Brain and Peripheral Distribution of Intranasal Radiolabeled PH94B in Laboratory Rats

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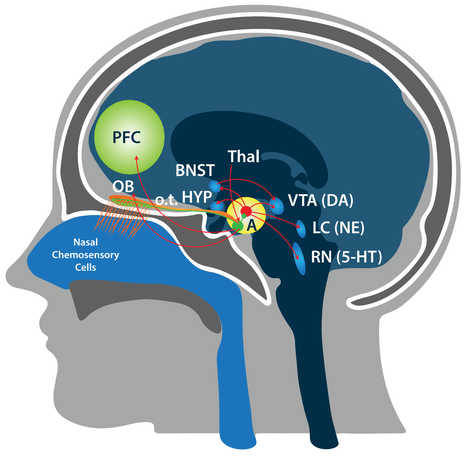
BACKGROUND

- PH94B (3β-hydroxy-androsta-4,16-dien-ol) is a synthetic neuroactive steroid from the androstane family of pherines, lacking androgenic properties and without affinity for steroidal hormone receptors[1]
- Although the precise mechanism of action is under active investigation, evidence from early studies suggests that PH94B engages olfactory chemosensory neurons that activate subsets of neurons in the olfactory bulbs that project to GABAergic forward inhibitory neurons in the limbic amygdala regulating fear and anxiety (**Figure 1**)
- Olfactory bulb connections to the limbic amygdala bypass the thalamus, providing neural input to the basal forebrain with shorter latency compared with other sensory afferent systems[2]

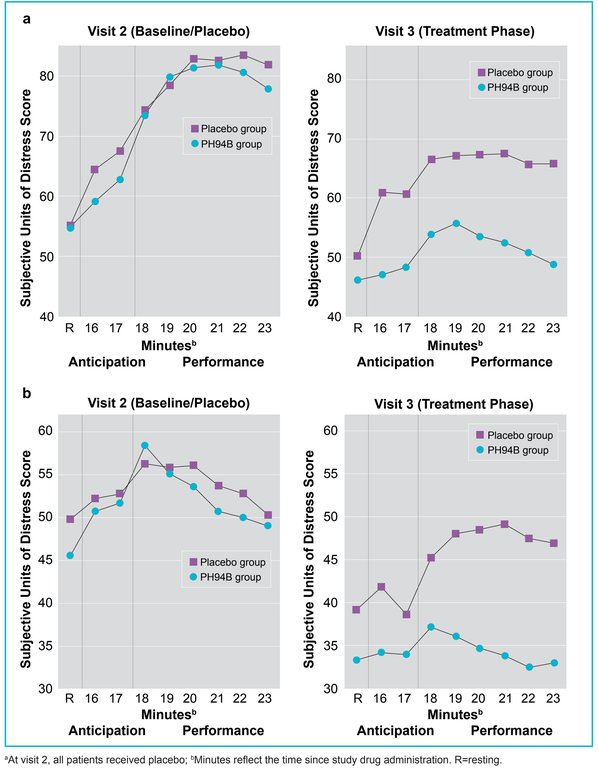
Figure 1. Olfactory Connections to the Limbic Amygdala and Related Areas



A, amygdala (green area, basolateral amygdala; red area; central amygdala); BNST, bed nucleus of the stria terminalis; DA, dopamine; 5-HT, serotonin; HYP, hypothalamus; LC, locus coeruleus; NE, norepinephrine; OB, olfactory bulb; o.t., olfactory tract; PFC, prefrontal cortex; RN, raphe nuclei; Thal, thalamus; VTA, ventral tegmental area.

