Neuroendocrinology Letters ISSN 0172-780X

ORIGINAL ARTICLE

New analogs of somatostatin: inhibiting effectively GH, glucagon and insulin levels

Boguslawa Baranowska,[1] Malgorzata Radzikowska,[1] Elzbieta Wasilewska-Dziubinska,[1] Artur Plonowski,[1] Krzysztof Roguski,[1] Anna Legowska[2] & Józef Przybylski[2]

1. Department of Neuroendocrinology, Medical Centre of Postgraduate Education, Fieldorfa 40, 04-158 Warsaw, Poland,
2. Faculty of Chemistry, Gdansk University, Poland

Correspondence to: Prof. Boguslawa Baranowska, M.D., Ph.D., Department of Neuroendocrinology, Medical Centre of Postgraduate Education, Fieldorfa 40, 04-158 Warsaw, Poland.
TEL/FAX: +48 22 610 3159
E-mail: zncmkp@polbox.com

Submitted: *February 9, 1999*
Accepted: *April 12, 1999*

Key words: **somatostatin analogs; growth hormone; insulin; glucagon**

*Neuroendocrinology Letters 1999; **20**:237–244 pii: NEL203499A09*

Abstract

The in vivo effects of three new analogs of somatostatin (ASS-51, ASS-52 and ASS-53 analogs) on GH, insulin and glucagon were studied in WKY rats. The solid phase method was used for the synthesis of ASS. Octreotide and ASS were given iv. in a dose of 0.05 μg/kg per animal in a time-dependent manner. ASS-52 and ASS-53 were longer acting and more potent somatostatin analogs when compared to octreotide in producing the inhibition of GH. ASS-51 was found to be the most potent and selective inhibitor of insulin and glucagon release. Our results show that the increased inhibitory effect and the higher selectivity of the new somatostatin analogs may result from the differences in their chemical structure.

ASS-52 is most active in inhibiting GH release. The mechanism by which ASS-52 inhibits preferentially GH release may involve the opioid system and the activation of GABA-ergic receptors.

In studies in vitro ASS-52 inhibited GH release from pituitary cells' culture.

Introduction

Somatostatin (somatotropin release-inhibiting factor SRIF)—tetradecapeptide discovered by Brazeau [1]—is known as a regulatory hormone which exhibits inhibitory effects on several organs and tissues, including the central nervous system, hypothalamus and pituitary gland, the gastrointestinal tract and the exocrine and endocrine functions of the pancreas [2]. Considerable progress has been achieved in recent years in the synthesis of highly potent and selective analogs of somatostatin. Veber et al. [21] carried out a conformational analysis and designed several analogs by replacing 9 of the 14 aminoacids of somatostatin with a single proline residue. Some of hexapeptide analogs, in particular cyclo(-Pro-Phe-DTrp-Lys-Thr-Phe), were reported to be highly active with regard to the inhibition of GH, insulin and glucagon [4]. The same group reported the synthesis of another series of analogs. Among a large number of cyclic hexapeptides synthesized, the analog cyclo(N-Met-Ala-Tyr-DTrp-Lys-Val-Phe) was found to have 50-100 times improved inhibitory activity on GH, insulin and glucagon release [4]. Bauer et al. [5] synthesized the analog DPhe-Cys-Phe-DTrp-Lys-Thr-Cys-Thr-ol (SMS 201-995), which suppressed the secretion of GH more selectively than that of insulin and glucagon [6].

It has been reported that some analogs of somatostatin have a selective inhibitory effect on insulin while having little effect on GH and glucagon [7]. The analogs with different properties may act selectively through different somatostatin receptors [8].

The aim of this study was to evaluate the in vivo effect of three newly synthesized analogs of somatostatin on growth hormone (GH), insulin and glucagon release, and to compare their activity with the biological activity of octreotide.

Materials and methods

I. The synthesis of the peptides

Somatostatin and new somatostatin analogs were synthesized at the Faculty of Chemistry, Gdañsk University in Poland. Octreotide was kindly supplied by Sandoz.

Materials and instruments.

