Latrepirdine (Dimebon) Modulates Stress-Mediated Behaviors in Rodents

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Background

- Latrepirdine (Dimebon) was under clinical investigation in 3 studies as a drug to delay cognitive decline in patients with Alzheimer's Disease
- Although no beneficial effect on cognition was noted, a pooled analysis from these studies indicated that latrepirdine may have had a positive significant effect on the neuropsychiatric inventory (NPI) (**Chau et al., 2015**)
- NPI measures severity and frequency of 12 subdomains of behavioral functioning (**Cummings 2020**)
- The clinical trials demonstrated a desirable safety profile of latrepirdine in patients after dosing for up to one year
- Clinical development of latrepirdine has not progressed partly because the drug target and neuropathways affected are not known
- **Objectives of this study were:**
  - Identify the drug receptor that accounts for the efficacy of latrepirdine
  - Identify the neuronal pathways that might be affected by the drug
  - Identify which domains of the NPI account for the NPI score
  - Describe a testable model for the mechanism of action of latrepirdine
  - Assess the properties of latrepirdine in appropriate models to determine if it would be suitable as a chronic drug to treat stress-related behaviors

In Vitro pharmacology

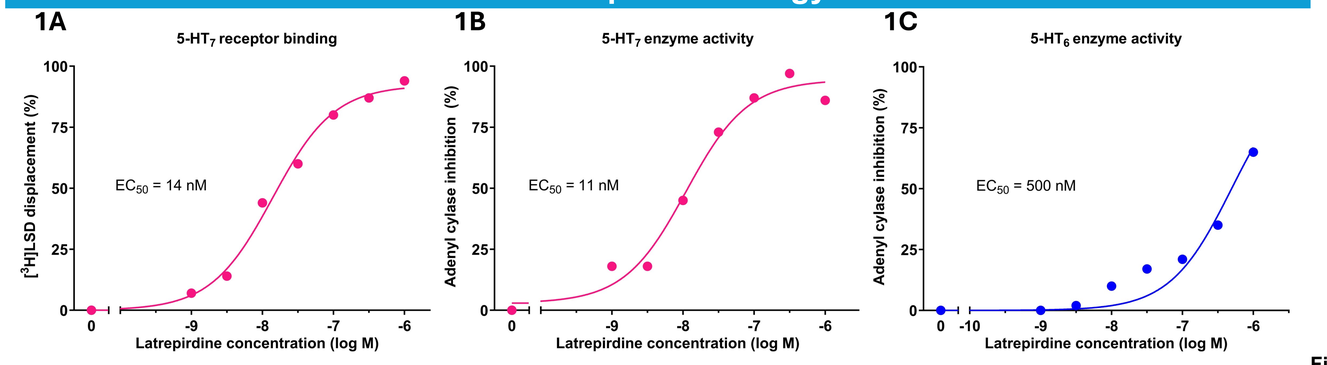

Figure 1: The potency of latrepirdine for the 5-HT_7 receptor was measured by displacement of LSD binding (A) and by inhibition of 5-carboxamidotryptamine-stimulated adenylyl cyclase activity (B). The potency of latrepirdine for the 5-HT_6 receptor was measured by inhibition of 5-carboxamidotryptamine-stimulated adenylyl cyclase activity in cells transfected with the human 5-HT_6 receptor (C). Measurements are in duplicates and a 3-parameter logistic curve.

Microdialysis

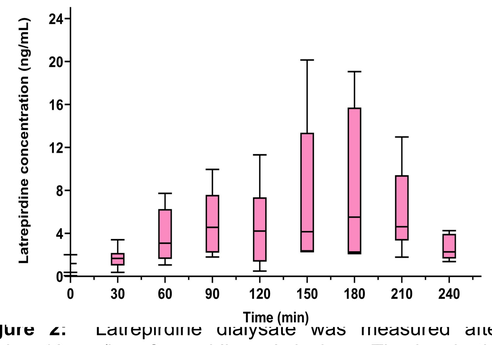

Figure 2: Latrepirdine dialysate was measured after dosing 10 mg/kg after sublingual dosing. The bar is the arithmetic mean, the box extends from the 25th to 75th percentiles and the whiskers from the 10 to 90 percentiles..

