A NOVEL CYCLIC UNDECAPEPTIDE, WY-40,770, WITH PROLONGED GROWTH HORMONE RELEASE INHIBITING ACTIVITY

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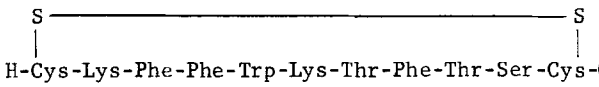
SUMMARY: A cyclic undecapeptide, Wy-40,770, has been synthesized by a combination of solid phase and conventional peptide synthesis methodology. The compound inhibits the release of growth hormone without significantly affecting glucagon levels in rats. Wy-40,770 shows growth hormone release inhibiting activity for four hours after s.c. injection.

The isolation and sequence determination of somatostatin by Brazeau et al (1) unexpectedly has opened a new chapter in the management of diabetes (2). Somatostatin possesses several biological activities (3) in addition to the inhibition of growth hormone release, including suppression of insulin (4) and glucagon (5). Luft et al (6) has shown that human growth hormone causes aggravation in the diabetes of hypophysectomized patients and Lundbaek et al (7) have suggested that abnormally high levels of growth hormone in diabetics lead to diabetic angiopathy.

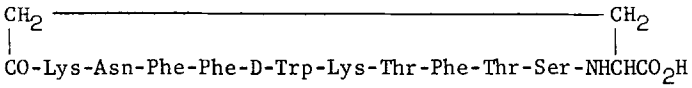
An analog of somatostatin which would lower specifically growth hormone could, on this basis, be potentially valuable for therapy of diabetes.

We recently reported (8) on an analog of somatostatin, des-Ala^1, Gly^2-Asn^5-somatostatin which shows good growth hormone and insulin release inhibiting activity <u>in vivo</u> without significant glucagon activity. We would like to report here the synthesis and biological activity of a cyclic undecapeptide, Wy-40,770 which lowers growth hormone without affecting glucagon.

Somatostatin



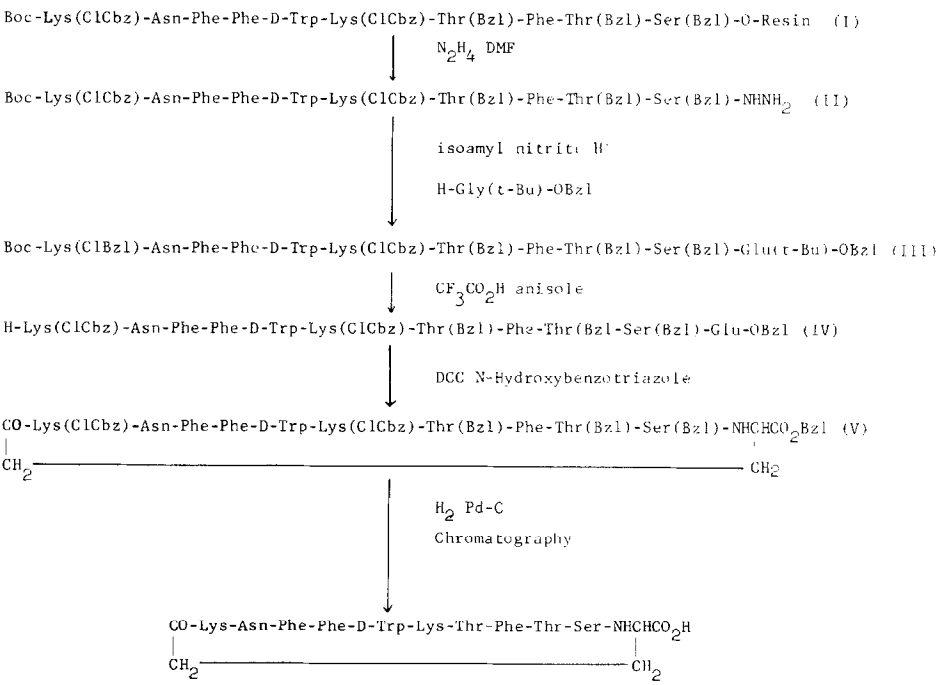
des-Ala^{1},Gly^{2},Asn^{5} - Somatostatin



