ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/11251255

Design, synthesis, and biological evaluation of novel, centrally-Acting thyrotropin-Releasing hormone analogues

ARTICLE *in* BIOORGANIC & MEDICINAL CHEMISTRY LETTERS · SEPTEMBER 2002

Impact Factor: 2.42 · DOI: 10.1016/S0960-894X(02)00368-2 · Source: PubMed

CITATIONS
19

READS
9

5 AUTHORS, INCLUDING:

Katalin Prokai-Tatrai
University of North Texas HSC at Fort Worth
94 PUBLICATIONS **1,189** CITATIONS
SEE PROFILE

Laszlo Prokai
University of North Texas HSC at Fort Worth
190 PUBLICATIONS **3,298** CITATIONS
SEE PROFILE

Available from: Katalin Prokai-Tatrai
Retrieved on: 13 January 2016

Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 2171–2174

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Design, Synthesis, and Biological Evaluation of Novel, Centrally-Acting Thyrotropin-Releasing Hormone Analogues

Katalin Prokai-Tatrai,[a,b] Pál Perjési,[a] Alevtina D. Zharikova,[a] Xiaoxu Li[a] and Laszlo Prokai[a,c,*]

[a]*Center for Drug Discovery, College of Pharmacy, University of Florida, Gainesville, FL 32610-0497, USA*
[b]*Center for Neurobiology of Aging, College of Medicine, University of Florida, Gainesville, FL 32610-0267, USA*
[c]*The McKnight Brain Institute of the University of Florida, Gainesville, FL 32610-0015, USA*

Received 23 January 2002; accepted 29 April 2002

Abstract—Novel, metabolically stable and centrally acting TRH analogues with substituted pyridinium moieties replacing the [His^2] residue of the endogenous peptide were prepared by solid-phase Zincke reaction. The 1,4-dihydropyridine prodrugs of these analogues obtained after reducing the pyridinium moiety were able to reach the brain and maintain a sustained concentration of the charged, degradation-resistant analogues formed after enzymatic oxidation of the prodrug, as manifested by the analeptic action measured in mice. Among the four analogues reported, compound **2a** showed the highest potency and longest duration of action in reducing the pentobarbital-induced sleeping time compared to the parent TRH. No binding to the endocrine TRH-receptor was measured for **2a**; thus, this compound emerged as a potent, centrally acting TRH analogue. © 2002 Elsevier Science Ltd. All rights reserved.

TRH (pGlu-His-Pro-NH_2, **1**) is the first hypothalamic releasing factor characterized, establishing the fundamental proof for the existence of a neuroendocrine regulation of pituitary functions by hypothalamic structures.[1,2] TRH induces a wide range of behavioral effects by either peripherally or centrally.[3] One of the best-known central nervous system (CNS) effects of **1** is the analeptic action; the reduction of barbiturate narcosis or haloperidol-induced catalepsy as a measure of dopaminergic stimulation.[4] A large number of TRH analogues have been synthesized to separate the endrocrine and CNS effects.[5] Analogues where [His^2] is replaced by residues having alkyl side chain are characteristic representatives of TRH analogues having increased CNS activity but decreased or absent hormonal activity.[6] This feature is desired concerning potential treatment of CNS disorders such as Alzheimer's disease, brain or spinal cord trauma and motorneuron disease.

TRH and its analogues, however, have poor access to the CNS due to their insufficient lipid solubility and the absence of specific transport systems present in the endothelial cells forming the blood–brain barrier (BBB).[7] Previously, we have reported a novel, but rather complex method for improving brain-targeting of [Leu^2]TRH.[8] In this approach, a progenitor sequence was embedded in a chemical targeting system that penetrated the BBB, retained and liberated the target TRH analogue in the CNS through multiple enzymatic transformations. For a simplification of this method, we reasoned that the design of analogues having specific moieties with ability to furnish CNS targeting could be explored. This approach may result in a much less complex system (synthetically and 'enzymatically'), as well as an enhanced CNS bioavailability due to reduction in molecular size. We also expected that metabolic stability compared to TRH could be greatly improved by our design. Therefore, we replaced the central basic His, believed to be an essential structural element to the full thyrotropin-releasing activity but not to CNS effects,[9] with pyridinium derivatives that, thereby, provide a permanent positive charge to the resulting tripeptide analogues (**2a–d**). The pyridinium moiety enabled us to make transient chemical modification (reduction of pyridinium to dihydropyridine)[10] to obtain prodrugs **3a–d** that, as neutral compounds, were expected to show an increased lipophilicity compared to that of **2a–d**; hence, CNS targeting could be achieved.

