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Synthesis and Biological Activity of New Octapeptide Analogues of Somatostatin with N-Terminal Modifications*

by A. Janecka[1] and M. Zubrzycka[2]

[1]*Department of Medicinal Chemistry and* [2]*Department of Clinical and Experimental Physiology, Institute of Physiology and Biochemistry, Medical University of Łódź, Lindleya 6, 90-131 Łódź, Poland*

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Fi e ne oc apep ide analog e of oma o ain, ba ed on RC-160, D-Phe-c(C -T -D-T p-L -Val-C)-Th -NH_2, con aining N- e minal modifica ion ha e been n- he i ed. N- e minal, e oc clic D-Phe a eplaced b a oma id nna al amino acid : D-Nal, D-3(2-naph h l)alanine; D-Pal, D-3-(3-p id l)alanine; D-Qal, D-3-(3- ino- l l)alanine; D-Cl-Phe, D-3-(4-chlo ophen l)alanine, and D-Cl_2-Phe, D-3-(3,4-dichlo o- phen l)alanine. The inhibi o effec of he e ne analog e on g o h ho mone (GH) elea e in a a mea ed. I a fo nd ha he analog e i h bic clic D-Nal and D-Qal e e le po en han RC-160 in inhibi ing GH elea e *in vivo*, hile all he analog i h a oma ic, monoc clic amino acid in po i ion 1 e e mo e po en han RC-160. The be el e e ob ained fo he analog e con aining h d ophilic D-Pal in po i ion 1. Thi analog e a 8.4 ime mo e po en han RC-160.

Key words: oma o ain analog e , olid pha e pep ide n he i , d e-ac i i ela ion hip

Na i e oma o a in (SRIF), Ala-Gl -c(C -L -A n-Phe-Phe-T p-L -Th -Phe-Th -Se -C) i a e adecapep ide ho mone, hich i p ima il in ol ed in nt o- an mi ion and nt omod la ion, eg la ion of cell p olife a ion, a ell a mod - la ion of endoc ine and e oc ine ec e ion incl ding g o h ho mone, in lin, and gl cagon. Di co e ed in 1973 b B a et *et al.* [1] a he p ta i e fac o e pon ible fo he inhibi ion of g o h ho mone (GH) elea e, oma o a in i ac i e ac o ma ch la ge and mo e di e g p of i e and f nc ion han nea l an o he ho mone. The ho t half-life of oma o a in ha challenged man e ea che o de- elop mo e table compo nd . S d al modifica ion of na i e oma o a in, ch a he inco po a ion of D-amino acid o pep idomime ic ha e led o he di co e of analog e i h e tended half-life and inc ea ed biological ac i i ie [2,3]. The de el- opmen of pep ide and non-pep ide analog e of oma o a in a e en i el e- ie ed in [4]. Th gh hb and of oma o a in analog e ha e been n he i ed o fa , onl h ee oc apep ide analog e : oc eo ide [5], lan eo ide [6], and RC-160 [7,8] (Fig. 1) a e in clinical d die and/o e.

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* Abbreviations of unnatural amino acids: Nal 3(2-naph h l)alanine; Pal 3-(3-p id l)alanine; Qal 3-(3- inol l)alanine; Cl-Phe 3-(4-chlo ophen l)alanine; Cl_2-Phe 3-(3,4-dichlo ophen l)alanine.

We ha e cho en RC-160 fo the modifica ion . In he p e en pape e epo t he de ign, n he i and *in vivo* biological ac i i of fi e ne oc apep ide analog e of RC-160.

D-Phe-C -Phe-D-T p-L -Th -C -Th -ol

S-----------------------------S

Oc eo ide

D-Nal-C -T -D-T p-L -Val-C -Th -NH_2

| |

S-----------------------------S

Lan eo ide

DPhe-C -Phe-D-T p-L -Th -C -Th -NH_2

| |

S-----------------------------S

RC-160

Figure 1. Chemical e of clinicall ef l oma o a in analog e .