WY-40,770

Synthesis: The cyclic undecapeptide, Wy-40,770, was synthesized by a combination of solid phase and conventional methodology. The peptidoresin (I), see Scheme A, was prepared on a chloromethylated resin (Lab Systems, Inc., SX-1, 0.75 mEq Cl/g). The first amino acid, Boc-Ser(Bzl)OH was esterified on the resin by Gisin's method (9). Removal of the Boc-group was accomplished in 40% trifluoroacetic acid in CH_2Cl_2 containing 0.5% dithioerythritol.

Synthetic Scheme



Wy-40,770

The following amino acid intermediates were attached on the polymeric ester, H-Ser(Bzl)-O-Resin, by the use of diisopropylcarbodiimide (D.I.C.) and one equivalent of N-hydroxybenzotriazole, Boc-Thr(Bzl)-OH, Boc-Phe-OH, Boc-Thr-(Bzl)-OH, Boc-Lys(ClCbz)-OH, Boc-D-Trp-OH, Boc-Phe-OH, Boc-Phe-OH, Boc-Asn-OH as the p-nitrophenyl-ester and Boc-Lys(ClCbz)-OH. We found that DIC is a superior carbodiimide since the by-product diisopropylurea is more soluble in organic solvents such as methanol and easier to remove from the solid phase. The peptidoresin (I) was treated with hydrazine (50 equivalents for 5 hours) to afford the decapeptide hydrazide (II) which was precipitated several times from DMF-water.

The decapeptide hydrazide (II) was coupled with α-benzyl-γ-tert-butyl-L-glutamate by Rudinger's method (10) to give the undecapeptide (III), which was then treated with trifluoroacetic acid in the presence of anisole to obtain the α-amino and γ-carboxyl group deprotected undecapeptide (IV). Cyclization of (IV) to (V) was accomplished by the method of Wieland (11). The cyclic protected undecapeptide (V) was not purified but it was deprotected by hydrogenation in the presence of 10% Pd-on charcoal. The soluble in 2M-aq AcOH material was chromatographed through a Sephadex G-25 column (2.5 x 150 cm) twice and eluted with 1M-aq. AcOH. The cyclic undecapeptide, Wy-40,770. emerged at 1.5 void volumes, see Table(I). Its elution rate through the Sephadex G-25 columns and the absence of any α-aminodansylated lysine after dansylation and acid hydrolysis makes unlikely the possibility of a dimer or a higher polymer.

Biological Testing: Plasma growth hormone, insulin and glucagon were measured in arginine stimulated rats under nembutal anesthesia 15 minutes after the undecapeptide, Wy-40,770, was administered subcutaneously (14). The procedure described below was followed to determine the duration of action of the compound. The undecapeptide, Wy-40,770 in physiological saline was injected into non-fasted male Charles River CD® rats at different doses subcutaneously (sc). Matched physiological saline sc injected rats

Table I

Properties of Wy-40,770

Amino Acid Analysis: Asp (1) 1.02; Thr (2) 1.96; Ser (1) 0.92, Glu (1) 0.99 Phe (3) 3.12, Lys (2) 2, Trp (1) 0.77, NH_3 (1) 1.16

$[\alpha]_D^{25}$ -30° ± 2 (C 0.5, 75% aq. AcOH)

TLC: R_{fA} = 0.47, R_{fB} = 0.71 one spot, chlorine spray

Amino acid analysis was run on a Durrun D 500 amino acid analyzer and hydrolysis of the peptide was carried out in 6N-HCl containing thioglycolic acid. Thin layer chromatograms were run on Avicel precoated glass plates (Analtech), system A, n-butanol-water-gl: AcOH, 4:5:1, v/v, system B, n-butanol-water-gl. AcOH-pyridine, 30:24:6:20, v/v.

served as control animals. Twenty minutes before the end of the test time they were given an intraperitoneal (ip) injection of nembutal at a dose of 50 mg/kg. Blood samples were obtained by cardiac puncture and the plasma was separated by centrifugation for the radioimmunoassay of growth hormone concentration. Double antibody radioimmunoassay was employed using growth hormone reagents from the NIAMDD (A. Parlow), commercial insulin reagents and Unger 30K anti-glucagon serum.