Amino acid derivatives (Z-D-Phe, Boc-Phe, Boc-D-Trp, Bpc-Lys(Tos), Boc-Thr(Bzl), Boc-Cys(Bzl) were obtained from the Peninsula Lab. Europe Ltd (St. Helen, England); trifluoroacetic acid (TFA), dicyclohexylcarbodiimine (DCC), methylene chloride (CH_2Cl_2), triethylamine (TEA), dimethylformamide (DMF), dimethyl sulfide (DMS), acetic acid and methanol were purchased from Fluka AG (Switzerland). All solvents and reagents were of analytical purity grade and were redistilled before use. Chloromethylated resin (copolystyrene - 1% divinylbenzene, 0.7 mmol of Cl/g of the resin) Bio-Beads SX was obtained from Bio-Rad Laboratories (USA). Sephadex G-15, LH-20 and Sepharose S (Fast Flow) were supplied by Pharmacia (Sweden). TLC was carried out on silica plates (Merck), and the spots were visualized with iodine, ninhydrin and the Ehrlich reagent.

The following solvent systems were used:

A: chloroform - methanol - water (80:50:5, v/v);

B: ethyl acetate - methanol - water (5:1:0.75, v/v);

C: 1-butanol - acetic acid - water - ethyl acetate (1:1:1:1, v/v);

D: 1-butanol - acetic acid - water - pyridine (15:3:12:10, v/v);

E: 1-butanol - acetic acid - water (4:1:5, v/v, upper phase).

For carrying out amino acid analysis the peptides (approximately 0.5 mg) were hydrolyzed with constant boiling hydrochloric acid (400 ml) containing phenol (20 ml) in evacuated sealed ampules for 18 h at 110°C. The analyses were performed on a Beckman Model 121 analyzer.

Optical rotations were measured with a Perkin-Elmer polarimeter accurately to ± 0.01°.

HPLC analyses of peptides were performed on a Beckman Model 338 chromatograph with a RFC18, 5 μm column (ODS 4.6 x 150 μm ultraphase plus 4.6 x 4.5 μm precolumn). The mobile phases for isocratic elution were 30%, 45% and 48% acetonitrile in 0.1% trifluoroacetic acid, flow rate 1.5 ml/minute.

Each analog gave a single peak. The purity of all peptides was 97-99% as determined by the integrated areas recorded at 223 nm.

Synthesis of the peptides.

The peptides were synthesized manually using the solid phase method [9]. First, chloromethylated resin was esterified with BocCys(Bzl) to a load of 0.46 mmol/g [9]. Then Z-D-Phe-Cys(Bzl)-Phe-DTrp-Lys(Tos)-Thr(Bzl)-Cys(Bzl)-R and $(CH2)_5C$(S-Bzl) $-CH_2$-CO-Phe-DTrp-Lys(Tos)-Thr(Bzl)-Cys(Bzl)-R were prepared by the solid phase method as described previously [10, 11, 12]. Coupling reactions were mediated either by DCC or DCC-HOBt methods [13]. The completeness of each coupling reaction was checked by the Kaiser test [14]. The protected peptides were cleaved from the resin using 50% solution of 2-amino-2-methyl-1,3-propandiol (ASS-51), ethanoloamine (ASS-52) and ethylenediamine (ASS-53), in

methanol, respectively. After evaporation of the solvent, the products were extracted by the means of hot DMF, precipitated with boiling water and left overnight at room temperature. The peptides were collected by filtration, washed with water, and dried in vacuum over P_2O_5. The products were purified by dissolution and reprecipitation with methanol-ethyl ether [1:3]. After the application of the sample, linear gradient elution (0.01 mole, pH 4.5 to 0.5 mole, pH 6.5) was carried out with ammonium acetate. The fractions comprising the major peak were pooled, lyophilized and desalted on Sephadex LH-20 (110 x 1.4) column. The purity of the final products was checked by the HPLC method. The chemical structure of newly synthesized analogs of somatostatin is presented in Table 1.

The procedure of synthesis of somatostatin analogs was registered at the Polish Patent Office on June 24, 1992 (No. P-295064).

II. Studies in vivo

All experiments were carried out on adult female Wistar-Kyoto rats, 4 weeks after ovariectomy, for elimination of the influence of sex hormones on GH release. The animals were kept in an air-conditioned room (light on from 7.00 to 19.00 h, at a temperature of 25±1°C), with free access to laboratory chow and tap water. The rats weighed 200-250 g. The experiments were performed in accordance with Guidelines for the Care and Use of Experimental Animals (Endocrine Society).

