$ versus control. COR, cortisol; EGNR, electrogram of nasal receptors of the chemosensory mucosa; EST-β, estrogen-17β; F, female; M, male; PRO, progesterone; TES, testosterone.

steroidal hormone tested ($p < 0.001$) (Figure 1b). Autonomic nervous system responses were significantly reduced in both sexes after fasedienol versus control administration: respiratory rate (males: 14.1 vs. 15.9 cycles/min, $p = 0.002$; females: 14.0 vs. 16.0 cycles/min, $p < 0.001$) (Figure 2); heart rate (males: 63.0 vs. 65.1 cycles/min, $p = 0.004$; females: 63.8 vs. 66.4 cycles/min, $p = 0.003$) (Figure 3); and electrodermal activity (males: 2.2 vs. 3.0 cycles/min, $p = 0.05$; females: 2.0 vs. 3.2 cycles/min, $p = 0.038$) (Figure 4). Autonomic nervous system effects after fasedienol occurred rapidly with mean latency of 452 milliseconds, 410 milliseconds,

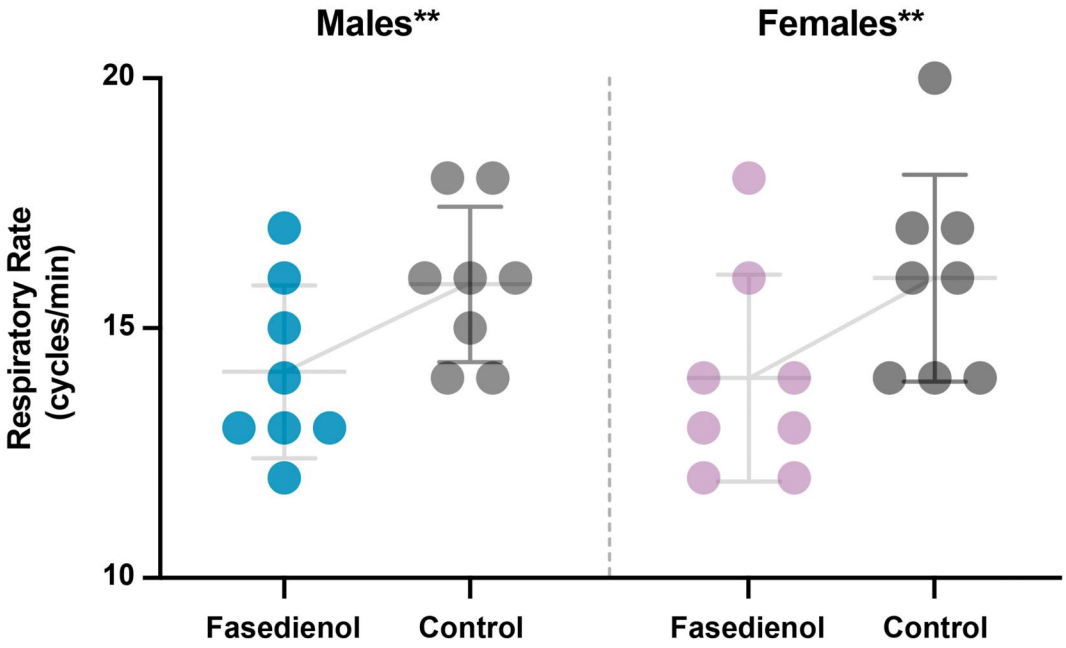

FIGURE 2 Respiratory rate in healthy male and female volunteers following intranasal administration of fasedienol versus control; the effect of each steroidal hormone tested on respiratory rate (data not shown) was similar to that observed with control administration. $^{**}p < 0.01$ versus control.