Methodology

Binding: Lysergic acid diethylamide (LSD) binds with high affinity to 5-HT_7 receptors. CHO-K1 cells were stably transfected with a plasmid encoding the human serotonin 5-HT_7 receptor. A 50 µg aliquot of membrane was incubated with 5.5 nM [^{3}H]LSD for 120 minutes at 25°C in a buffer consisting of 50 mM Tris-HCl, pH 7.4, 10 mM MgCl2, 0.5 mM EDTA. Reaction was stopped by filtration. Membranes were filtered and washed 3 times, and the filters were counted to determine [^{3}H]LSD specifically bound. The K_d of LSD for the 5-HT_7 site was 7.4 nM. Non-specific binding was 90% and estimated in the presence of 10 µM serotonin. B_{max} was 0.95 pmol/mg protein. Competition for the LSD-labelled 5-HT_7 site was measured in the presence of increasing concentrations of latrepirdine. The vehicle was 1% DMSO in buffer solution.

Adenylyl Cyclase and 5-HT_7 Receptors: 5-carboxamidotryptamine (5-CT) potently stimulates adenylyl cyclase activity by activating 5-HT_7 receptors. CHO-K1 cells were stably transfected with a plasmid encoding the human serotonin 5-HT7 receptor. Test compound and/or vehicle is incubated with the cells (2 x 10^6/ml) in HBSS buffer, 5 mM HEPES, 0.1% BSA, 100 µM IBMX, pH 7.4 and incubated at 37°C for 20 minutes. The reaction was evaluated for cAMP levels by time-resolved fluorescence resonance energy transfer TR-FRET. Reaction mix was incubated with increasing concentrations of the test compound, latrepirdine, and the inhibition of 5-CT-stimulated cAMP accumulation was determined. The vehicle was a 0.40% DMSO solution in buffer.

Adenylyl Cyclase and 5-HT_6 Receptors: Human recombinant serotonin 5-HT_6 receptors stably expressed in HeLa cells. Test compound and/or vehicle were incubated with the cells (2 x 10^6/ml) in HBSS buffer, 5 mM HEPES, 0.1% BSA, 100 µM IBMX, pH 7.4 and incubated at 37°C for 20 minutes. The reaction was evaluated for cAMP levels by time-resolved fluorescence resonance energy transfer TR-FRET.. Reaction mix was incubated with increasing concentrations of the test compound, latrepirdine, and the inhibition of 5-HT-stimulated cAMP accumulation was determined. The vehicle was a 0.40% DMSO solution in buffer.

Microdialysis: Dialysate from brain (prefrontal cortex) was collected and the amount of latrepirdine was determined in samples from rat (n=60). Sprague Dawley rats were implanted with MQ-NM-PAN 6/4 probes in PFC for ultraslow ISF collection, and a jugular vein cannula for blood collection. After the collection of one pre-dose ISF sample (30-minute duration), the test compounds were administered (intravenous; IV or sublingual). ISF samples from the PFC were collected for 4 hours after treatment administration. Serial jugular vein plasma was also be collected at baseline, 30, 60, 120, 180 and 240 minutes after dosing. All samples were stored at -80°C awaiting analysis for compound levels by Charles River Laboratories using LC/MS-MS.

Elevated Plus Maze: Male Wistar rats were maintained in a room with controlled temperature (21-22°C) and a reversed light-dark cycle (12h/12h; lights on: 17:30 – 05:30; lights off: 05:30 – 17:30) with food and water available ad libitum. EPM apparatus was a PVC maze covered with Plexiglas and subdivided into four equal exploratory arms (40 x 10 cm), which were all interconnected by a small platform (10 x 10 cm). The apparatus was placed 65 cm above the floor. Two arms were open, and two others were closed with wall (high: 10 cm). The test was performed under reversed light-dark cycle and under 100 – 150 lux lighting condition. Yohimbine (i.p.) was administered to all the treatment groups, 30 mins prior to the test. Latrepirdine (i.m.), fluvoxamine (i.m.), dextromethorphan (i.m.) were administered 60 minutes prior to the test and diazepam (i.p.) was administered 30 mins prior to the test.

Resident Intruder Test: Male Swiss albino mice (25-40 grams, Resident animals) were housed individually with an ovariectomized female Swiss albino mice for a period of three weeks.. On day 1 and 2 of the study, female mouse was removed from the resident cage and intruder was placed in the home cage of resident animal for a period of 10 minutes. During this 10 minutes' exposure, the aggressive behavior (like tail rattling, chasing, biting, lateral attack, clinch attack) of resident animal was noted as duration of attack. On day 4 of the study, test item / vehicle / sodium valproate was administered to the resident animal and same intruder is exposed to the same resident animal for a period of 10 minutes and the duration of attack was noted.

