(S2042) PH10: A new intranasal neuroactive steroid with rapid onset of antidepressant effect but a mechanism of action different from GABA-A allosteric modulator antidepressants

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1. Introduction

Endogenous neurosteroids or their synthetic analogs derived from cholesterol via-pregnanolone or pregnenolone (i.e.: allopregnanolone, pregnanolone, 3a5aTHDOC and 3a5bTHDOC) are known to produce antidepressant effects through allosteric modulation of GABA-A receptors. These neurosteroids are associated with sedation and other side effects consistent with their direct effect on GABA receptors.

In contrast, PH10 (pregn-4-en-20-yne-3-one) is a synthetic neuroactive steroid derived from pregnenolone that is under investigation by VistaGen Therapeutics for its rapid antidepressant effect when administered intranasally in low microgram dose (Figure 1). The aim of the present in vitro study was to assess the potentiation effect or inhibition effect of PH10 on GABA-A receptors.

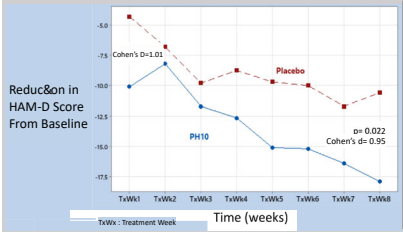

Figure 1. Mean reduction of HAM-D scores induced by 6.4µg intranasal PH10 administered twice daily during 8 consecutive weeks.
Effect size at end of first week of treatment: Cohen's D = 1.01
Effect size at end eighth week of treatment: Cohen's D = 0.95

HAM-D Findings at One Week of Treatment with PH10: Effect Size

Mean Reduction on HAM-D:

6.4 µg PH10 → 10.1
Placebo → 4.2

Effect Size PH10 vs Placebo: Cohen's D= 1.01 (large effect)

2. Methods

This was an in vitro study using a human ion channel cell-based ion flux assay to compare the modulatory effect of PH10 to a benzodiazepine (diazepam) and the GABA antagonistic effects of PH10 to the GABA antagonist bicuculline as well as to assess any GABA agonist properties of PH10 compared to GABA itself. This study was carried out by Eurofins Discovery.

To help differentiate the mechanism of action of PH10 from benzodiazepines, we studied whether PH10 had a positive modulatory effect on GABA receptors using in vitro patch recording electrophysiology in cells transfected with human GABA-A α1/β2/γ2 receptors.

Peak inward currents in response to the addition of GABA in the presence of a single concentration of test compound were measured. All recordings were obtained from a holding potential of -60 mV. The test substances were: PH10 (µM: 0.0316; 0.1; 0.316; 1.0; 3.16 and 10), GABA (µM: 0.1; 0.3; 1.0; 3.0; 10 and 30), Diazepam (PAM) (µM 0.003; 0.01; 0.03; 0.1; 0.3 and 1.0), and GABA antagonist Bicuculline (µM: 0.032; 0.16; 0.8; 4.0; 20 and 100). Each concentration of test compound was applied to the perfusate during 2 sec, followed by a 60 sec wash (Figure 2). This was repeated with the next ascending concentration of test compound (Table 1).

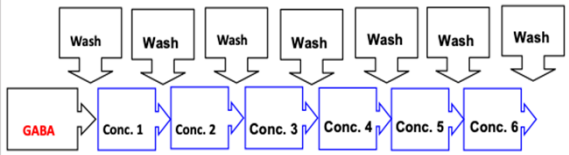

Figure 2. Schematic drawing of the design used to administer test substances and control to the cells transfected with GABA receptors

Compound	Target	Mode	Concentration(s) (µM)
GABA (EC_{100})	GABAA α1/β2/γ2	Agonist	30
GABA (EC_{80})	GABAA α1/β2/γ2	Antagonist	15
GABA (EC_{20})	GABAA α1/β2/γ2	PAM	2
GABA	GABAA α1/β2/γ2	Agonist	0.1, 0.3, 1, 3, 10, 30
Bicuculline	GABAA α1/β2/γ2	Antagonist	0.032, 0.16, 0.8, 4, 20, 100
Diazepam	GABAA α1/β2/γ2	PAM	0.003, 0.01, 0.03, 0.1, 0.3, 1
PH10	GABAA α1/β2/γ2	Agon, Antagon, PAM	0.0316, 0.1, 0.316, 1, 3.16, 10

Table 1. Concentration of Test Substances Studied

3. Results

Results showing a potentiation or an inhibition effect lesser than 25% were considered non-significant. GABA, bicuculline and PAM induced robust activity on the GABA receptor cell assay. On the contrary, PH10 had no significant effect on GABA potentiation at doses up to 10 µM compared to the 300% potentiation induced by diazepam (Figure 3, Table 3 and Table 4). An EC50 could not be calculated for PH10, whereas the EC50 of diazepam was 72 nM. Additionally, PH10 had no agonist or antagonist effects on GABA receptors as compared to the effect of GABA (EC50= 4.7 µM) and bicuculline (EC50= 1.6 µM), respectively (Table 2).

Therefore, if PH10 does not produce its pharmacological effect by directly binding to GABA receptors, what is the mechanism of action? We have previously reported that that PH10 increases the amplitude of inward currents in patch recorded male and female human nasal chemosensory cells and intranasal spray administration of PH10 to clinically healthy men and women volunteers decreases parasympathetic nervous system tone and increases the frequency of electrodermal activity events.

