Lipophilization of Somatostatin Analog RC-160 Improves Its Bioactivity and Stability

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Purpose. Acromegaly is a symptomatically disabling condition, resulting from a growth hormone (GH) secreting pituitary tumor. The somatostatin analog RC-160 is known to potently inhibit hypersecretion of GH, from pituitary adenomas. However, the therapeutic potential of RC-160, is limited by its short serum half life. To overcome this limitation, fatty acids with carbon chain lengths ranging from 4 to 18 were conjugated to RC-160.

Methods. The GH-inhibitory activity of these lipopeptides, as well as their binding profile to somatostatin receptors, on the rat pituitary adenoma cell line GH3 was studied *in vitro*. The relative stability of lipophilized RC-160 towards degradation by crude papaya protease was also determined.

Results. The long chain lipopeptides, like myristoyl-RC-160 (carbon chain length = 14) were found to exhibit greater receptor affinity and GH-inhibitory activity, as compared to their counterparts of lower chain lengths. However, the receptor affinity and GH-inhibitory activity of stearoyl-RC-160 (carbon chain length = 18), was found to lower than RC-160 and its lipophilized derivatives. Unlike RC-160, the myristoylated derivative was found to have significantly greater resistance to protease and serum degradation ($p < 0.01$).

Conclusions. Lipophilization of RC-160 with long chain fatty acids improves its stability and GH-inhibitory activity. The activity of lipophilized RC-160 seems to increase with increasing hydrophobicity of the lipopeptide, and reaches a maxima at myristoyl-RC-160 for GH3. Hence, optimizing the hydrophobicity should be an important consideration governing the design and synthesis of bioactive lipopeptides.

KEY WORDS: acromegaly; somatostatin; RC-160; lipophilization; GH-inhibitory activity; protease-resistance.

INTRODUCTION

Acromegaly is virtually always caused by a GH secreting pituitary tumor. It causes disfigurements of the face, hands and feet in adults, and in children it causes gigantism (1). Subcellular mechanisms support the notion of an intrinsic pituitary defect in this disease, with elevated growth hormone (GH) and insulin-like growth factor (IGF-I) levels that affect the cardiovascular and respiratory system as well as the proliferation of neoplastic cells. Surgery, even with external beam adjuvant therapy is successful in less than 60% of the patients, and is associated with side effects. However, an alternate effective and safe long-term medical treatment is essential for about 35% of the patients in whom the pituitary tumor cannot be removed by transsphenoidal surgery. The recent advent of peptidomergic therapy has revolutionized the approach to manage patients. The somatostatin analog, octreotide normalizes GH and IGF-I levels in upto 60% of the patients, and reduces tumor burden in 90% of the patients (2).

Somatostatin, a cyclic neuropeptide was originally characterized as a potent inhibitor of GH secretion. However, it also inhibits the secretion of multiple hormones like insulin, glucagon and TRH etc. (3). The anti-secretory activity of the somatostatin analog RC-160 (D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2) has been demonstrated in a number of experimental models of endocrine tumor cells *in vitro* and *in vivo* (1).

The anti-secretory effects of somatostatin are mediated through high affinity, G-protein coupled receptors on target cells (4), which belong to five distinct subtypes (SSTRx, x = 1–5). The somatostatin agonist RC-160 has been extensively investigated for its anti-secretory activity. Hofland *et al.* (1994) showed that, RC-160 was more potent than octreotide and BIM23014, in inhibiting GH secretion from cultured human and rat GH-secreting pituitary tumors *in vitro*. The biological half life of these somatostatin analogs (about 2 hours in serum) is greater than somatostatin, yet their use requires frequent administration as these peptides are rapidly degraded by proteolytic enzymes present in the cell membrane and blood (6). Furthermore, the hydrophilic nature of most peptide drugs severely limits their permeability across lipophilic biomembranes. The current strategy to circumvent this limitation has been to devise long-acting sustained release preparations by entrapping peptides within poly(DL-lactide-coglycollide) microspheres, or by using miniosmotic pumps to ensure sustained release of the therapeutic peptide (6).