New analogs of somatostatin (ASS): ASS-51, ASS-52, ASS-53, as well as octreotide were administered intravenously (into the jugular vein) in a dose of 0.05 μg. The levels of serum GH were assessed at 10, 30, 60, 120, 180 minutes for the 0.05 μg dose in five groups of rats. Serum insulin and glucagon were determined 60, 120 and 180 minutes after the injection of 0.05 μg of analogs. The same determinations were done after placebo (saline) injection. All groups in each experiment were studied at the same time, between 9.00 and 12.00 h.

The effects of ASS-52 on GH release were also evaluated after injections of enkephalin and also after GABA-ergic receptor blockers (bicuculline). Enkephalin was administered in a dose of 10 μg as well as it was pre-treated with ASS-52, 45 minutes after injection of ASS-52. Bicuculline was injected in a dose of 750 μg as well as it was pre-treated with ASS-52, 45 minutes after injection of ASS-52. The blood was withdrawn 60 minutes after ASS-52 injection for GH measurements.

III. RIA measurements

The following RIA kits were used:

GH: The standards of rGH and antisera anti rGH were supplied by the National Hormone and Pituitary Program NIH (Baltimore, Maryland, USA). Iodination of GH was performed with IODO-GEN (Pierce, USA), sensitivity -0.2 μg/l; inter/intra assay variabilities: 5.2% and 6.0%, respectively; **Insulin:** Swierk, Poland: sensitivity - 2 mU/l; inter/intra assay

Table 1. Chemical structure of somatostatin analogs.

Octreotide

DPhe-Cys-Phe-DTrp-Lys-Thr-Cys-Thr(ol)

S — S

ASS 51 (m.w. 925,15)

CH_2OH

CH_2CO-Phe-DTrp-Lys-Thr-Cys-NH-C-CH_3

CH_2OH

S — S

ASS 52 (m.w. 881,09)

CH_2CO-Phe-DTrp-Lys-Thr-Cys-NH-CH_2-CH_2OH

S — S

ASS 53 (m.w. 880,12)

CH_2CO-Phe-DTrp-Lys-Thr-Cys-NH-CH_2- CH_2-NH_2

S — S

Table 2. Serum GH concentration (mg/l) after iv. injection of 0.05 µg somatostatin of analogs (time response). The values are given as means ± SEM. a indicates the values below the sensitivity limit (0.2 µg/l). (* p< 0.05; ** p< 0.01; *** p< 0.001 vs placebo)

Compound	0 min.	10 min.	30 min.	60 min.	120 min.	180 min.
Placebo n = 10	3.9 ± 0.2	3.9 ± 0.2	3.8 ± 0.2	4.0 ± 0.2	3.8 ± 0.3	3.7 ± 0.2
Octreotide n = 10	3.9 ± 0.2	0.67 *** ± 0.03	0.44*** ± 0.06	0.34*** ± 0.06	0.34*** ± 0.06	0.4*** ± 0.03
ASS-51 n = 10	3.9 ± 0.2	1.4** ± 0.4	0.7** ± 0.3	0.4*** ± 0.3	0.5** ± 0.3	0.3*** ± 0.1
ASS-52 n = 10	4.0 ± 0.3	1.3** ± 0.1	0.4*** ± 0.2	<0.2a	<0.2a	0.2*** ± 0.01
ASS-53 n = 10	3.9 ± 0.2	2.3* ± 0.3	0.5*** ± 0.3	<0.2a	<0.2a	0.75** ± 0.2

variation: 3.0% and 5.0%, respectively; **Glucagon:** Serono Diagnostics, Italy: sensitivity - 14.5 ng/l; inter/intra assay variation 8% and 9%, respectively.

Studies in vitro

Effects of ASS-52 on GH release from pituitary cells' culture

The procedures of pituitary tissue dissociation, cell preparation and cell culture were based on methods described previously [15, 16, 17, 18].

Briefly, pituitary glands were obtained from three month-old (weight approx. 200 g) female Wistar–Kyoto rats, anesthetized by vetbutal injection and decapitated. They were washed twice with DMEM pH 7.3 with 0.2% glucose, 2 mmol glutamine/l, 0.3% bovine serum albumin (BSA), penicillin (50U/ml) and streptomycin (50 µg/ml) and processed for culture immediately. They were enzymatically dispersed during 20 minutes incubation at 37°C in 0.1% trypsin in PBS buffer (without Ca^{2+} and Mg^{2+}) followed by 20 minutes of incubation in 0.1% DNase I (deoxyribonuclease I from bovine pancreas, type IV) in DMEM pH 7.3 with 0.3% BSA, penicillin (50 U/ml) and streptomycin (50 µg/ml). The glands were finally mechanically dispersed on a sieve (50 mesh) and washed twice by centrifugation for 10 minutes at 50 g with culture medium (DMEM pH 7.3 with 0.2% glucose, 2 mmol glutamine/l, 0.1% BSA and 10% fetal calf serum (FCS)). The pituitary cells were counted in a hemocytometer and assessed for viability by exclusion of trypan blue (>85%).