- In a previously reported phase 2 study in patients diagnosed with Social Anxiety Disorder according to DSM-IV criteria,[3] treatment with PH94B nasal spray induced a rapid and significant decrease in both public speaking performance anxiety and social interaction anxiety compared with the effect of placebo nasal spray as measured by Subjective Units of Distress scores (**Figure 2**)
- Upon eligibility confirmation, participants underwent 2 challenges
  - Patients received single-blind placebo, rested 15 minutes, then picked a topic for giving a speech
  - After 2 minutes preparation (anticipation), participants gave a 5-minute speech without notes to 3 unfamiliar clinic staff (performance)
  - After a 30 minute break, participants received a second single-blind placebo treatment, followed 15 minutes later by a social interaction challenge that followed the same format as the speech paradigm
  - Patients with moderate to severe anxiety during the 5-minute performance phase of either challenge (ie, ≥75 on at least one Subjective Units of Distress Score) were brought back for double-blind treatment with PH94B or placebo at Visit 3, repeating the challenges employed at Visit 2[3]

Figure 2. Public Speaking Challenge a) and Social Interaction Challenge b) in Women with Social Anxiety Disorder as Measured by Subjective Units of Distress Scores (PH94B, N=45; Placebo, N=46)[a]



[a]At visit 2, all patients received placebo; [b]Minutes reflect the time since study drug administration. R=resting.

METHODS

- The objective of the present study was to determine the brain and peripheral tissue distribution following a single intranasal dose administration (10 µCi) of radiolabeled PH94B ([^{14}C]-PH94B) to naïve male and female rats
- Male and female Long-Evans rats 10-13 weeks old and weighing 231-325 g at initiation of dosing were used
- After randomized group assignment, rats were acclimated for 7 days in paired or group housing; food and water were available *ad libitum*
- The dosing formulation was administered once on Day 1 via intranasal instillation, with approximately half of the total dose volume (approximately 25 µL) delivered to each naris
- One male and one female rat were euthanized at each time point (15 min, 60 min, and 6, 24, 72, 168, 336, and 504 hours) after intranasal dosing of radiolabeled PH94B ([^{14}C]-PH94B) and subjected to whole-body autoradiography
- Whole-body sagittal plane sections approximately 30 µm thick were obtained, exposed to phosphor imaging screens, and scanned
- Quantification, relative to the calibration standards, was performed by image densitometry
- Statistical analysis was not performed; however, means and standard deviations were calculated

RESULTS

- Whole body [^{14}C]-PH94B concentrations for hours 0 through 168 and radiography images at 15 minutes, 1 hour, and 6 hours after radiolabeled PH94B ([^{14}C]-PH94B) intranasal administration in male and female rats are presented in **Figures 3, 4, 5, and 6**)
- Overall, tissue distribution was minimal (**Figures 3, 4, 5, and 6**), with the highest concentrations of radioactivity observed in the nasal turbinates at 15 minutes post dose
- At later time points, the highest concentrations of radioactivity were observed in the cecum and intestinal contents (**Figures 3 and 4**)
- There were minimal levels of measurable radioactivity in the central nervous system (CNS; olfactory lobes, cerebrum, cerebellum, and spinal cord) tissues at 15 minutes post dose; at 1 hour post dose, radiolabeled PH94B was below the limit of quantification (BLQ) in CNS tissues (**Figures 5 and 6**)
- By 24 hours post dose, most radioactivity was cleared from the animal and quantifiable radioactivity was present only in the intestines and contents, and the nasal turbinates (male animal only; **Figures 3 and 4**)
- At 168 hours post dose, all tissues had [^{14}C]-PH94B levels that were BLQ or not detectable (**Figures 3 and 4**)
  - The lower limit of quantification (LLOQ) was determined to be <13 ng equivalents test article/g

Figure 3. Select Individual Quantitative Whole-Body Autoradiography [^{14}C]-PH94B Concentration Values (g), Male Rats