Open Space Swim Test: Male Swiss albino mice were individually placed in a makrolon cage (41 x 25 x 18 cm) containing 10 cm water (22°C) from which they could not escape, for 15 minutes daily, for 5 consecutive days (Day 1 to Day 5) and on Days 8, 10, 12, 15, 16, 19, 22, 24 and 26. The control group was administered with vehicle from Day 8 to Day 26 while the other group was treated with latrepirdine i.p. daily from Day 8 to Day 15 and from Day 23 to Day 26 and with vehicle from Day 16 to Day 22 (washout period). Swim test were done 1-hour post-administration. On day 15, swim test was also done at 6 hours post treatment. All swimming sessions were video-recorded, and the behavior of animals was analyzed using a video-tracking system (Panlab: SMART).

Marble Burying Test: CD-1 mice were placed individually in a cage for a 20 min test session, one hour after latrepirdine (i.p.) / diazepam (p.o.) administration, each. The apparatus consists of transparent polycarbonate cages (30 cm x 18 cm x 19 cm) containing a 5 cm layer of fine sawdust bedding and 20 glass marbles (diameter: 1.5 cm) spaced evenly along the walls of the cage.On termination of the test session, the animals were removed from the cage and the number of marbles buried in the sawdust were recorded by the experimenter.

Data Analysis: All data were fitted and analyzed using GraphPad Prism version 9.5.1 for Windows

Behavior models: Single dosing

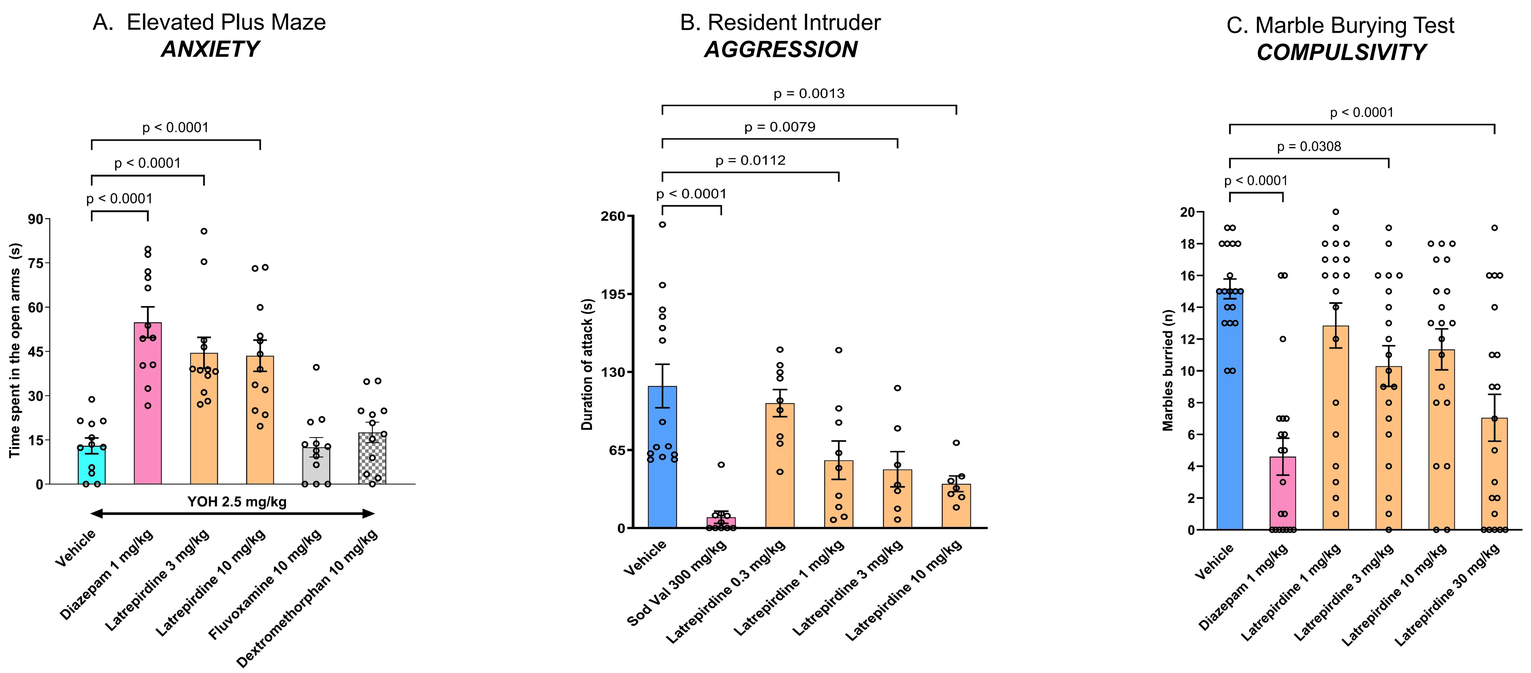

Figure 3: Effects of Latrepirdine on, **(A)** Yohimbine induced anxiety in the Elevated Plus Maze in Wistar rats (n = 12 per group); **(B)** Aggression in the Resident-Intruder Assay in Swiss albino mice, (male; n = 7 – 14); **(C)** Duration of immobility after repeated daily administration for 19 days in the Open Space Swimming Test in Swiss albino mice (n = 12); (D) Number of marbles buried in CD-1 mice (n = 20); (C) Number of marbles buried in CD-1 mice (n = 20).