The pituitary cells (0.2 x 10^6 /ml) were incubated in 24-well culture plates for up to 48 h in a humidified incubator in an atmosphere of 95% air and 5% CO_2 at 37°C. The culture plates were washed with twice the volume of the serum-free medium with 30 µg ascorbic acid/l 30 minutes before every experiment. The neuropeptides were dissolved in saline at concentrations 1 mmol/l. They were diluted with serum-free medium with 30 µg ascorbic acid/l to final nanomolar concentrations.

For short-term effects, the somatostatin and its analoges were added after 48 h of culture and the medium was collected 30, 60, 120, 240 and 480 minutes thereafter. The collected medium was stored at -20°C until assayed for PRL, LH and GH.

All media and chemicals were purchased from Sigma (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany), culture dishes from Corning (Bibby Sterilin Ltd, Staffordshire, UK), GHRH from Bachem, and new somatostatin analogs from the Department of Chemistry—Gdañsk, Poland. Octreotide was kindly supplied by Sandoz.

For the statistical analysis the unpaired Student test and the analysis of variance were used, as appropriate.

Results

Serum GH concentrations in response to octreotide, and in response to ASS (ASS-51, ASS-52, ASS-53), in the 0.05 µg dose in a time response manner, are presented in Table 2. The maximal inhibition of GH was found at 60 minutes, and the inhibitory effect was observed still after 180 minutes.

Table 3. Plasma insulin concentration (mU/l) after iv. injection of 0.05 µg of somatostatin analogs. The values are given as means ± SEM. (* p< 0.05; ** p< 0.01; *** p< 0.001)

Compound	0 min.	60 min.	120 min.	180 min.
Placebo n = 10	47.6 ± 0.2	47.6 ± 0.2		
Octreotide n = 10	46.2 ± 0.3	11.3 ± 1.0***	45.0 ± 1.8	48.3 ± 1.9
ASS-51 n = 10	48.2 ± 0.2	10.3 ± 1.2***	18.8 ± 1.5***	37.5 ± 1.5*
ASS-52 n = 10	47.3 ± 0.2	39.6 ± 0.5	39.6 ± 1.5	44.8 ± 1.5
ASS-53 n = 10	49.3 ± 0.3	17.4 ± 0.9***	23.4 ± 1.4**	40.2 ± 1.8

Table 4. Plasma glucagon concentration (ng/l) after iv. injection of 0.05 µg of somatostatin analogs. The values are given as means ± SEM. (* p< 0.05; ** p< 0.01; *** p< 0.001)

Compound	0 min.	60 min.	120 min.	180 min.
Placebo n = 10	140.8 ± 15.5	146.8 ± 15.3		
Octreotide n = 10	148.2 ± 14.8	85.4 ± 5.2**	77.2 ± 6.8**	65.3. ± 7.1**
ASS-51 n = 10	150.7 ± 14.2	38.3 ± 3.5***	40.1 ± 7.1***	80.2 ± 6.8**
ASS-52 n = 10	146.6 ± 11.3	110.8 ± 10.2*	100.7 ± 10.3*	120.4 ± 9.4*
ASS-53 n = 10	148.2 ± 13.6	55.6 ± 6.8***	50.3 ± 8.1***	72.5 ± 5.8**

