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The Wave of the Future

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and the Seventeenth American
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Edited by

Michal Lebl
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Springer-Science+Business Media, B.V.

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Proceedings of the Second International and the
Seventeenth American Peptide Symposium
June 9–14, 2001, San Diego, California, U.S.A.

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A C.I.P. Catalogue record for this book is available from the Library of Congress.

ISBN 978-94-010-3905-5 ISBN 978-94-010-0464-0 (eBook)
DOI 10.1007/978-94-010-0464-0

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Originally published by American Peptide Society in 2001.
Softcover reprint of the hardcover 1st edition 2001

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Glucagon-Like Peptide-1 Analogs with Significantly Improved *in vivo* Activity

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Introduction

Glucagon-like peptide-1 (GLP-1), a potent and strictly glucose-dependent insulino-tropic agent, has received increasing attention as a possible new treatment for type 2 diabetes. Although its effectiveness in type 2 diabetes patients has been demonstrated in clinical evaluations, the potential use of the native GLP-1 as a therapeutic agent is greatly hampered by its short plasma half-life. Physiologically, GLP-1 is rapidly degraded by endoproteases. Here we report that a series of novel human GLP-1 (hGLP-1) analogs have been designed and synthesized, which have greatly improved plasma half-life and significantly enhanced *in vivo* activity.

Results and Discussion

One of the enzymes that are responsible for the fast degradation of GLP-1 *in vivo* is DPP-IV, which cleaves the amide bond between Ala⁸ and Glu⁹ at the N-terminus of hGLP-1 [1]. To prevent this enzymatic cleavage, we replaced Ala⁸ with some unnatural amino acids, including *N*-methyl-D-alanine (N-Me-D-Ala), 1-aminocyclopentane-1-carboxylic acid (A5c), and aminoisobutyric acid (Aib). These sterically hindered amino acids make the peptide bond between positions 8 and 9 less accessible to the enzyme, yielding analogs with greater DPP-IV resistance (compounds **1**, **2**, and **3**, Table 1).

Knowing that the amide bond between Lys³⁴ and Gly³⁵ of hGLP-1(1-36)NH₂ may also be cleaved *in vivo* [2], we further substituted the C-terminal Gly³⁵ residue with Aib or β -alanine (β -Ala) with the goal of protecting the peptide bond. The resulting analogs bearing modifications at both positions 8 and 35 (compounds **4–8**, Table 1) have much longer plasma half-life than mono-substituted compounds **1**, **2** and **3**. These

Table 1. hGLP-1 receptor binding affinity and rat plasma half-life.

Peptide	hGLP-1 ^a K _i (nM)	Rat plasma T _{1/2} (h)	Sequence
hGLP-1(7-36)NH ₂	1.09	0.84	
1	1.13	4.35	[N-Me-D-Ala ⁸]hGLP-1(7-36)NH ₂
2	7.23	4.86	[A5c ⁸]hGLP-1(7-36)NH ₂
3	0.64	4.52	[Aib ⁸]hGLP-1(7-36)NH ₂
4	0.95	9.76	[Aib ^{8,35}]hGLP-1(7-36)NH ₂
5	1.26	8.34	[Aib ⁸ , β -Ala ³⁵]hGLP-1(7-36)NH ₂
6	1.39	17.6	[Aib ^{8,35} ,Phe ³¹]hGLP-1(7-36)NH ₂
7	1.77	7.40	[Aib ⁸ ,Phe ³¹ , β -Ala ³⁵]hGLP-1(7-36)NH ₂
8	2.12	8.91	[Aib ^{8,35} ,Arg ^{26,34} ,Phe ³¹]hGLP-1(7-36)NH ₂

^a The assays were done in CHO-K1 cells expressing the human recombinant GLP-1 receptor.

novel hGLP-1 analogs with modifications at positions 8 and 35 also retain receptor potency of the native hGLP-1 (Table 1). Replacement of Trp31 by chemically more stable Phe does not significantly influence receptor affinity (compounds **6** and **7**).

The *in vivo* studies of this new series of hGLP-1 analogs in normal Sprague-Dawley rats demonstrated that the efficacy of the analogs, in terms of the glucose-dependent stimulation of insulin secretion, is highly correlated with their *in vitro* plasma half-life [3]. Among these analogs, compound **4** enhanced the insulin response to elevated glucose with a calculated ED₅₀ at 16.0 pmol/kg, compared to that of the native hGLP-1(7-36)NH₂ at 121 pmol/kg [4]. This 7.6-fold increase in efficacy is likely due to its enhanced enzymatic stability, resulting in an increased circulating half-life. In studies utilizing the *db/db* mouse, intraperitoneal administration of compound **4** at 5–50 nmol/kg to 5-week old animals produced a dose-dependent reduction in blood glucose monitored over a 5-h period [4].

In conclusion, we have designed and synthesized a novel class of GLP-1 analogs that have substantially enhanced plasma half-life, while retaining full receptor potency of the native hormone. The representative analog, compound **4**, is significantly more efficacious than hGLP-1 *in vivo*, and is effective in lowering blood glucose in the *db/db* mouse model of type 2 diabetes.

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