RESULTS AND DISCUSSION

Growth hormone was lowered by Wy-40,770 at doses as low as 12 µg/kg, although the inhibition was significantly less than that resulting from somatostatin administration at 10 µg/kg (Table II). Plasma glucagon was not lowered by the undecapeptide even at levels as high as 3,000 µg/kg, and insulin was marginally and inconsistantly lowered.

Growth hormone, in addition to being lowered by low doses of Wy-40,770 15 minutes after peptide administration, was also suppressed by higher levels of the somatostatin analog for periods as long as four hours after dosage (Table III). The prolonged growth hormone suppression by Wy-40,770 is in marked contrast with somatostatin, which has growth hormone lowering action of less than 30 minutes.

Table II

Effects of Wy-40,770 on Suppression of Growth Hormone, Insulin, and Glucagon at 15 Minutes

Exp.	Peptide	Dose µg/kg	Plasma Hormone Levels (M±SEM) GH ng/ml	Insulin µU/ml	Glucagon pg/ml
A	Control	-	247 ± 52	194 ± 17	30 ± 3
	Wy-40,770	3000	61 ± 7*	152 ± 13	29 ± 5
B	Control	-		183 ± 19	13 ± 3
	Wy-40,770	3000		93 ± 9*	9 ± 2
C	Control	-	315 ± 22		
	Somatostatin	10	53 ± 5*		
	Wy-40,770	12	192 ± 29*		

*$p < 0.01$ compared to saline controls by analysis of variance.
10 rats per group in experiments A and B, 8 rats per group in experiment C.

Table III

Prolonged Suppression of Growth Hormone Release by WY-40,770

Peptide	Dose µg/kg	N	Time hrs. after injection	Plasma Growth Hormone ng/ml, M ± SEM
Control	-	9	2	80 ± 27
Wy-40,770	1500	9	2	18 ± 4†
Control	-	10	4	182 ± 49
Wy-40,770	1500	9	4	32 ± 11‡
Control	-	10	5	85 ± 13
Wy-40,770	1000	9	5	138 ± 33

†$p < 0.05$
‡$p < 0.02$
compared to saline control by Student t test.

The undecapeptide, Wy-40,770, encompasses the ten central amino acid residues of the somatostatin sequence with the tryptophan residue in the D-configuration, in a 35-membered cyclic structure through the γ-carboxyl

group of glutamic acid and the α-amino group of lysine. The high activity of Wy-40,770 against growth hormone release suggests that the disulfide bond of somatostatin and the size of the ring is not essential. We reported earlier that linear analogs of somatostatin with two alanine residues (12) or two tyrosine residues (13) substituting the two cysteines show low but significant *in vitro* activity. It seems that restriction of the conformational mobility and the hydrophobic structure of the side chain of glutamic acid, enhance binding to the receptor and biological response. The presence of D-tryptophan instead of the L-isomer contributes further to the affinity for the receptor. The L-tryptophan analog of Wy-40,770 which we report elsewhere (14) exhibits much less potency.

The prolonged *in vivo* activity of Wy-40,770 most likely is a combination of several factors, the most important of which are the presence of the γ-amide bond of the glutamic acid and the D-tryptophan residue in the center of the molecule. Each substitution alone does not prolong the activity of somatostatin analogs (15). Our analog, Wy-40,770, because of its high specificity and duration of biological activity, could find applications for the suppression of excessive growth hormone secretion.

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