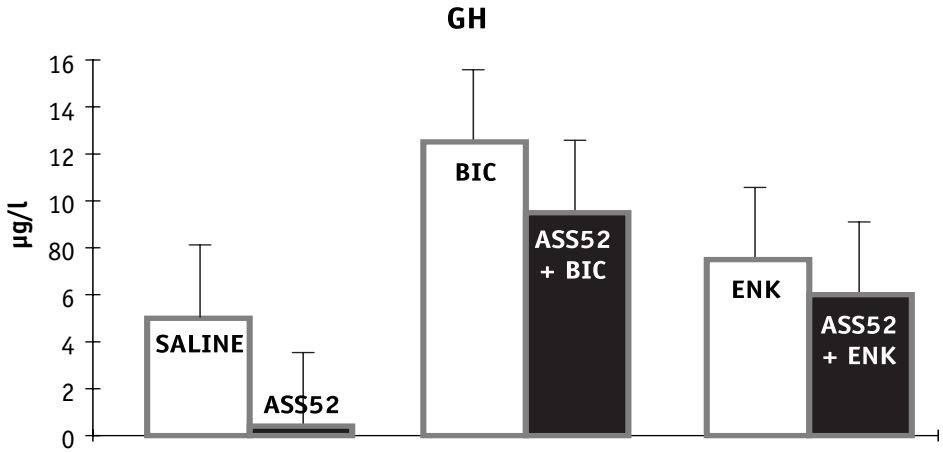

Fig. 1. Serum GH concentrations after enkephalin injection in a dose of 10 µg and after combined therapy (ENK+ASS-52), 45 minutes after injection of ASS-52 in a dose of 0.05 µg; (mean ± SEM).

after BIC, BIC+ASS52
saline vs. ASS52 p<0.01
ASS52 vs. ENK p<0.001
ASS52 vs. ASS52+ENK p<0.01
saline vs. ENK p<0.05
saline vs. BIC p<0.001
ASS52 vs. ASS 52+BIC p<0.001

ASS-52 and ASS-53 appeared to be the most potent analogs for GH release inhibition.

Plasma insulin concentrations after ASS and octreotide injection are shown in Table 3. ASS-51 was most active in insulin inhibition at 60 and 120 minutes.

Plasma glucagon levels are presented in Table 4. ASS-51 was more potent than octreotide in glucagon inhibition.

The effect of opioid peptide—enkephalin and GABA-ergic blockade with bicuculline on the GH response to ASS-52—was shown in Fig. 1. Enkephalin administered in a combined therapy with ASS-52 significantly decreased the inhibitory effect of ASS-52 on GH ($p<0.01$). Bicuculline (BIC) increased significantly GH release ($p<0.001$) and the combined therapy BIC+ASS-52 markedly diminished inhibiting action of ASS-52 on GH ($p<0.001$).

Table 5. Effects of ASS-52 on GH release from cultured pituitary cells. GH concentration in culture media in µg/l. a indicates the values below the sensitivity limit (0.2 µg/l).

Time (min.)	ASS-52 1 nM	Control 10 nM	100 nM	1 mM	
30	1.0	0.5	1.0	0.2	1.8
60	0.5	0.7	0.8	< 0,2a	3.0
120	0.5	2.5	2.7	1.0	4.0
240	2.7	2.7	0.9	0.9	3.0

Studies in vitro

Effects of ASS-52 on GH release from cultured pituitary cells are shown in Table 5.

ASS-52 in a dose-dependent manner inhibited GH release from pituitary cells. Maximal effects were observed after 60 minutes in a dose of 1mM.

Discussion

Somatostatin (somatotropin release-inhibiting factor, SRIF)—a tetradecapeptide isolated from ovine hypothalamus—has inhibitory properties not only with respect to GH secretion but also to insulin, glucagon, and gastrin release [1,3]. Various studies in animals and humans have demonstrated that somatostatin can decrease GH, TSH, PRL and ACTH release from the pituitary [19, 20]. The therapeutic effectiveness of somatostatin is limited because of its short half-life in plasma and the wide diversity of action. A large number of long acting and more selective analogs were synthesized as yet. Analogs with octapeptide structure proved to have longer action and greater selectivity than the native hormone in vivo assays [5, 6, 21, 22]. The sequence Phe-Trp-Lys-Thr in C terminal is very important for interacting with the receptors, while the rest of the molecule is responsible for maintenance of the bioactive conformations [21]. The Cys-a-Cys fragment serves as a conformational constraint allowing the tetrapeptide to attain a bioactive conformation [21]. It was suggested that such constraint not only resulted in the observed reduced sensitivity to metabolic cleavage by peptidases but also provided a long duration of action. The higher activity may be due also to the interaction of the additional benzene ring with the receptor. Octreotide (SMS 201-995 - Sandostatin) appears to be one of the more potent among a number of somatostatin analogs [22, 23, 24, 25]. Its effect on GH secretion was investigated in the animal [26] and human cell models [27]. In the rat C-cell cultures, octreotide (10^{-7}M) inhibited the accumulation of cyclic AMP induced by GH-releasing peptide (GHRP) [28]. This analog is characterized by greater potency than the native somatostatin, longer duration of action, greater metabolic stability and higher selectivity in GH inhibition as compared with insulin and glucagon. These properties were achieved by the optimization of a weakly active cystine-bridged hexapeptide containing the essential residues of the native somatostatin [29]. Octreotide, a selective octapeptide somatostatin analog, is protected against degradation by a D-phenylalanyl residue at the N terminal and by the amino alcohol at the C-terminal. Octreotide is more potent in vitro and in vivo than the native hormone in GH secretion inhibition, it has much longer action and is much more selective in inhibition of GH secretion than insulin and glucagon [5, 6].