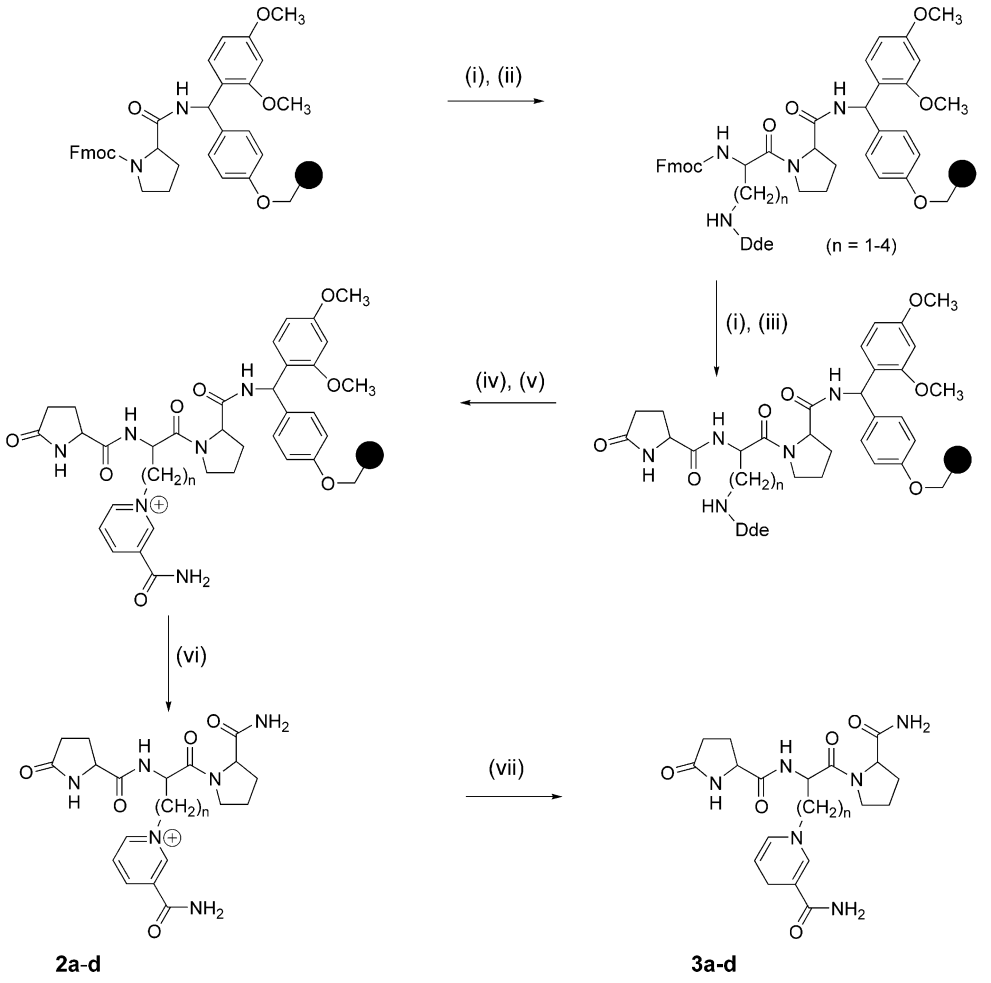
*Corresponding author: Tel.: +1-352-392-3421; fax: +1-352-392-3421; e-mail: lprokai@grove.ufl.edu