Table 2. Estimated EC50/IC50 Summary

Compound	Target	Mode	Estimated EC_{50}/IC_{50} (µM)
PH10	GABAA α1/β2/γ2	Agonist	>10
PH10	GABAA α1/β2/γ2	Antagonist	>10
PH10	GABAA α1/β2/γ2	PAM	N/C*

*N/C: estimated EC_{50} values for PH10 could not be calculated

Table 3. Comparison of effect of PH10 and Agonists of GABA receptors

Compound ID	Client Compound ID	Concentration (µM)	(%) control		
			n1	n2	Mean
US034-0010316-1	PH10	0.0316	2.36	3.23	2.80
US034-0010316-1	PH10	0.1	4.74	5.85	5.30
US034-0010316-1	PH10	0.316	2.54	3.03	2.79
US034-0010316-1	PH10	1	2.65	2.44	2.55
US034-0010316-1	PH10	3.16	3.68	3.92	3.80
US034-0010316-1	PH10	10	2.08	3.23	2.65
Time-Matched Vehicle Control	0.33% DMSO		3.52	3.00	3.26
Time-Matched Vehicle Control	0.33% DMSO		2.65	2.64	2.65
Time-Matched Vehicle Control	0.33% DMSO		1.51	1.72	1.62
Time-Matched Vehicle Control	0.33% DMSO		3.88	2.99	3.43
Time-Matched Vehicle Control	0.33% DMSO		3.87	3.21	3.54
Time-Matched Vehicle Control	0.33% DMSO		2.57	1.95	2.26
Positive Reference Control	GABA	0.1	1.70	1.65	1.68
Positive Reference Control	GABA	0.3	2.43	2.85	2.64
Positive Reference Control	GABA	1	7.14	7.86	7.50
Positive Reference Control	GABA	3	31.74	31.12	31.43
Positive Reference Control	GABA	10	80.87	79.87	80.37
Positive Reference Control	GABA	30	100.00	100.00	100.00

Table 4. Comparison of effect of PH10 and Antagonists of GABA receptors

Compound ID	Client Compound ID	Concentration (µM)	(%) inhibition		
			n1	n2	mean
US034-0010316-2	PH10	0.0316	-9.90	0.49	-4.70
US034-0010316-2	PH10	0.1	-13.45	-14.39	-13.92
US034-0010316-2	PH10	0.316	-19.87	-19.44	-19.66
US034-0010316-2	PH10	1	-7.33	-10.33	-8.83
US034-0010316-2	PH10	3.16	-14.36	-16.62	-15.49
US034-0010316-2	PH10	10	-22.60	-18.36	-20.48
Time-Matched Vehicle Control	GABA EC_{80}	15	-4.00	1.26	-1.37
Time-Matched Vehicle Control	GABA EC_{80}	15	-7.54	-6.31	-6.93
Time-Matched Vehicle Control	GABA EC_{80}	15	-8.59	-4.89	-6.74
Positive Reference Control	Bicuculline	0.032	-0.10	-0.63	-0.36
Positive Reference Control	Bicuculline	0.16	-6.56	8.32	0.88
Positive Reference Control	Bicuculline	0.8	25.38	23.05	24.22
Positive Reference Control	Bicuculline	4	80.34	80.23	80.28
Positive Reference Control	Bicuculline	20	97.44	95.99	96.72
Positive Reference Control	Bicuculline	100	98.81	98.90	98.86

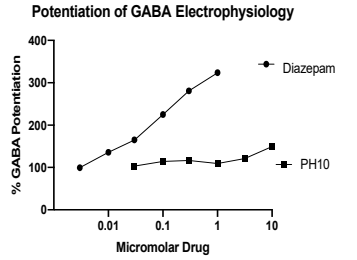

Figure 3. PH10 does not potentiate GABA receptors

4. Conclusions

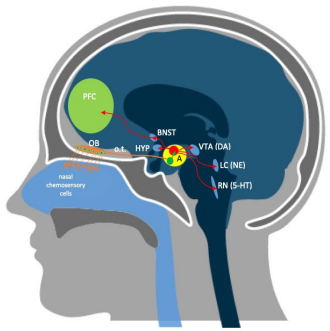

Figure 4. Schematic diagram showing the proposed neural regulation of amygdala function by PH10 and its influence on other neural structures. OB: olfactory bulb, OT: olfactory tract, A: amygdala, HYP: hypothalamus, BNST: bed nucleus of stria terminalis, PFC: prefrontal cortex, LC: locus coeruleus, VTA: ventral tegmental area, RN: raphe nucleus

While PH10 may regulate GABA circuits in the limbic amygdala, the results presented here show that PH10 does not directly bind to or modulate GABA receptors at concentrations ≤10 µM, which differentiates its mechanism of action from benzodiazepines. Importantly, the concentration of PH10 in plasma is below the limits of detection by conventional HPLC assays (1 ng/mL or 3.7 nM). Thus, any small potentiation that may occur in vitro at a concentration <10 µM is of no physiological consequence after intranasal administration of the proposed clinical dose (3.2 µg).

We propose that the mechanism of action of PH10 is through the neural regulation of forward inhibitory GABAergic neurons in the central amygdala rather than through a direct local effect on GABAergic receptors (Figure 4). Therefore, neural circuits starting from nasal chemosensory neurons stimulating olfactory bulb neurons that project axons to the medial amygdala and onto GABAergic neurons in the central amygdala could explain the anxiolytic effect of *pherine* PH10.

The results presented here suggest that PH10's rapid anxiolytic effects are mediated by a novel mechanism of action without benzodiazepine-like side effects or abuse liability.

5. References

1. Monti L, Nicolini H, Liebowitz M and Hanover R. A Placebo controlled trial of PH10: Test of a new rapidly acting intranasally administered antidepressant. British J. Pharmaceutical and Medical Res. 2019. 4(6), pp 2157-2168.
2. Monti L, Liebowitz MR. Neural circuits of anxiolytic and antidepressant pherine molecules. *CNS Spectrums*, 2020; https://doi.org/10.1017/S109285292000190X.