In the present paper we describe the relationship between the chemical structure and biological activity of the three newly synthesized somatostatin analogs. Differences in the chemical structure of these analogs, as compared with octreotide, consist of the reduction of the number of amino acids and the changes in N- and C-terminals. In the ASS-51, ASS-52 and ASS-53 analogs, the cyclic hydrocarbon group derived from b,b-pentamethyl-b-mercaptopropionic acid was introduced in position 1. In the ASS-51 analog the N-1.1-dihydroxymethylethyl group was substituted for Thr(ol). In ASS-52 the N-2 hydroxyethyl group was incorporated into the C-terminal. In ASS-53 the N-2 aminoethyl group was substituted into the C-terminal. Our results showed that the analogs ASS-51, ASS-52 and ASS-53 containing the b,b-pentamethyl-b-mercaptopropionic acid derivates in the N-terminal possessed a marked inhibitory effect on GH release. ASS-52 was most active and most selective in the inhibition of GH. This analog demonstrated a lower activity in insulin and glucagon inhibition.

The relationship between the chemical structure and biological activity was demonstrated by other authors. Cai et al. [22, 23] pointed out the importance of the C-terminal and N-terminal residues in a series of somatostatin analogs. The removal of the hydroxyl group from C-terminal residue of the analogs lowered their activity. This may suggest the presence of a hydrogen bond between the hydroxyl group of the receptor-bound hormone and the receptor. The incorporation of $ProNH_2$ at C-terminal prevented the formation of a hydrogen bond not only between the molecules between the C-terminal residue and the receptor, but also within the molecule between the C-terminal and N-terminal peptide bonds. Analogs possessing $TrpNH_2$ showed high potency. The aromatic side chain of Trp 12 may be involved in building up of a hydrophobic area through staking of aromatic rings which may promote the formation of the active conformation. In this series of analogs the most active ones DPhe-Cys-Tyr-DTrp-Lys-V- Cys-$ThrNH_2$ and DPhe-Cys-Tyr-DTrp-Lys-Val-Cys-Trp-NH_2 were respectively 200 and 135 times more potent in GH inhibition than the native somatostatin. These results were similar to those obtained with octreotide which inhibited GH secretion much more selectively than insulin and glucagon secretion. Nutt et al. [30] demonstrated that the cyclic hexapeptide structure stabilized the biologically active conformation in the L-Trp. The interaction with the receptor is highly dependent on the spheric environment at the tryptophan residue.

Our results indicate that the strong inhibitory effect on GH was observed after the administration of the new somatostatin analog ASS-52 in a dose of 0.05 μg. We previously demonstrated that the infusion of a dose of 0.05 μg produced the maximal inhibiting effects but higher doses produced an opposite (stimulating) effect on GH release with the only exception of ASS-52, which caused inhibition at higher doses [31]. The observed paradoxical action may be explained partly by the different responsiveness of somatostatin and partly by an exaggerated response of GH secretion to GHRH.

We showed that opioid - enkephalin as well as the GABA-blocker - bicuculline partly decreased the inhibiting effects of ASS-52 on GH release.

Some evidence is accumulated that somatostatin and GABA are colocalized in the periventricular hypothalamic area [32, 33]. These findings suggest that GABA modulates somatostatin neuronal activity and seems to play an important role in the regulation of GH secretion. In our studies we observed that bicuculline decreased inhibiting effects of ASS-52 on GH.

It has been reported that somatostatin and its analogs may be able to bind with opiate receptors and to displace ^{3}H-naloxone or ^{3}H-dihydromorphine from opiate receptors in crude rat brain homogenate [34]. We found that enkephalin significantly decreased inhibiting effects of ASS-52 on GH release.