0960-894X/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(02)00368-2

Essentially, the transport of **3a–d** into the CNS should be followed by an enzymatic oxidation to **2a–d** that cannot leave the CNS due their permanent positive charge and highly hydrophilic nature. While the previously reported[8] brain-targeting systems for [Leu^2]TRH required very time-consuming and cumbersome synthetic procedures, **2a–d** reported here were prepared by semi-automated solid-phase peptide synthesis (SPPS) utilizing Fmoc chemistry. Introduction of the pyridinium moiety was also carried out by solid-phase reaction; the Zincke reaction[11] was done on the resin-bound tripeptide containing central residue having side chain amino group (Scheme 1). Briefly, the pre-loaded Fmoc-Pro-Rink Amide-MBHA resin was deprotected with 20% (v/v) piperidine in DMF, followed by coupling with Fmoc-diaminopropionic/butyric acid, Fmoc-ornithine or Fmoc-lysine, respectively, protected with 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl (Dde)[12] in their side chain amino group. Once the pyroglutamic acid had been attached, the Dde group was removed by two 5-min treatments of the resin with 2% hydrazine hydrate in DMF unmasking the side chain amino group for the Zincke-reaction. To the resin suspended in DMF, 5 equiv of Zincke-type salt[13] [*N*-(2,4-dinitrophenyl)nicotinamide chloride] was added in the presence of catalytic amount of pyridine and the mixture was kept at 60 °C. The reaction was usually complete within 4–5 h. Then, the peptide analogues **2a–d** were cleaved from the resin using TFA/water (98:2, v/v) and purified by semi-preparative gradient HPLC on octadecylsilica reversed phase. To obtain the prodrugs **3a–d**, **2a–d** were reduced with $Na_2S_2O_4$.[10,14] The reaction was monitored by HPLC with UV detection at 254 nm (for **2a–d**) and 355 nm (for **3a–d**).

In vitro stability studies in rat brain homogenate (20%, w/v) and plasma revealed that prodrugs **3** converted to **2** generating thereby the central [pyridinium^2] residue. The half-lives ($t_{1/2}$) were around 6 and 20 min in brain homogenate and plasma, respectively. On the other hand, the novel analogues (**2a–d**) were very stable in biological media (less than 10% degradation in 2 h), while TRH itself had $t_{1/2}$ around 16 min in brain homogenate and 11 min in plasma. The replacement of His also abolished the endocrine activity of **2a–d**, based on the diminished binding affinity to the receptor labeled by [^{3}H][3-Me-His^2]TRH in pellets obtained from rat brain.

A pharmacological paradigm was used to assess the potency of the new analogues as CNS agents adminis-



Scheme 1. Synthesis of TRH analogues **2a–d** and their prodrugs (**3a–d**): (i) 20% (v/v) piperidine in DMF, 10 min; (ii) PyBOP/HOBt: Fmoc–NH–CH[$(CH_2)_n$–NH-Dde]COOH/DiPEA (1:1:1:2); (iii) PyBOP/HOBt/pyroglutamic acid/DiPEA (1:1:1:2); (iv) 2% (w/v) hydrazine in DMF, 2 × 5 min; (v) *N*-(2,4-dinitrophenyl)nicotinamide (5 equiv), 60 °C, 5 h; (vi) TFA/H_2O (98:2, v/v); (vii) $Na_2S_2O_4$ in water, pH 7.

tered to the animals in their prodrug forms in vivo. The antagonism on the barbiturate-induced anesthesia was explored to survey the extent of the activation of cholinergic neurons by the test compounds. Ten to 16 Swiss-Webster mice (30 ± 2 g) were used in each group. Test compounds were dissolved in degassed DMSO. The vehicle alone (1.5 mL/kg body weight) or equimolar doses of **1** as control and prodrugs **3a**–**3d** (15 μmol/kg body weight) were injected through the tail vein (iv). After 10 min, each animal received an intraperitoneal (ip) injection of sodium pentobarbital at a dose of 60 mg/kg bodyweight. The sleeping time was recorded as the time elapsed from the onset of the loss of righting reflex until the reflex was regained.