Pharmacokinetic profiles of the long-acting sustained release formulations for both octreotide (Sandostatin LAR™) and lantreotide (Somatuline LP™) have been extensively studied (7–8). Both these formulations are effective, but they have certain drawbacks. Somatuline produces an initial "burst" in drug release whereas Sandostatin produces an initial "delay" in peptide release. The administration of these anti-secretory agents has also been limited by their pleotropic nature and side effects like inhibition of gall bladder emptying which leads to increased incidence of cholesterol gallstones, short-term inhibition and/or delay of insulin release in response to meals, decrease in glucose tolerance in some patients (9). The acute administration of somatostatin has found to produce receptor desensitization which results in diminished therapeutic response and induction of tolerance (9). Hence, there is a need for further modification of these somatostatin analogs to increase their therapeutic efficacy and stability.

Lipophilization of bioactive peptides, is an alternate strategy which can confer peptides with improved stability, bioavailability, absorbability as well as augments their ability to permeate biomembranes without loss of activity (10,11). Unlike sustained release formulations, lipophilization can also impart enhanced receptor selectivity to peptides. The attachment of fatty acids to peptides like VIP leads to the generation of highly potent and selective peptide analogs (10–12), which have provided novel tools in drug design for neurodegenerative diseases and in various forms of cancer.

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The objective of the present study was to evaluate the effect of lipophilization, on the GH-inhibitory activity of RC-160, and to determine whether such derivatization of RC-160 could confer better resistance to protease degradation, resulting in increased half life and enhanced ability to inhibit GH secretion from the rat pituitary adenoma cell line GH3 *in vitro*. The study also attempted to gain insight into effect of varying peptide hydrophobicity (indicated by the carbon chain length of the conjugated fatty acid) on the biological activity of lipophilized RC-160. This was accomplished by the attachment of fatty acids of carbon chain lengths ranging from 4 to 18, to RC-160. Thereafter, the GH-inhibitory activities of these lipopeptides were studied

MATERIALS AND METHODS

Chemicals

Rink amide resin and FMOC amino acids were purchased from Bachem California, U.S.A. All growth media, antibiotics were purchased from Gibco BRL, U.S.A. Foetal calf serum (FCS) was obtained from Biological Industries, Israel. The rat GH standards, as well as the specific and sensitive antisera against rat GH was a kind gift from National Institute of Digestive and Kidney diseases (NIDDK), NIH, U.S.A.

Synthesis, Purification and Characterization of Peptides

Peptide synthesis was carried out, according to the manual solid phase strategy, employing optimum side chain protection (13). The saturated fatty acid was coupled to the peptide while it was bound to the resin, using a combination of DIPCDI and HOBT, normally employed for extension of the peptide chain. The intramolecular disulfide bond was formed by dissolving 4 mg of the crude peptide in 3 ml DMSO (14), in a 12:1 v/v of acetic acid water buffer (pH = 6, adjusted by ammonium bicarbonate). The reaction mixture was stirred overnight. The oxidation reaction was monitored on an HPLC system, equipped with a semipreparative C_{18} reverse phase column (Nihon Waters, Japan.), using gradients established between 0.1% triflouroacetic acid (TFA) and acetonitrile containing 0.1% TFA (v/v). The parent peptide was characterized by N-terminal sequencing and mass spectrometry. The molecular weight of the lipophilized peptides and the parent peptide was ascertained by electron spray mass spectrometry.

Cell Culture

The rat pituitary adenoma cell line GH3 was obtained from National Center of Cell Science, Pune, India. It was maintained in MEM supplemented with 2 mM glutamine, 2.2 g/l sodium bicarbonate, 1 mM sodium pyruvate, 100 units/ml penicillin, 50 µg/ml streptomycin, 40 µg/ml gentamycin, 15% donor horse serum and 2.5% FCS, at 37°C in a humidified atmosphere containing 5% CO_2 in air.

