Anxiolytic Effects of Noribogaine on Novelty Stress in Rats and Zebrafish

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Abstract

Recent molecular evidence suggests that noribogaine is relevant to novel therapeutic approaches to treat addiction and other brain ailments. Here, we report the effects of noribogaine in established paradigms relevant to novelty stress and anxiety in two animal models.

(1) Adult female Sprague-Dawley rats (10 per group) were subjected to Functional Observational Battery (FOB) testing following a single oral administration of noribogaine at doses of 25, 50 and 100 mg/kg or vehicle (0.975% MC, 1.75% Dextrose and 0.175% Tween 80 in purified water). Each rat was evaluated at predose, 1, 2, 6 and 24 hours post dose. The treated rats displayed transient and dose-dependent reductions in frequency of rearing, exploratory activity and arousal between 1 and 6 hours pose dose (peaked at 2 hours) in the open field test without observed neuromuscular or autonomic effects. In the home cage, noribogaine had no impact on activity, alertness, and posture.

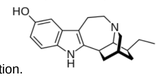
(2) Adult zebrafish (*Danio rerio*, 15 per group) were subjected to the novel tank test following drug immersion treatment of noribogaine at concentrations of 1, 5 and 10 mg/L or water only. Each group was observed for 5 minutes. Noribogaine produced robust anxiolytic-like behavior in fish as revealed by the prolonged time spent in the top half compartment, increased number of transitions to top, and the absence of freeze-bouts usually associated with fear and anxiety in control fish. These effects were not associated with locomotor impairment or stereotypic behaviors.

The data obtained from rats and zebrafish show that noribogaine modulates the components of the acute stress response usually associated with emotionality and anxiety in these models. The mechanism of action for noribogaine is most likely related to the effects targeted to a highly conserved neurological structure(s) in these species, such as hypothalamic-pituitary-adrenal (HPA) axis.

Introduction

Noribogaine is the primary human metabolite of ibogaine, an alkaloid derived from the African shrub, *iboga (Tabernanthe iboga).* Ibogaine has demonstrated beneficial effects for substance abuse in humans and preclinical studies. It was confirmed noribogaine had excellent permeability across the blood brain barrier and maintained high levels in the brain following an intra-peritoneal injection of ibogaine in rats. Unlike ibogaine, noribogaine did not produce tremors and ataxia in rodents. A study in healthy volunteers revealed safety and tolerability of single oral doses of 3-60 mg noribogaine, and that a slow half-life (28-49 hours) was noted with large volume of distribution[A]. In a recent single ascending dose study in opioid-dependent participants, noribogaine (60–180mg) was well tolerated and demonstrated a concentration-dependent increase in QT interval[B]. A multiple-dose safety study conducted by DemeRx is ongoing.

Molecular characterization indicates that noribogaine is a polypharmacological drug which targets multiple neurotransmitter system including nicotinic ionotropic receptors, serotonin and dopamine transporters and the opioid receptors. With additive and potentially synergic activities at its principal central targets, such as the kappa opioid receptor[C], noribogaine appeared remarkably well-fit to target the emotional, motivational, and stress-related components of addiction.



Paradigms promoting and measuring novelty stress in animals such as open field test in rats and the novel tank diving test in zebrafish have been considered as the pillar of anxiolytic drug research for many years.

The objective of the study was to evaluate the apparent anxiolytic and/or calmative effects of noribogaine following novelty stress in rats and zebrafish.