As shown in Figure 1, a significant decrease in the sleeping time was obtained by analysis of variance (ANOVA) followed by post hoc Dunnett's test ($p < 0.05$) against the control group (animals injected with the vehicle; sleeping time 69.4 ± 13.5 min) for all of the test compounds except **3c**. TRH (**1**) and **3a** produced essentially the same sleeping times (26.0 ± 10.3 and 19.3 ± 5.9 min, respectively), while the increase of the side chain length had negative impact on the potency of the test compounds.

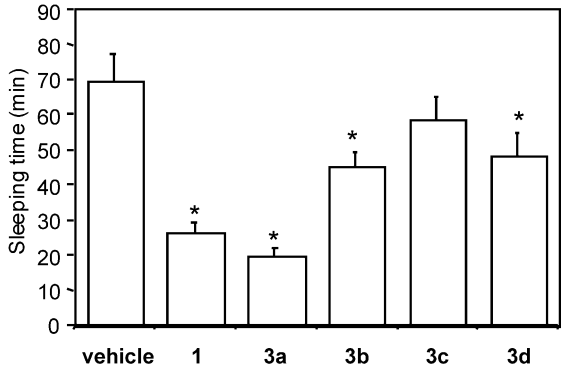


Figure 1. Analeptic effects of **2a**–**d** injected iv in their prodrug forms (**3a**–**d**) and TRH at equimolar dose of 15 μmol/kg body weight when pentobarbital (ip, 60 mg/kg body weight) was administered 10 min after the injection of the vehicle, **1** or **3a**–**d**. Asterisks indicate statistically significant differences ($p < 0.05$) from the control group (vehicle).

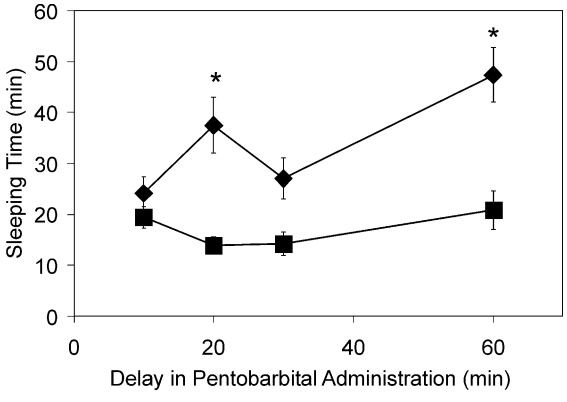


Figure 2. Pharmacological comparison of the duration of analeptic action at equimolar dose (15 μmol/kg body weight, iv) of **1** (◆) and **3a** (■) upon varying the time for pentobarbital post-administration (ip, 60 mg/kg body weight). Asterisks indicate statistically significant differences (ANOVA followed by Dunnett's test, $p < 0.05$) compared to the experiment when cholinergic challenge was made 10 min after the injection of **1**.

The most potent prodrug (**3a**) was further studied and compared to **1** for the duration of the analeptic action by increasing the time after which the cholinergic challenge was made in the animals injected with **1** or **3a** (Fig. 2). The analeptic activity of **1** showed a decrease (in overall trend), when the time between injections was increased. A loss over 50% of CNS activity was observed at 60 min, compared to when pentobarbital injection was made after 10 min. Compound **3a** actually reached its highest analeptic activity 20–30 min after administration and still remained comparable even 60 min post-administration to the maximum effect reached by **1** in our experiment. We reasoned that the CNS-effect of **1** dissipated due to its metabolic instability and depletion from the site of action as time elapsed, while **2a** remained trapped in the brain and its enzymatic degradation was slow. The great difference in biological half-lives between **1** and **2a** we obtained in the biological tissues convincingly supports this explanation. The longer half-life of **3a** in plasma compared to that in brain could also have contributed to a CNS sequestration of **2a** after systemic administration.

