ANTIDEPRESSANT-LIKE ACTIVITY OF AMOP-H-OH ('SAZETIDINE-A') IN THE FORCED SWIM TEST IS MEDIATED BY HIGH AFFINITY NICOTINIC ACETYLCHOLINE RECEPTORS.

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Introduction

- Both preclinical and clinical data suggest a role for nicotinic acetylcholine receptors (nAChRs) in depression.
- Both nAChR agonists and antagonists reduce depressive symptoms in humans and have antidepressant-like effects in rodent models.
- This study evaluated the action of the selective α4β2 partial agonist AMOP-H-OH (6-[5-(Azetidin-2-ylmethoxy) pyridin-3-yl]hex-5-yn-1-ol (aka Sazetidine-A)) in the forced swim test in mice.

Methods

Animals

- Male BALB/cJ or C57BL/6J mice from Jackson Laboratory (Bar Harbor, ME) were housed in groups of four and maintained on a 12hr/12hr light/dark cycle. Room temperature was maintained at 20-23°C with relative humidity at approximately 30%. Chow and water were provided *ad libitum* for the duration of the study. All procedures were approved by PsychoGenics' Institutional Animal Care and Use Committee.
- BALB/cJ mice were used for all studies with the exception of the mecamylamine dose response study that used the C57BL/6J strain.

Forced Swim Test

- Mice were individually placed into clear glass cylinders (15 cm tall x 10 cm wide, 1 L beakers) containing 23±1°C water 12 cm deep (approximately 800 mL). The time the animal spent immobile was recorded over a 6 min trial. Immobility was defined as the absence of all movement except those required by the mouse to keep its head above the water.

Measurement of AMOP-H-OH levels in plasma and brain

- Mice were treated with 1 or 3mg/kg of AMOP-H-OH and plasma and brains were collected 0.25, 0.5, 1, 2, and 4 hours after injection. Levels of AMOP-H-OH were measured by HPLC (Enthalpy Analytical, Raleigh-Durham, North Carolina).

Drugs

- All drugs were administered i.p. at a concentration of 10ml/kg in a saline or vehicle. AMOP-H-OH was synthesized in-house and all other compounds were purchased from Sigma.

Statistical Analysis

- Data were analyzed using analysis of variance (ANOVA) followed by Fisher's PLSD post hoc test when appropriate. An effect was considered significant if $p<0.05$.

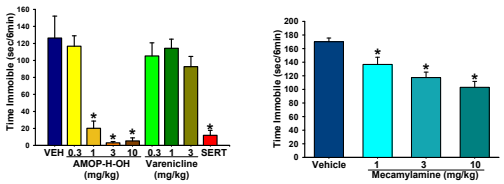
Summary

- AMOP-H-OH produced a robust reduction in immobility in the forced swim test in comparison to weaker effects seen with the non-competitive nAChR antagonist mecamylamine and the α4β2 partial agonist varenicline.
- The antidepressant-like effect of AMOP-H-OH in forced swim was completely reversed by mecamylamine and the high affinity nAChR antagonist dihydro-β-erythroidine (DHβE), but not by the α7 nAChR antagonist methyllycaconitine (MLA).
- The antidepressant-like effect of AMOP-H-OH in forced swim was completely absent in knockout mice lacking the β2 subunit of the nAChR.
- AMOP-H-OH was long lasting in the forced swim test with efficacy observed up to 4 hours after treatment, an effect that was also completely reversed by mecamylamine.
- Brain levels of AMOP-H-OH reached only low levels 15 min after administration and were at or below detection level at the later time points. Therefore, the long lasting effects of AMOP-H-OH in forced swim may be mediated by a metabolite rather than directly by AMOP-H-OH.
- Additional experiments are underway to understand the dissociation between plasma/brain levels of AMOP-H-OH and duration of action.

Conclusions

- The behavioral actions of AMOP-H-OH in the forced swim test are mediated by α4β2 receptors.
- Our results support the development of α4β2 ligands for the treatment of depression. The superior efficacy of AMOP-H-OH in forced swim compared to clinical candidate compounds suggests that the AMOP-H-OH class of compounds may also provide novel opportunities for the development of drugs to treat depression.

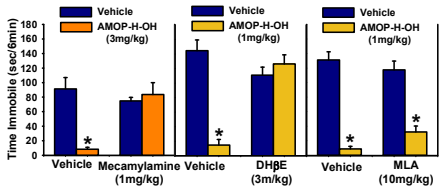
Figure 1: AMOP-H-OH has superior antidepressant-like efficacy in forced swim compared to varenicline or mecamylamine.



AMOP-H-OH (0.3, 1, 3, or 10mg/kg), varenicline (0.3, 1, or 3mg/kg), mecamylamine (1, 3, or 10mg/kg), sertraline (SERT, 20mg/kg) or vehicle (VEH) was administered to mice 30 min before forced swim testing. AMOP-H-OH produced a potent antidepressant-like effect in mice, whereas varenicline did not significantly reduce immobility. Mecamylamine showed a small but significant antidepressant-like effect and sertraline produced the expected reduction in immobility.