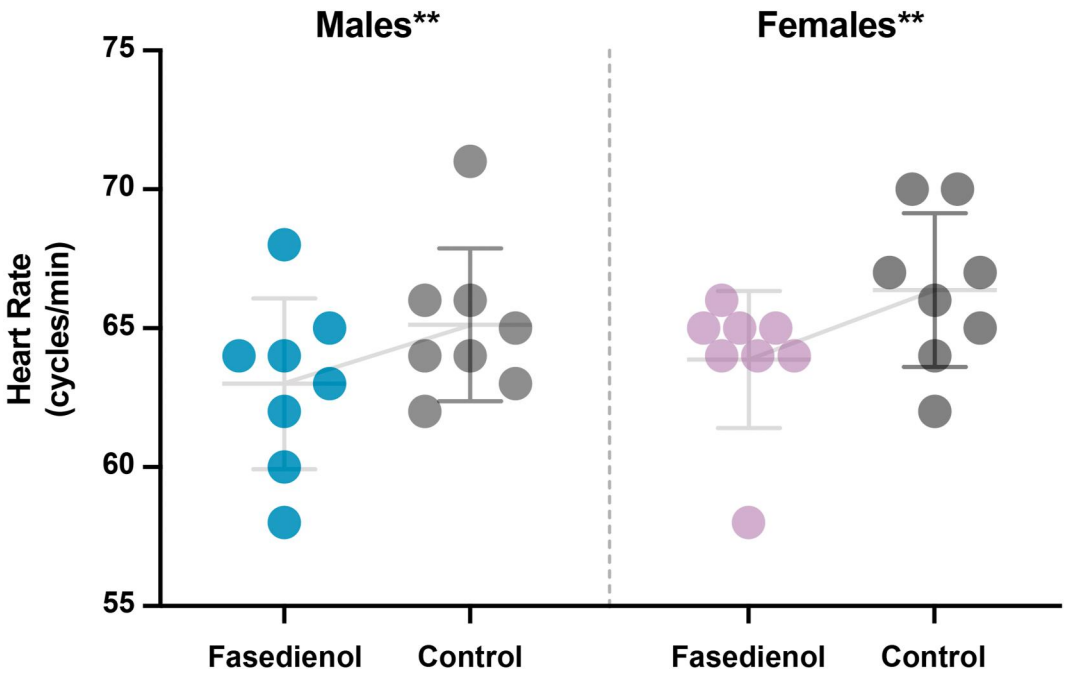

FIGURE 3 Heart rate in healthy male and female volunteers following intranasal administration of fasedienol versus control; the effect of each steroidal hormone tested on heart rate (data not shown) was similar to that observed with control administration. $^{**}p < 0.01$ versus control.

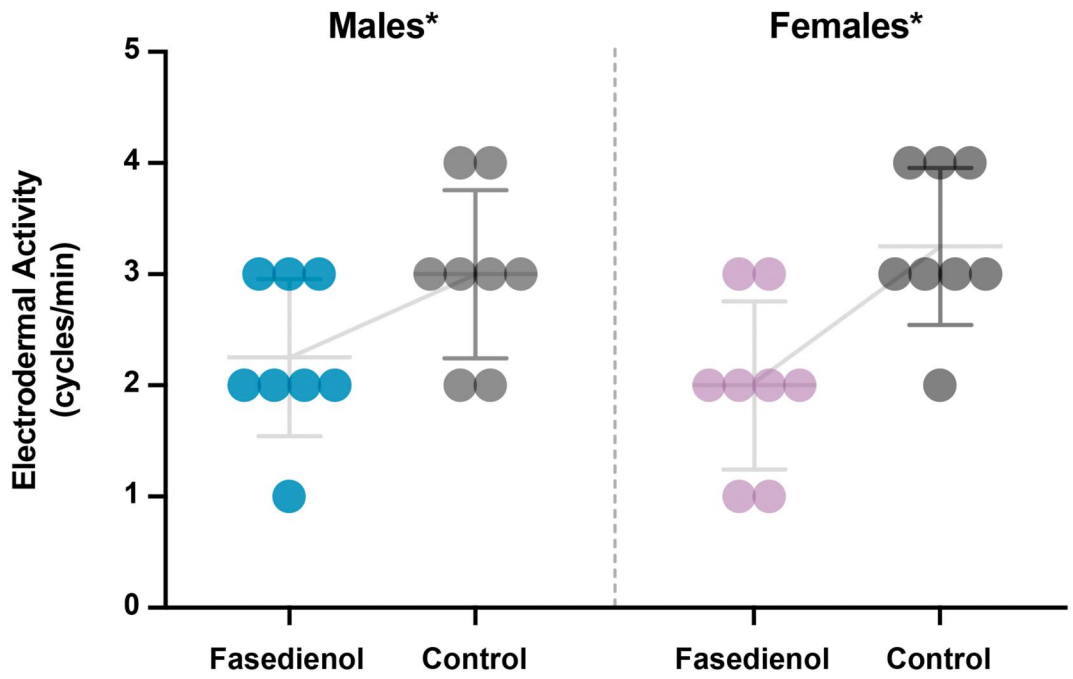

FIGURE 4 Frequency of electrodermal activity events in healthy male and female volunteers following intranasal administration of fasedienol versus control; the effect of each steroidal hormone tested on electrodermal activity (data not shown) was similar to that observed with control administration. $^{*}p \leq 0.05$ versus control.

and 10 s for respiratory, cardiac, and electrodermal effects, respectively, and returned to baseline levels 30 min after fasedienol administration. In contrast, heart rate, respiratory rate, and electrodermal activity changes after steroid administration were similar to those of controls.