EXPERIMENTAL

Starting materials. The amino acid de i a i e : Boc-Phe, Boc-D-Phe, Boc-C (4-MeB l), Boc-D-T p, Boc-L (2-Cl-Z), Boc-Th (B l), and al amino acid : Boc-D-Cl-Phe and Boc-D-Cl_2-Phe e e cha ed f om Chem Impe In . (USA). Boc-D-Nal, Boc-D-Qal, Boc-D-Pal, BHA e in, and TBTU (2-[1H-ben o ia ol-1- l]-1,1,3,3- e amo h om m e a o obo a e) e e cha ed f om Bachem (S i e land).

Peptide synthesis. The analog e e e a embled ma all ing anda d olid-pha e p oce e on BHA e in (0.6 me i g $^{-1}$) in 0.25 mmol cal ing *tert*- o ca bon l (Boc) g p fo N$^{\alpha}$-amino p o ec ion and TBTU a a conden ing eagen . The c pling ime a 180 min. A 3-fold e ce of p o-ec ed amino acid a ed.

Oc apep ide e e clea ed f om he e in ppo i h h d ogen o ide (HF), con aining he ca -enge : ani ole and di hio ei ol, fo 60 min. a 0°C. The pep ide e e c cli ed in 90% AcOH (500 ml) i h a ligh e ce of I_2 (15 min.). E ce I_2 a hen emo ed b he addi ion of a co bic acid [9]. Af e c cli a ion, di lfide pep ide e e bjec ed o gel fil a ion on a 3 × 110 cm Sephade G-10 col mn in 5% AcOH, follo ed b ch oma og aph on Sephade LH-20, in he ol en em H_2O:n- OH:CH_3COOH:MeOH 90:10:10:8. The i of he final p od c a checked b anal ical HPLC (Beckman In men , USA) on a C_{18} V dac col mn (3.6 × 250 mm) in a linea g adien 30 80% of B (A: 0.1% TFA in H_2O and B 80% ACN in H_2O + 0.1% TFA). The ified pep ide e e cha ac e i ed b FAB-MS.

GH potency assay. A l male Long-E an a eighing 350 400 g e ed in all e pe imen . The a e e anae he i ed i h odi m pen oba bi al (60 mg/kg of bod eigh , admini e ed in a-pe i oneall), and 30 min. la e he oma o a in analog e o aline e e injec ed b aneo l . Blood ample e e d a n f om he j g la ein 15 min. af e injec ion, and pla ma a epa a ed and adioimm noa a ed fo GH acco ding o Me e [10]. The po encie e e e p e ed a he pe cen age of oma o a in ac i i .

RESULTS AND DISCUSSION

The ...heic oc...apep...ide ...epo...ed he...e a...e ba...ed on RC-160 and con...ain...f nc-...ional f...agmen... Phe-D-T...p-L ...-Thr... (co...e ponding ...o ...e id...e 7 10 of oma...o-...a...in), ...hich a...e fo...nd ...o be an e...en...ial pha...macopho...e of oma...o...a...in and i... analog...e [11]. The confo...ma...ional con...ain... endo...ed b... ...he di...lfide b...idge al-lo... ...he main...f nc...ional f...agmen... of ...he analog...e ...o a...ain a bioac...i...e confo...ma-...ion. Ho...e...e..., i... a fo...nd ...ha... ...he apep...ide ...u...d...e con...i...ing onl... of ...he e...o elemen... ...ho...e lo... biological ac...i...i... . The compo...nd c[C ...-Phe-D-T...p-L ...-Thr...-C ...]-NH_2 e...hibi...ed onl... 1.4% of oma...o...a...in ac...i...i... *in vivo* [12]. Inco...po...a...ion of D-Phe a... ...he N-...e...mi... and Thr...-ol o... Thr...-NH_2 a... ...he C-...e...mi... g...ea...l inc...ea...ed ...he GH...elea...e inhibi...o... effec... [5]. Ba...ed on ...he e...e...l...e decided ...o ...eplace e...oc...clic N-...e...minal D-Phe...e id...e b... a...oma...ic, mono- and bic...clic...nna...al...e i-...e : D-Nal, D-Cl-Phe, D-Cl_2-Phe, D-Pal and D-Qal. Ne... analog...e ...e...e ...he-...i...ed b... a...anda...d ...olid-pha...e me...ho...d. The ...u...de p...o...d...c ...e...e ob...ained in abo...t 60 80% ...ield, on ...he ba...i... of anal...ical HPLC. Di...lfh...d...l pep...ide ...e...e o...idi...ed ...i...h iodine and p...i...ified b... gel fil...a...ion on Sephade... G-10, follo...ed b... ch...oma...o-g...aph... on Sephade... LH-20. The p...i... of ...he pep...ide ...a... checked b... anal...ical HPLC. In all ca...e...he p...i... a... fo...nd ...o be abo...t 97%, ba...ed on UV ab...o...bance a... 214 nm. The p...i...ified oc...apep...ide ...ho...ed e...pec...ed mole...la...ion (Table 1).