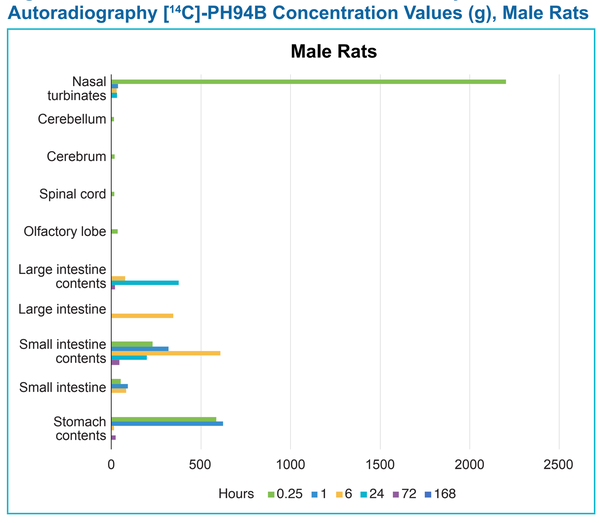
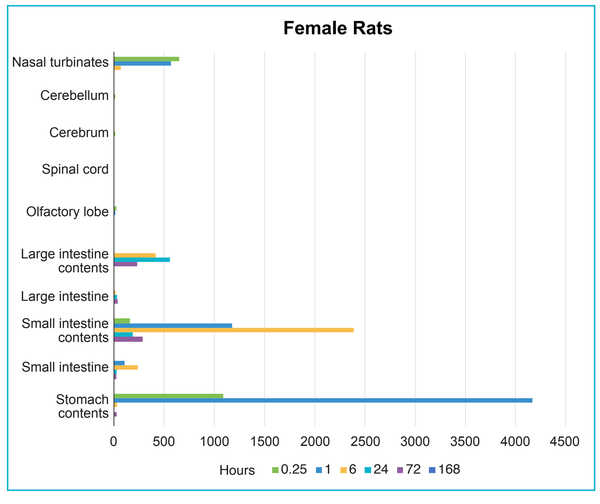


Figure 4. Select Individual Quantitative Whole-Body Autoradiography [^{14}C]-PH94B Concentration Values (g), Female Rats



- [^{14}C]-PH94B was largely confined to the nasal passages, with minimal or undetectable radiolabeled [^{14}C]-PH94B uptake in the CNS tissue at all time points—from 15 minutes to 504 hours after intranasal administration
- [^{14}C]-PH94B uptake in peripheral tissue (eg, blood plasma (not shown), kidney, and liver) was likewise minimal or undetectable (**Figures 5 and 6**)

Figure 5. Whole-Body Autoradiography of Male Rats After a) 15 Minutes, b) 1 Hour, and c) 6 Hours of [^{14}C]-PH94B Exposure