In studies in vitro we observed that ASS-52 inhibited GH release from pituitary cells' culture.

For a full estimation of biological activity of new somatostatin analogs, binding experiments should be introduced in the future and affinity binding of these analogs for 5 human affinity subtypes, opioid receptors and GABA receptors should be evaluated.

Conclusions

In experiments "in vivo"

1. The newly synthesized analogs ASS-52 and ASS-53 are potent and selective for the inhibition of GH release, while the ASS-51 analog is active in the inhibition of insulin and glucagon secretion and these effects may depend on the chemical structure of these analogs.

In experiments "in vitro"

1. ASS-52 inhibited GH release from the pituitary cell culture.

Acknowledgments

The study was supported by grants CMKP 501-1-2-25-22/98 and 501-2-2-25-39/99.

REFERENCES

1 Brazeau P, Vale W, Burgus R, Ling N, Butcher M, River J, et al. Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science 1973; **179**:77–79.

2 Lamberts SWJ, Krenning EP, Klijn JGM, Reubi JC. The clinical use of somatostatin analogues in the treatment of cancer, Baillier's Clin. Endocrinol Metabol 1990; **4**:29–49.

3 Veber DF, Fredinger RM, Schwenk-Perlow D, Palaveda WJ Jr, Holy RW, Strachan RG, et al. A potent cyclic hexapeptide analogue of somatostatin. Nature 1981; **292**:55–58.

4 Veber DF, Saperstein R, Nutt RF, Freidunger RM, Brady SF, Curby P, et al. A super active cyclic hexapeptide analogue of somatostatin. Life Sci 1984; **34**:1371-1378.

5 Bauer W, Briner K, Doepfner W, Haller R, Huguenin R, Marbach P, et al. SMS 201–995: a very potent and selective octapeptide analogue of somatostatin with prolonged action. Life Sci 1982; **31**:1133–1140.

6 Marbach P, Bauer W, Briner U, Doepfner W, Petcher T, Pless J. Structure-function relationships of somatostatin analogs. Horm Res 1988; **29**:54–58,

7 Brown M, Rivier J, Vale W. Somatostatin analogues with selected biological activities. Metabolism 1976; **25**:1501–1503.

8 Brown M, Vale W and Rivier J. Insulin selective somatostatin analogues. Diabetes 1977; **26**:360.

9 Gisin BF. The preparation of Merrifield-resins through total estrification with caesium salts. Helv Chem Acta 1973; **56**:1476–1482.

10 Manning M, Coy E and Sawyer WH. Solid-phase synthesis of [4-threonine]-oxytocin. A more potent and specific oxytocic agent than oxytocin. Biochemistry 1970; **9**:3925–3930.

11 Manning M. Synthesis by the Merrifield method of a protected nonapeptide amide with the aminoacid sequence of oxytocin. J Am Chem Soc 1968; **90**:1348–1349.

12 Merrifield RB. Solid phase peptide synthesis. I. Synthesis of a tetrapeptide. J Am Chem Soc 1963; **85**:2149–2154.

13 Konig W and Geiger R. Eine neue Methode zur Synthese von Peptiden: Aktivierung der Carboxylgruppe mit Dicyclohexylcorbodiimid unter Zusatz von 1-Hydroxy-benzotriazolen. Chem Ber 1970; **103**:788–793.

14 Kaiser E, Colesott RL, Bossinger CD and Cook PI. Colour test for detection of free terminal amino groups in the solid-phase synthesis of peptides. Anal Biochem 1970; **34**:595–598.

15 Artz E, Buric R, Stelzer G, Stalla J, Sauer J, Renner U, et al. Interleukin involvement in anterior pituitary cell growth regulation: effects of IL-2 and IL-6. Endocrinology 1993; **132**:459–467.

16 Renner U, Newton CJ, Pagotto U, Sauer J, Arzt E, Stalla GK. Involvement of interleukin-1 and interleukin-1 receptor antagonist in rat pituitary cell growth regulation. Endocrinology 1995; **136**:3186–3193.

17 Dieguez C, Ford SM, Peters JR, Hall R, Scanlon MF. The effect of thyroid hormone deprivation *in vivo* and *in vitro* on growth hormone (GH) responses to human pancreatic (tumor) GH-releasing factor (1-40) by dispersed rat anterior pituitary cells. Endocrinology 1985; **116**:1066–1070.