Table 1. Ph...icochemical da...a of ...he ne... analog...e .

Analog...e	Yield (%)	Rf[a] (HPLC)	MW Calc.	MW Fo...nd
RC-160	88	8.74	1032.36	1033.45
1	76	10.43	1082.47	1083.57
2	73	9.81	1066.77	1068.01
3	75	10.67	1101.37	1102.45
4	68	5.32	1034.26	1035.33
5	57	6.27	1083.53	1084.42

[a]HPLC on a V...dac C_{18} co...l...mn, 3.6 × 250 mm; ...ol...en... A 0.1% TFA in H_2O, ...ol...en... B, 80%ACN in H_2O +0.1% TFA; g...adien... 30 80% B in 30 min.

The na...i...e oma...o...a...in po...e...e...a... ...ide...ange of biological ac...i...i...ie... In ...he...u...d...e-ac...i...i... ...ela...ion...hip ...u...die...e concen...a...ed on ...he inhibi...ion of ...he...elea...e of GH. The inhibi...o... effec... of all ne... oc...apep...ide analog...e on GH...elea...e *in vivo* in od...m pen...aba...bi...ol-anae...he...i...ed ...a... a mea...u...ed. The ...e...l...of ...he inhibi...ion of GH...elea...e fo... all ne... analog...a...e ...ho...n in Table 2. Th...ee...o...t of fi...e ne... analog...s ...ho...ed g...ea...e...po...enc... in inhibi...ing GH ...ec...e...ion *in vivo* ...han oma...o...a...in. The mo...t po...en... analog...e of ...hi...e...ie... a... fo...nd ...o be compo...nd 4, D-Pal-c[C ...-Phe-D-T...p-L ...-Thr...-C ...]-Thr...-NH_2, ...hich a... 8.4 ...ime...mo...e po...en... ...han oma...o...a...in. Pep...ide...s 2 and 3, con...aining D-Cl-Phe and D-Cl_2-Phe a... po...i...ion 1, ...e...e 5.4 and 4.8 ...ime...mo...e po...en... ...han oma...o...a...in, ...e...pec...i...el... Analog...e 1 and 5 ...i...h bic...clic

e id e a he po i ion 1 e e bo h le po en han oma o a in. I eem ha monoc clic, h d ophilic N-e mina i mo e ad an age o fo po enc of oma o a in analog e . The mode a e diffe ence in ac i i of all ne analog e ma g ge ha he e oc clic N-e minal e id e i no di ec l in ol ed in he ecep o ecogni ion, b mo e likel i a pa of a con ained opolog , hich main ain he p ope o ien a ion of he Phe-D-T p-L -Th pha macopho e.

Table 2. S d e and GH inhibi o ac i i ie of oma o a in analog e .

Analog e	S d e	GH inhibi ion [%]
RC-160	D-Phe-c[C -Phe-D-T p-L -Th -C]-Th -NH_2	100
1	D-Nal-c[C -Phe-D-T p-L -Th -C]-Th -NH_2	56
2	D-Cl-Phe-c[C -Phe-D-T p-L -Th -C]-Th -NH_2	540
3	D-Cl_2-Phe-c[C -Phe-D-T p-L -Th -C]-Th -NH_2	480
4	D-Pal-c[C -Phe-D-T p-L -Th -C]-Th -NH_2	840
5	D-Qal-c[C -Phe-D-T p-L -Th -C]-Th -NH_2	72

Ackno ledgmen

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