PH94B: A new intranasal neuroactive steroid with rapid onset of anxiolytic activity but a mechanism of action different from benzodiazepines

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Abstract

PH94B (androsta-4,16-dien-3b-ol) is a neuroactive steroid* with demonstrated anxiolytic activity but without significant side effects. Nasal spray administration of 1.6 to 3.2 micrograms PH94B rapidly (15 min) decreased performance anxiety significantly better than placebo in subjects diagnosed with social anxiety disorder (SAD) (Figure 1). And yet the side effects were similar to placebo and there was no sedation. Most anxiolytics that work acutely are GABAergic drugs such as benzodiazepines. Here we present evidence that the anxiolytic effect of PH94B is not mediated by a direct effect on GABA receptors, but instead involves activation of human nasal chemosensory neurons that then broadcast signals to indirectly regulate GABAergic neurons in the central amygdala.

Introduction

Benzodiazepines such as diazepam and alprazolam mediate their anti-anxiety effects by acting as positive allosteric modulators at gamma-amino butyric acid (GABA) receptors to make them more responsive to GABA, thereby increasing the inhibitory neuronal activity in the brain. Benzodiazepines are drugs that can be abused with serious dependency and withdrawal issues.

In addition to synthetic benzodiazepines, endogenous neurosteroids derived from cholesterol via-progesterone (i.e.: allopregnanolone, pregnanolone, 3a5aTHDOC and 3a5bTHDOC) are known to be potentiating modulators with a direct effect on GABA-A receptors. Almost all neurosteroids (endogenous or synthetic) with potent direct effects on GABA-A receptors derive from pregnanolone or pregnenolone. In contrast, PH94B (androsta-4,16-dien-3b-ol) is a synthetic neuroactive steroid (NAS) derived from an androgen (but without androgenic properties). The aim of the present in vitro study was to assess the potentiation effect or inhibition effect of PH94B on GABA receptors.

*Neuroactive steroids (NAS) refer to steroids that, independent of their origin, are capable of modifying neural activities by binding to membrane receptors.

Methods

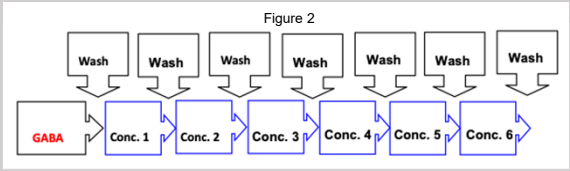
This was an in vitro study to compare the modulatory effect of PH94B to a benzodiazepine (diazepam) and the GABA antagonistic effects of PH94B to the GABA antagonist bicuculline as well as to assess any GABA agonist properties of PH94B compared to GABA itself. This study was carried out by Eurofins Discovery. Figure 2 shows a schematic diagram of the study design.

In order to help differentiate the mechanism of action of PH94B from benzodiazepines, we studied whether PH94B had a positive modulatory effect on GABA receptors using in vitro patch recording electrophysiology in cells transfected with human GABAA 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: PH94B (μM: 0.03; 0.1; 0.3; 1.0; 3.0 and 10), GABA (μM: 0.1; 0.3; 1.0; 3.0; 10 and 30), Diazepam (μM 0.003; 0.01; 0.03; 0.1; 0.3 and 1.0), GABA antagonist Bicuculline (μM: 0.032; 0.16; 0.8; 4.0; 20 and 100) (Table 1). Each concentration of test compound was applied to the perfusate during 2 sec, followed by 60 sec wash. This was repeated with the next ascending concentration of test compound.

Table 1

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
PH94B	GABAA α1/β2/γ2	Agon, Antagon, PAM	0.0316, 0.1, 0.316, 1, 3.16, 10

Figure 2



Results

Results showing a potentiation or an inhibition effect lesser than 25% were considered non-significant. As shown in Table 2, 3 and 4, GABA, bicuculline and diazepam (PAM) induced robust activity on the GABA receptor cell assay. PH94B had no significant effect on GABA potentiation at doses up to 10 μM compared to the 300% potentiation induced by diazepam. An EC50 could not be calculated for PH94B, whereas the EC50 of diazepam was 72 nM. Additionally, PH94B 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, 3 and 4). Finally, as shown in Figure 3, unlike benzodiazepines PH94B did not potentiate the GABA receptor in the assay. Therefore, if PH94B does not work by directly binding to GABA receptors, what is the mechanism of action? We have demonstrated previously that PH94B induces species specific and concentration dependent increase in intracellular calcium (ED50= 1 μM) in isolated human male and female chemosensory neurons located in the nasal epithelium (Figure 4). Intranasal spray administration of PH94B to human volunteers produces dose dependent depolarization of the electrogram recorded from the surface of the nasal chemosensory mucosa.