Behavior model: Repeat dosing

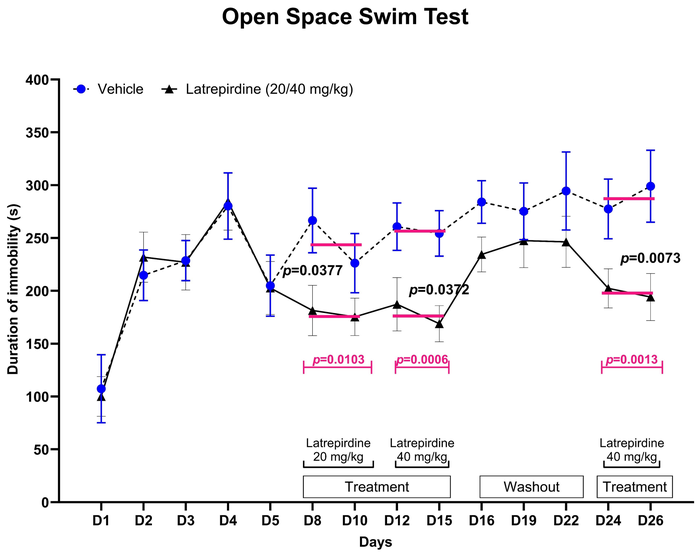

Figure 3: Effects of Latrepirdine on duration of immobility after repeated daily administration for 19 days in the Open Space Swimming Test in Swiss albino mice (n = 12).

Proposed Model

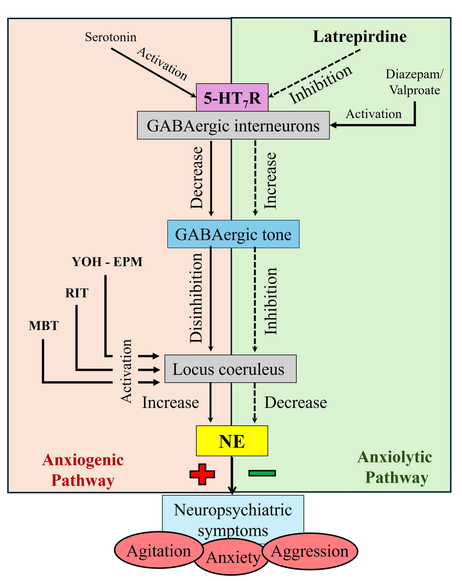

Figure 4: Proposed Mechanism – 5-HT_7 receptors are expressed on GABAergic interneurons in the locus coeruleus and other brain nuclei. These receptors may inhibit GABAergic tone. 5-HT_7 antagonist like latrepirdine may block 5-HT_7 and therefore elevate GABAergic tone in the LC, reducing arousal.

Conclusions

- Free brain levels at efficacious doses suggests that latrepirdine achieves its effects by antagonizing the 5-HT_7 receptor
- The brain levels measured at efficacious doses are close to those achieved in clinical studies with latrepirdine dosed at 20 mg three times a day **(Chew et al., 2016)**
- At these doses, latrepirdine demonstrated a significant change in the neuropsychiatric inventory (NPI), a scale used several different neuropsychiatric behaviours
- In this study, latrepirdine is effective in models that are locus coeruleus dependent suggesting the drug may work by reducing noradrenergic tone (**Morris et al. 2020**)
- Domains of NPI that are likely contributing to the clinical changes may be related to stress-activated locus coeruleus behaviours such as anxiety, agitation and aggression
- Latrepirdine possesses good qualities to be a CNS drug such as reversibility, no desensitization after repeat dosing, and no rebound effect.
- BioXcel proposes that latrepirdine may work by blocking the 5-HT_7 receptor-mediated suppression of GABAergic interneuron activity si,mi;ar to reported effects in the raphe (Kusek et al., 2015)

References

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Disclosures: All authors are employees and hold equity in BioXcel Therapeutics

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