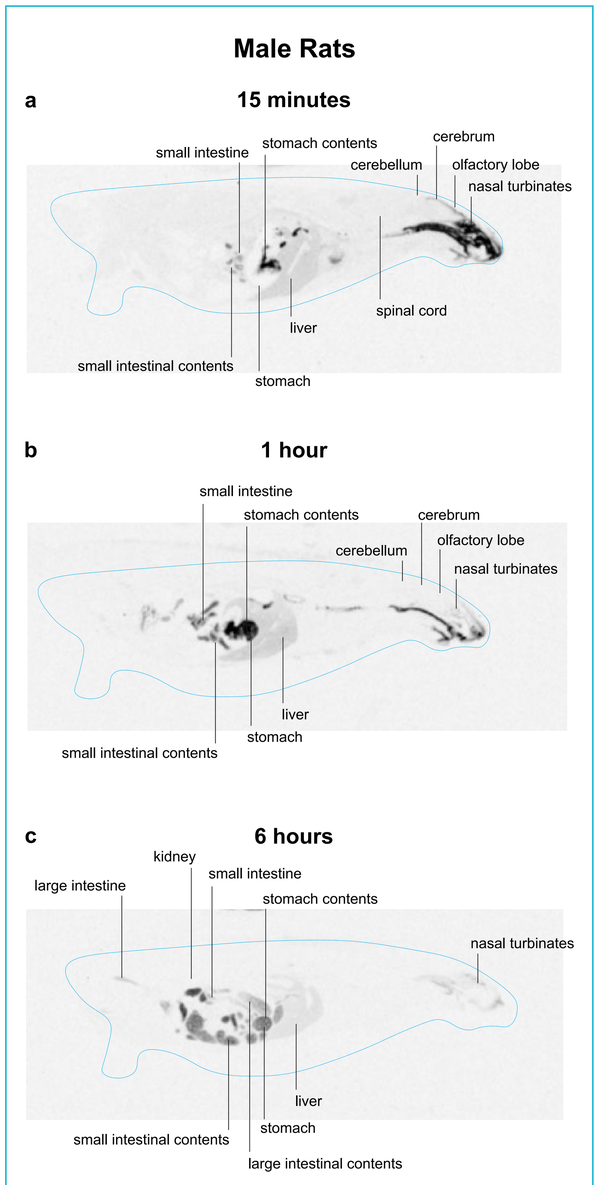
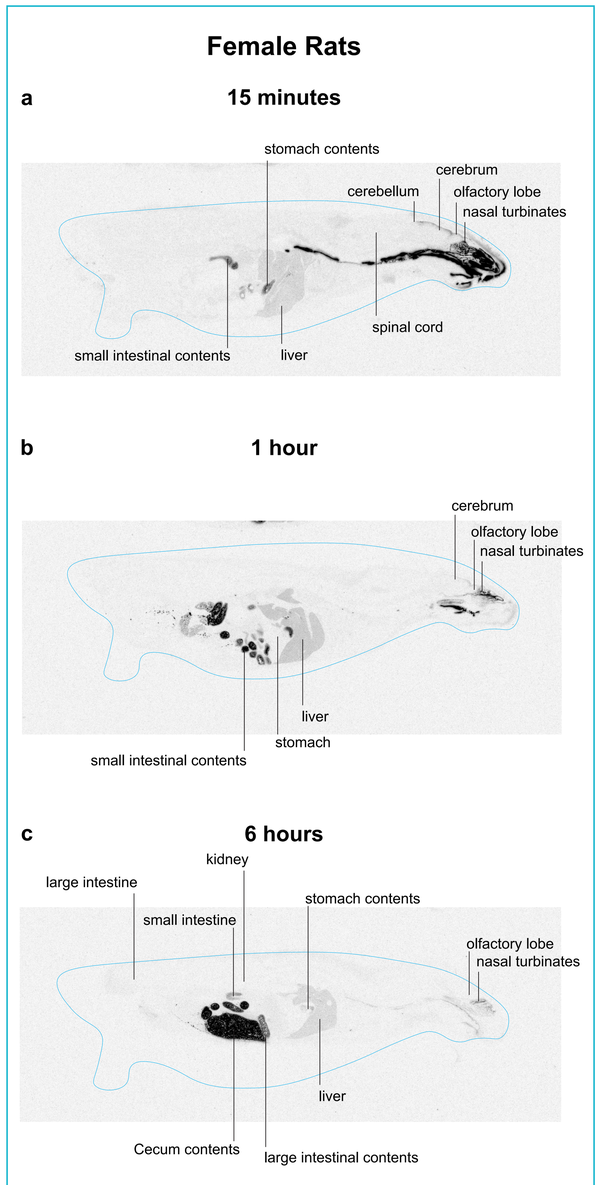


Figure 6. Whole-Body Autoradiography of Female Rats After a) 15 Minutes, b) 1 Hour, and c) 6 Hours of [^{14}C]-PH94B Exposure



CONCLUSIONS

- A single intranasal administration of 10 µCi [^{14}C]-PH94B in rats was partially absorbed and quickly eliminated from the body
- Distribution of radioactivity in tissues, including the CNS, was minimal
- These data further support the proposed mechanism of action whereby PH94B binds to receptors in nasal chemosensory neurons, rather than in the CNS, limiting transport of PH94B to the circulatory system and minimizing blood-brain barrier penetration
- These data, in combination with data from in vitro binding studies showing that PH94B does not directly activate GABA-A receptors,[2] support the growing body of evidence suggesting that PH94B induces its anxiolytic effects without the need for CNS penetration or systemic uptake, avoiding side effects and safety concerns associated with current therapies (eg, benzodiazepines and antidepressants)
- In conclusion, we hypothesize that PH94B stimulates nasal chemosensory neurons that in turn activate subgroups of neurons in the olfactory bulbs that project to amygdala neurons regulating fear and anxiety circuits[2]
- The efficacy, safety, and tolerability of PH94B in human subjects suffering from social anxiety disorder is currently being evaluated by VistaGen Therapeutics, Inc., in the PALISADE phase 3 clinical program (PALISADE-1 [NCT04754802] and PALISADE-2 [NCT05011396])

References

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Disclosures

Louis Monti: Employee and stockholder of VistaGen Therapeutics, Inc.
Ross A. Baker: Employee of VistaGen Therapeutics, Inc.
Mark A. Smith: Employee and stockholder of VistaGen Therapeutics, Inc.

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