Data are mean ±SEM (AMOP-H-OH, n=9-10/group; mecamylamine (n=9-18/group).
**p<0.05 vs. vehicle*

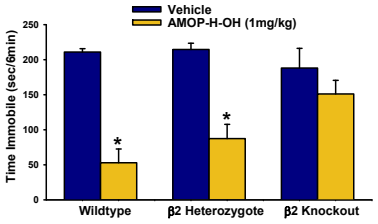
Figure 2: The antidepressant-like effects of AMOP-H-OH in the forced swim test are mediated by high affinity nAChRs.



Mice received a 5 min pretreatment with the nAChR antagonists mecamylamine (1mg/kg), dihydro-beta-erythroidine (DHβE, 3mg/kg), or methyllycaconitine (MLA, 10mg/kg) followed by AMOP-H-OH (1 or 3mg/kg, i.p.). Mice were tested in forced swim 30 min after AMOP-H-OH administration. Both the broad nAChR antagonist mecamylamine and the high affinity antagonist DHβE completely reversed the antidepressant-like effect of AMOP-H-OH in the forced swim test. The α7 antagonist MLA did not reverse the antidepressant-like effects of AMOP-H-OH.

Data are mean ±SEM (n=9-10/group).
**p<0.05 vs. vehicle*

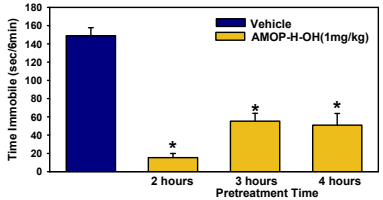
Figure 3: AMOP-H-OH is inactive in forced swim in knockout mice lacking the β2 subunit of the nAChR.



Wildtype, β2 subunit knockout, or β2 subunit heterozygous mice were administered either AMOP-H-OH (1mg/kg) or vehicle 30 min before forced swim testing. Whereas wildtype mice showed the expected decrease in immobility following AMOP-H-OH treatment, β2 subunit knockout mice showed no antidepressant-like response to AMOP-H-OH. β2 subunit heterozygous mice showed a behavioral response to AMOP-H-OH similar to that seen in wildtype mice.

Data are mean ±SEM (n=5-8/group).
**p<0.05 vs. vehicle of same genotype*

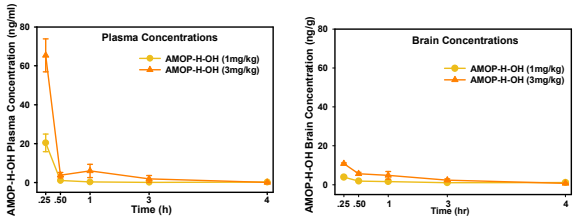
Figure 4: The antidepressant-like effects of AMOP-H-OH in the forced swim test are long lasting.



AMOP-H-OH (1mg/kg) was administered to mice 2, 3 or 4 hours before forced swim testing. The effect of AMOP-H-OH was long lasting with significant reductions in immobility observed up to 4 hrs after treatment.

Data are mean ±SEM (n=10-12/group).
**p<0.05 vs. vehicle*

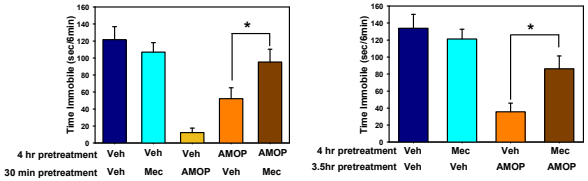
Figure 5: Plasma and brain levels of AMOP-H-OH show disassociation with duration of action in the forced swim test.



AMOP-H-OH (1 or 3mg/kg) was administered to mice and brain and plasma levels were measured 0.25, 0.5, 3, and 4 hrs after administration. Although AMOP-H-OH was active in the forced swim test up to 4hr after administration, drug levels were undetectable in both plasma and brain at 4hrs. These results suggest that AMOP-H-OH may produce a behaviorally active metabolite or act via desensitization of receptors.

Data are mean ±SEM, n=3/group

Figure 6: The long lasting effects of AMOP-H-OH in the forced swim test are mediated by nAChRs.



Mice were administered vehicle (Veh) or AMOP-H-OH (AMOP) (3mg/kg) and 3.5 later were administered Veh, mecamylamine (Mec) (1mg/kg) or AMOP-H-OH (3mg/kg). Mice were tested in forced swim 4 hrs after the first drug administration. Mecamylamine blocked the reduction of immobility by AMOP-H-OH though brain levels of AMOP-H-OH were no longer detectible.

Data are mean ±SEM (n=10/group).
**p<0.05 AMOP-Veh vs. AMOP-Mec*

Mice were administered vehicle (Veh) or mecamylamine (Mec) (1mg/kg) and 0.5 hr later were administered Veh, or AMOP-H-OH (AMOP) (3mg/kg). Mice were tested in forced swim 4 hrs after the first drug administration. Mecamylamine blocked the effects of AMOP, demonstrating that AMOP-H-OH's long lasting antidepressant-like actions are mediated by nAChRs.

Data are mean ±SEM (n=10/group).
**p<0.05 Veh-AMOP vs. Mec-AMOP*