Materials and Methods

(1) Functional Observational Battery (FOB) Testing in Female Rats

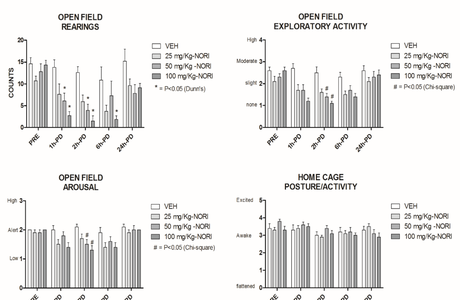
- Groups of female Sprague-Dawley rats (10 rats /group) were administered by single oral gavage vehicle/control (0.975% MC, 1.75% Dextrose and 0.175% Tween 80 in purified water) or noribogaine at doses of 25, 50, and 100 mg/kg. Dose volume was 10 mL/kg for all animals including controls. Rats were 8-10 weeks old upon initiation of treatment.
- General behavior changes for each rat were assessed using FOB testing on 5 occasions: predose, at 1, 2, 6 and 24 hours post-dose. FOBs were performed on each occasion at four stages: when the animals were in their home cage; while handling the animals; when the animals were freely moving in an open filed; and when the animals received diverse stimuli for reactivity evaluation. Body temperature and neuromuscular strength were measured for each animal. Throughout the study the observers performing the FOB test were not aware of the specific treatment administered to the animals. All examinations were performed and selected observations were grouped according to functional domains of the nervous system as follows:
  - *Behavioral domain*: posture and activity in home cage; ease of removal from the cage; handling reactivity; arousal in open filed; rearing in open filed; exploratory activity in open field; touch response; and abnormal/stereotyped behavior.
  - *Neurological/sensorimotor and neuromuscular domain*: vision test; touch response; auditory test; tail pinch response; eye blink response; flexor reflex; extensor thrust reflex; pinna reflex; proprioceptive positioning; righting reaction; hindlimb foot splay; involuntary motor movement (convulsion and tremors); gait; forelimb and hindlimb grip strength.
  - *Autonomic domain*: lacrimation; salivation; pupil response to light; palpebral closure; defecation; urination; piloerection; exophthalmos; body temperature.
- Data analysis was performed as follows and a significance level of $p < 0.05$ was reported for all statistical tests.
  - Qualitative observations were reported per individual and as incidences per group per grading for each group of animals. The chi square statistical test and the Fisher's exact test were used to determine whether significant differences had occurred between control and treated animals.
  - Numerical data obtained during the conduct of the study were subjected to calculation of means and standard deviations. The data were analyzed for homogeneity of variance using Levene Median and for normality using Kolmogorov-Smirnov tests. Homogeneous data were analyzed using the Analysis of Variance and the significance of intergroup differences between control and treated animals was assessed using Dunnett's test. Heterogeneous data were analyzed using Kruskal-Wallis test and the significance of intergroup differences was assessed using Dunn's test. The ANOVA Rank Sum test was used for the data of rearing, urination and defecation if low count measurements occurred.

(2) Novel Tank Test (NTT) in Zebrafish

- A total of 60 adult wild type short-fin zebrafish (50:50 male:female ratio) were used and tested in novel tank diving test. Drug exposure was performed by submerging individual zebrafish in a 1-L plastic beaker for 20 minutes prior to testing. Submersion drug administration in zebrafish is usually reported in mg/L or ppm and offers a systemic exposure. Noribogaine was set at concentrations of 1, 5 and 10 mg/L; control fish were exposed to drug-free water for the same treatment period.
- Behavioral testing was performed between 11:00 and 15:00 h using tanks with water adjusted to room temperature (25°C). The apparatus was a 1.5-L trapezoidal tank (15 cm height x 28 cm top x 23 cm bottom x 7 cm width; aquatic habitats, Apopka, FL) maximally filled with water and divided into two equal virtual horizontal portions by a line marking the outside walls. Noribogaine or water treated zebrafish were introduced in the observation tank and observed for 5 minutes.
- Each zebrafish was scored for the latency to reach the top half of the tank, time spent in top, number of transitions to top, number and duration of freezing bouts. Freezing was defined as a total absence of movement, except for the gills and eyes, for greater than 2 seconds. Fish behavior was recorded to a computer using a USB webcam and subsequently analyzed by Ethovision XT8.5, assessing distance traveled (m), velocity (m/s) and meandering endpoints.
- Data analysis was performed as follows: one-way analysis of variance (ANOVA) was used for numerical data (in seconds, in number of events), followed by the Bartlett's test for equivalence of variance and the Dunnett's posttest for multiple comparisons at each dose group versus the control group. Data were expressed as mean ± SEM. Two-way ANOVA was performed on repeated measures at different time intervals and Bonferroni post-test was performed. When data were not suitable for previously described statistical approaches, sub-grouping and/or ranking was performed, and data were arranged in contingency tables where P value calculation was performed using Chi-square trend analysis. The accepted value for significance was P<0.05 and higher significance was indicated where it applied.