Receptor Binding Assay

^{125}I-[Tyr^1]somatostatin-14 (DU PONT NEN, U.S.A.), of specific activity 2000 Ci/mmol was used to measure somatostatin-14 binding sites on whole cell preparations (15). Cells were seeded in 24 well plate culture plates (5×10^4 cells/well) for 36 hours. The cells were washed once in binding buffer consisting of RPMI-1640, 0.1% BSA 10 mM $MgCl_2$, 1 mM EGTA, 0.25 mM PMSF and 10 µg/ml aprotinin. Cells were incubated with 0.5 nM of ^{125}I-[Tyr^1]-somatostatin-14 in the presence or absence of various concentrations of RC-160 or lipopeptides, for 2 hours, at 4°C. The cells were subsequently washed 4 times with ice cold binding buffer and lysed using 0.3N NaOH. The radioactivity of the cellular lysate was measured using a gamma counter (LKB Wallac, Finland). All measurements were carried out in duplicate and repeated 3 times.

Growth Hormone Determination

GH3 cells cultured to 70% confluence were harvested using 0.05% trypsin-EDTA solution and replated in MEM supplemented with 15% donor horse serum and 2.5% FCS, in 6 well plates at a density of 10^5 cells/well. The plates were incubated for 36 hours at 37°C to allow complete reattachment of the cells. Thereafter, the medium was changed and the peptides were added in varying concentrations. The cells were incubated with the peptides for 72 hours. The peptides were added every 12 hours, during the 72 hour incubation. Serum free MEM containing 0.1% DMSO was added to the control wells. At the end of the incubation the medium was removed and centrifuged for 5 minutes at 600 X g and the supernatants were stored at −20°C until analysis.

The rat GH concentrations, in the culture supernatant was measured by RIA, which was a based on the procedure of Singh *et al.*, (1993). The rat GH standards, as well as the specific and sensitive antisera against rat GH were obtained from NIDDK, U.S.A. Radioiodination of rat GH was carried out by the Iodogen method (17). The antibody bound antigen was separated from the free antigen by precipitation by 25% PEG-8000 with 20% normal horse serum being used as the carrier. MEM containing 15% donor horse serum and 2.5% FCS was also used an additional control in the rGH RIA. All measurements were carried out in duplicate and repeated 3 times.

Solubility Studies

Myristoyl-RC-160 was dissolved in 50 mM phosphate buffered saline containing 10% DMSO, at a concentration of 500 µg/ml. This solution was added a microlitre after every minute to 1 ml of 50 mM phosphate buffered saline. A total of 10 additions were made. Precipitation was quantified due to increase in light scattering in a UV spectrophotometer (Shimadzu Corporation, Japan) at a wavelength of 700 nm (18). All measurements were carried out in duplicate and repeated 3 times.

Protease Sensitivity

The peptide was dissolved in buffer consisting of 0.1 M ammonium bicarbonate and I M EGTA (pH = 8.0), containing 1% DMSO. Crude papaya protease, was added to the peptide solution (ratio 1:50 w/w). The reaction mixture was incubated at 37°C, for 36 hours (19). The progress of the digestion was monitored, using a C_{18} reverse phase column, on an HPLC system. All measurements were repeated 3 times.

Statistical Analysis

All data are expressed as the mean ± SEM. Mean values between the treatment group and the control group were analyzed by ANOVA (Micro Cal Origin Ver. 2.0). Data were considered significant when the value of p was less than 0.05.

RESULTS

Synthesis, Purification and Characterization of Lipophilized RC-160

RC-160 and its lipophilized derivatives were synthesized by the manual solid phase peptide synthesis, using the FMOC strategy (13). The peptides were purified on an HPLC system using a reverse phase C_{18} column (14) and characterized by electron spray mass spectrometry. There was a good agreement between the predicted molecular weights and the molecular weight actually obtained (Table 1).