18 Soto JL, Castrillo JL, Dominguez F, Dieguez C. Regulation of the pituitary-specific transcription factor GMF-1/Pit-1 messenger ribonucleic acid levels by growth hormone-secretagogues in rat anterior pituitary cells in monolayer culture. Endocrinology 1995; **136**:3863–3870.

19 Hall R and Gomez-Pan A. The hypothalamic regulatory hormones and their clinical applications. Adv Clin Chem 1976; **18**:173–212.

20 Schally AV, Coy DH, Murphy WA, Redding TW, Comaru-Schally AM, Hall R, et al. Physiological and clinical studies with somatostatin analogues and pro-somatostatin. In: Raptis S, Rosenthal J and Gerich E, editors. 2nd International Symposium on Somatostatin. Athens, Greece. Tübingen: Attempto Verlag; 1984. p.186–196.

21 Veber DF, Holly FW, Nutt RF, Bergstrand SJ, Brady SF, Hirschmann R, et al. Highly active cyclic and bicyclic somatostatin analogues of reduced ring size. Nature 1979; **280**:512–514.

22 Cai RZ, Szoke B, Lu R, Fu D, Redding TW and Schally AV. Synthesis and biological activity of highly potent octapeptide analogs of somatostatin. Proc Natl Acad Sci USA 1986; **83**:1896–1900.

23 Cai RZ, Szoke B, Fu D, Redding TW, Colaluca J, Torres-Aleman J, et al. Synthesis and evaluation of activities of octapeptide analogues of somatostatin. In Hruby VJ, Koppel KD, Deber CM, editors. Ninth American Peptide Symposium, Toronto, Pierce Chemical Co, Rockford IL, 1986; p. 627–630.

24 Bauer W. Octreotide drug development of constrained somatostatin analogue. Proceedings of 11th International Congress of Pharmacology, Amsterdam July 1–6, 1990. Europ J Pharmacol 1990; **83**:55.

25 Pless J. Chemie und Pharmakologie von Sandostatin (Chemical structure and pharmacological profile of Sandostatin. Proceedings of the "Octreotide Symposium"; Mai 4–5, 1990; Frankfurt. Z Gastroenterol 1990; **28**:4–7.

26 Iguchi H, Natori S, Nawata H. Effect of GRF and somatostatin on 7B2 secretion by rat 641 cells. Endocr Jap 1989; **6**:787–793.

27 Hattori N, Schimatsu A, Sugita M, Kumagai S and Imura H. Immunoreactive growth hormone (GH) secretion by human lymphocytes: augmented release by exogenous GH. Biochem Biophys Res Commun 1990; **168**:396–401.

28 Hoflich M, Sherubl H, Rane F, Hoffman J, Ziegler R. Regulation of adenylate cyclase in rat C-cells. Proceedings of 34 Symp of German Soc of Endocrinol; March 14–17, 1990; Hannover. Acta Endocrinol (Copenh.) 1990; 122 Ab. 35.

29 Reubi JC. New specific radioligand for one subpopulation of brain somatostatin receptors. Life Sci 1985; **36**:1829–1836.

30 Nutt RF, Saperstein R and Veber DF. Structural and conformational studies regarding tryptophan in a cyclic hexapeptide somatostatin analogue. Proceedings of the Eight American Peptide Symposium. In: Hruby VJ and Rich D, editors. Pierce Chemical Co, Rockford IL, 1983. p. 345–348.

31 Baranowska B, Wasilewska-Dziubiñska E, Radzikowska M, Plonowski A, Roguski K, Lêgowska A, et al. The biological activity of new analogues of somatostatin. Europ J Endocrinol 1994; **130** suppl.2:26.

32 Schmechell DE, Viorey BG, Fritzpatrick D, Slade RP. GABA-ergic neurons of mammalian cerebral cortex: widespread subclass defined by somatostatin content. Neurosci Lett 1984; **47**:227–232.

33 Kakucska I, Tappaz ML, Gaal G, Stoeckel ME, Makara GB. GABA-ergic innervation of somatostatin-containing neurosecretory cells of the anterior periventricular hypothalamic area; a light and election microscopy double immunolabelling study. Neurosci 1988; **25**:585–593.

34 Dutta AS. Somatostatin analogues as opiate (μ-receptor) antagonists. In: Small Peptides, Chemistry, Biology and Clinical Studies. Timmerman H, editor. Amsterdam, London, New York, Tokyo: Elsevier; 1993. p.333–342.