Table 2

Compound	Assay/Target	Mode	Estimated EC_{50}/IC_{50} (μM)
GABA	$GABA_A$ (α1/β2/γ2) Human Ion Channel Cell Based Agonist Ion Flux Assay	Agonist	4.7
Bicuculline	$GABA_A$ (α1/β2/γ2) Human Ion Channel Cell Based Antagonist Ion Flux Assay	Antagonist	1.6
Diazepam	$GABA_A$ (α1/β2/γ2) Human Ion Channel Cell Based PAM Ion Flux Assay	PAM	0.072
PH94B	$GABA_A$ α1/β2/γ2	Agonist	>10
PH94B	$GABA_A$ α1/β2/γ2	Antagonist	>10
PH94B	$GABA_A$ α1/β2/γ2	PAM	N/C*

N/C: Estimated EC_{50} values could not be calculated

Figure 3

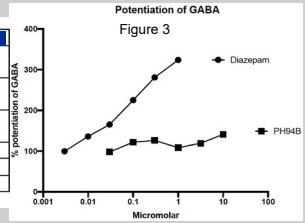


Table 3. Agonist

Compound ID	Client Compound ID	Concentration (μM)	(%) control		
			n1	n2	mean
US034-0010316-1	PH94B	0.0316	2.36	3.23	2.80
US034-0010316-1	PH94B	0.1	4.74	5.85	5.30
US034-0010316-1	PH94B	0.316	2.54	3.03	2.79
US034-0010316-1	PH94B	1	2.65	2.44	2.55
US034-0010316-1	PH94B	3.16	3.68	3.92	3.80
US034-0010316-1	PH94B	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, Antagonist

Compound ID	Compound ID	Concentration (μM)	% inhibition		
			n1	n2	mean
US034-0010316-1	PH94B	0.0316	-4.73	-3.08	-3.90
US034-0010316-1	PH94B	0.1	-4.42	-0.26	-2.34
US034-0010316-1	PH94B	0.316	-7.27	-1.63	-4.45
US034-0010316-1	PH94B	1	8.56	1.85	5.20
US034-0010316-1	PH94B	3.16	8.03	4.63	6.33
US034-0010316-1	PH94B	10	8.04	2.39	5.22
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

Conclusions

While PH94B may regulate GABA circuits in the limbic amygdala, the results presented here show that PH94B 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 PH94B 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 mM is of no physiological consequence after intranasal administration of the proposed clinical dose (3.2 μg).

We propose that the mechanism of action of PH94B 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 5). 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* PH94B.

The results presented here suggest that PH94B's rapid anxiolytic effects are mediated by a novel mechanism of action without benzodiazepine-like side effects or abuse liability.

Figure 1

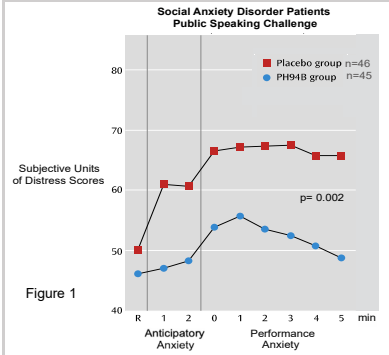


Figure 4

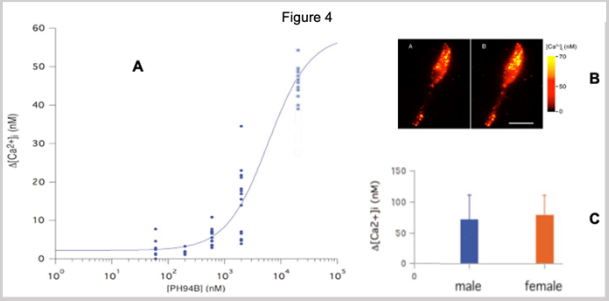
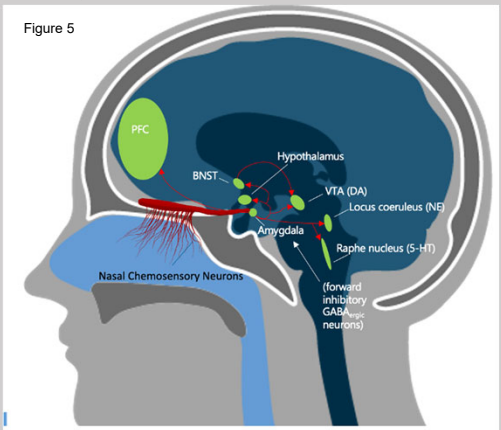


Figure 5



PFC: prefrontal cortex, BNST: bed nucleus of the stria terminalis, VTA: ventral tegmental area, DA: dopamine, NE: norepinephrine, 5-HT: serotonin

References

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