When asked about their experience, 13 of 16 (81%) participants reported feeling more relaxed and less tense and nervous 20 min after fasedienol administration; no subjects reported similar behavioral changes after control or steroidal hormone administration.

4 | DISCUSSION

Intranasal administration of 0.4 μg fasedienol significantly increased EGNR amplitude and significantly lowered autonomic reflex activity compared with control.

Past reports indicate that steroidal hormones can modulate nasal chemosensory cell responses (Albertazzi, 2002; Cherian et al., 2014; Kanageswaran et al., 2016). In the present study, however, intranasal steroidal hormone administration (0.4 μg each of progesterone, 17β-estradiol, testosterone, or cortisol) resulted in EGNR and ANS responses that were similar to control, and in direct contrast to the rapid onset responses induced by 0.4 μg of intranasal fasedienol.

Electrogram activity after fasedienol administration was consistent with receptor activation as reported previously (Monti & Liebowitz, 2022), and the induced changes in ANS activity were consistent with a sympatholytic effect leading to physical calmness and relaxation.

An earlier study where 0.2 μg fasedienol was administered to healthy volunteers suggested sexual dimorphism in the response to fasedienol, with a greater magnitude of ANS effects observed in females than in males. However, this study and a previous clinical study in patients with SAD showed similar effect sizes for fasedienol compared with control, in both males and females (Liebowitz et al., 2016). The potential threshold dose for fasedienol may be lower in women, but the 0.4 μg dose of fasedienol used here was equally effective in both sexes.

Changes in ANS activity occurred rapidly, with behavioral changes reported within 20 min of fasedienol administration, but not after administration of steroidal hormones or control. Results suggest that rapid onset of effects from fasedienol counteract heightened sympathetic activation and reduce symptoms of anxiety following intranasal administration, in contrast to effects of both steroidal hormones and control. Preclinically, delivery of fasedienol is associated with minimal tissue distribution, including to the CNS (Monti et al., 2022). In phase 2, fasedienol attenuated social interaction and public speaking anxiety in adults with SAD within 15 min of intranasal administration and was well tolerated (Liebowitz et al., 2014). In a recently reported phase 3 study, administration of single-dose fasedienol prior to a stressful public speaking challenge significantly reduced anxiety, as measured by both clinicians and participants (Liebowitz et al., 2023). Because of its rapid onset of action, tolerability, and lack of systemic exposure, fasedienol may fill the need for a patient-determined, as-needed, acute treatment that provides immediate relief of anxiety associated with SAD.

5 | LIMITATIONS

This study is limited by the small sample size and the lack of a validated metric for objectively assessing behavioral effects associated with the study treatments. In addition, it should be noted that these results describe EGNR and ANS activity in healthy adult volunteers; future studies will address responses to fasedienol in patients with SAD.

6 | CONCLUSION

In healthy volunteers, microgram level intranasal fasedienol, but not steroidal hormones, rapidly and significantly increased the amplitude of the local EGNR and reduced respiratory, cardiac, and electrodermal activity. These results suggest that nasal chemosensory neuron activation by fasedienol was followed by ANS activity changes consistent with decreased sympathetic tone. Fasedienol effects were rapid, with reported behavioral changes suggestive of anxiolysis. Specifically, in response to the investigator's request for feedback, participants volunteered that they "felt less tense and more relaxed at the end of the study session" (ie, 20 min after fasedienol nasal spray administration). Although these findings warrant further study, overall results underscore the potential of fasedienol to provide acute treatment of anxiety symptoms in patients with SAD.

AUTHOR CONTRIBUTIONS

Study design: Louis Monti. Study investigator: Louis Monti. Enrolled subjects: Louis Monti. Collection and assembly of data: Louis Monti. Data analysis: Louis Monti. Data interpretation: All authors. Manuscript preparation: All authors. Manuscript review and revisions: All authors. Final approval of manuscript: All authors.

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CONFLICT OF INTEREST STATEMENT

Louis Monti, Rita Hanover, Ester Salmán, Ross Baker, Jaakko Lappalainen, and Mark Smith were employees or consultants of Vistagen Therapeutics, Inc. at the time the manuscript was written, and may own stock/options in the company.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, [Louis Monti], upon reasonable request.

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