In conclusion, **2a** has emerged among the metabolically stable, non-endocrine TRH analogues as a new lead compound for the potential treatment of CNS disorders. Additional studies to assess neuropharmacodynamic properties of this compound, as well as development of novel analogues based on **2a** as a template are in progress.

Acknowledgements

The authors wish to thank to Olena Glushakova for her technical assistance in animal experiments. This research has been supported by a grant from the National Institutes of Health (MH59380).

References and Notes

1. Schally, A. V.; Bowers, C. Y.; Redding, T. W.; Barrett, J. F. *Biochem. Biophys. Res. Commun.* **1966**, *25*, 165.
2. Böler, J.; Enzmann, F.; Folkers, K.; Bowes, C. Y.; Schally, A. V. *Biochem. Biophys. Res.* **1969**, *37*, 705.
3. (a) Keller, H. H.; Bartholini, G.; Plestsche, A. *Nature* **1974**, *248*, 528. (b) Metcalf, G., Jackson, I. M. D., Eds. *Thyrotropin-Releasing Hormone: Biomedical Significance*. New York Academy of Sciences: New York, 1989.
4. (a) Schmidt, D. *Commun. Psychopharm.* **1977**, 469. (b) Sharp, T.; Tulloch, I. F.; Bennett, G. W.; Marsden, C. A.; Metcalf, G.; Dettmar, P. W. *Neuropharmacology* **1984**, *23*, 339. (c) Horita, A. *Life Sci.* **1998**, *62*, 1443.
5. (a) Nutt, R. F.; Holly, F. W.; Homnick, C.; Hirschmann, R.; Veber, D. F.; Arison, B. H. *J. Med. Chem.* **1981**, *24*, 692. (b) Somlai, C.; Balaspiri, L. *J. Prakt. Chem.-Chem. Ztg.* **1994**, *336*, 525.
6. Szirtes, T. L.; Kisfaludy, L.; Palosi, E.; Szporny, L. *J. Med. Chem.* **1984**, *27*, 741.

7. (a) Zlokovic, B. V.; Segal, M. B.; Begley, D. J.; Davson, H.; Rakic, L. *Brain Res*. **1985**, *358*, 191. (b) Zlokovic, B. V.; Lipovac, M. N.; Begley, D. J.; Davson, H.; Rakic, L. *J. Neurochem*. **1988**, *51*, 252.
8. (a) Prokai, L.; Ouyang, X.; Wu, W. M.; Bodor, N. *J. Am. Chem. Soc*. **1994**, *116*, 2643. (b) Prokai, L.; Prokai-Tatrai, K.; Ouyang, X.; Kim, H.-S.; Wu, W. M.; Zharikova, A. D.; Bodor, N. *J. Med. Chem*. **1999**, *42*, 4563.
9. Coy, D. H.; Hirotsu, Y.; Redding, T. W.; Coy, E. J.; Schally, A. V. *J. Med. Chem*. **1975**, *18*, 948.
10. Prokai, L.; Prokai-Tatrai, K.; Bodor, N. *Med. Res. Rev*. **2000**, *20*, 367 and references cited therein.
11. (a) Zincke, T. *Justus Liebigs Ann. Chem*. **1903**, *330*, 361. (b) Zincke, T.; Heuser, G.; Moller, W. *Justus Liebigs Ann. Chem*. **1904**, *333*, 296.
12. Bycroft, B. W.; Chan, W. C.; Chhabra, S. R.; Hone, N. D. *J. Chem. Soc., Chem. Commun*. **1993**, 778.
13. *N*-(2,4-Dinitrophenyl)nicotinamide chloride was prepared by heating the mixture of 1-chloro-2,4-dinitrobenzene (1.1 equiv) and nicotinamide (1 equiv) at 100 °C for 2 h. After cooling, MeOH was added and the product was precipitated with ether. This procedure was repeated three times. Recrystallization from MeOH/EtOAc resulted in a pale-yellow solid, mp 186–187 °C.
14. Prokai-Tatrai, K.; Prokai, L.; Bodor, N. *J. Med. Chem*. **1996**, *39*, 4775.