Results

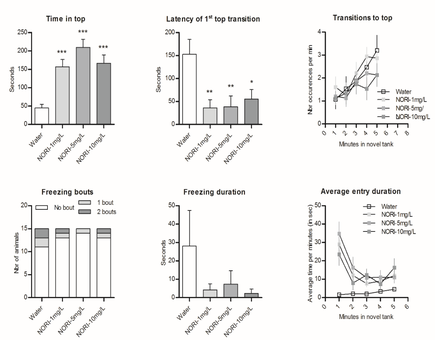
Figure 1. Dose-dependent and transient anxiolytic effects of noribogaine in rats during open field assessment



A) The number of rearings were counted during the 30 minutes observation in open field.
B) The following categories of exploratory activity in open field "none, "slight", "moderate", and "high" were graded from 1 to 4 for each animal.
C) The following categories of arousal in open field "low, "alert", and "high" were graded from 1 to 3 for each animal.
D) The following signs of posture/activity in home cage F flattened; L lying on side; A asleep; S sitting normally awake; I standing alert and sniffing; E abnormally excited were graded from 0 to 5 for each animal.

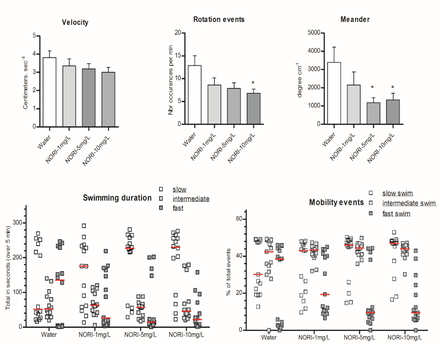
Data in the graphs are mean values ± SEM and statistical tests are indicated in figure insets

Figure 2. Effects of noribogaine on anxiogenic behavior in adult zebrafish using NTT test



Effect of 20 min acute noribogaine treatment (1, 5 and 10 mg/L) on anxiety/fear-related endpoints of zebrafish (n = 15) in comparison to water treated animals.
Data presented in mean ± SEM were subjected to ANOVA and where applicable, ranked by Dunnett's multiple comparison test.
* P<0.05, ** P<0.01 and *** P<0.001 vs water-treated fish.

Figure 3. Effects of noribogaine on locomotion and swimming pattern in adult zebrafish using NTT test



Effect of 20 min acute noribogaine treatment (1, 5 and 10 mg/L) on swimming behavior of zebrafish (n = 15) in comparison to water treated animals.
Data presented (A, B, C) in mean ± SEM were subjected to ANOVA and where applicable, ranked by Dunnett's multiple comparison test. * P<0.05, ** P<0.01 and *** P<0.001 vs water-treated fish.
Each data point are shown (E, F) as indicated in the graphicals and the median bar is shown in red.

Conclusions

1. Noribogaine dose-dependently decreases the manifestations of emotionality and arousal of female rats in the open field test and the peak effects were noted at 2 hours post dose that is consistent with Tmax values noted from other studies; similarly, noribogaine suppresses the fear-like and anxious behavior of zebrafish challenged by the novel tank diving test.

2. Noribogaine demonstrates anxiolytic activity in rats and zebrafish which is not associated with sedation, motor impairment, or stereotypies.

3. The mechanism of the non sedative anxiolytic effects of noribogaine seen in rats and in zebrafish could be mediated by highly evolutionarily conserved neurobiological structures.

References

[A] Glue, P, et al. 2015. PMID: 25279818
[B] Glue, P, et al. 2016. DOI: 10.1002/cpdd.254
[C] Maillet, EL et al. 2015. PMID: 26302653

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The novel tank diving test in Zebrafish was conducted at the ZENEREI LLC research facility.