Binding of RC-160, and Lipophilized RC-160 on Somatostatin Receptors on GH3

Scatchard analysis indicated the presence of only one class of specific binding sites on GH3. The K_d and B_{max} (per 5×10^4 cells) values for the binding sites were found to be 69.5 nM and 4.41 nmoles respectively. The presence of specific somatostatin receptors on GH3 was confirmed by displacement assays, using excess of cold peptide. RC-160 as well as its lipophilized counterparts could effectively displace ^{125}I-[Tyr^1]-somatostatin-14, bound to cells, in a dose dependent manner (Fig. 1). The IC_{50} indicates the concentration of the peptide which causes a 50% inhibition of binding of ^{125}I-[Tyr^1]-somatostatin-14 to the above cell lines (Table 2A).

The receptor affinity of lipophilized RC-160 seems to increase with peptide hydrophobicity in GH3. The IC_{50} values for pamitoyl-RC-160 and myristoyl-RC-160 are significantly lower than RC-160 ($p < 0.001$). However, stearoyl-RC-160 has lower receptor affinity than both myristoyl-RC-160 and RC-160 itself ($p < 0.001$), in the cell line GH3. The receptor affinity of butanoyl RC-160 was higher than RC-160 ($p < 0.01$), but lower than stearoyl-RC-160 ($p < 0.001$).

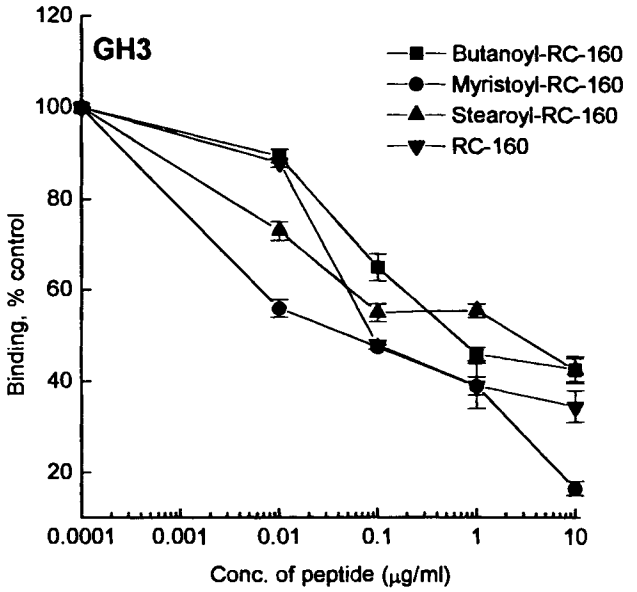


Fig. 1. Displacement of bound ^{125}I-[Tyr]^1-somatostatin from cells by RC-160 and its lipophilized derivatives, in the rat pituitary adenoma cell line GH3. Cells were incubated with 0.5 nM of ^{125}I-[Tyr]^1-somatostatin and the cold peptides at the concentrations detailed above, for 2 hours at 4°C. The excess radioactivity was washed off with binding buffer, subsequently the cells were lysed with 0.3N NaOH and cell associated radioactivity was read in a gamma counter.

Effect of RC-160 and Lipopeptides on GH Secretion from Cultured Rat GH-Secreting Pituitary Adenoma Cells

Figure 2A and B show the GH-inhibitory activity of lipophilized-RC-160, as compared to RC-160 in GH3. The concentration of peptide which causes a 50% inhibition of GH secretion (IC_{50}) is listed in Table 2B. The first observation was that the long chain lipopeptides are far more potent in inhibiting GH release as compared to the short chain lipopeptides. Myristoyl-RC-160 and pamitoyl-RC-160 were the most potent inhibitors of GH secretion from GH3 *in vitro* (Fig. 2A and B). Butanoyl-RC-160 and stearoyl-RC-160 were significantly less potent in inhibiting GH secretion, as compared to pamitoyl-RC-160 or myristoyl-RC-160 ($p < 0.01$).

The inhibition of GH secretion by RC-160 and the lipopeptides *in vitro*, seems to be mediated by the presence of specific

Table 1. Characterization of Lipophilized RC-160, of Differing Carbon Chain Lengths

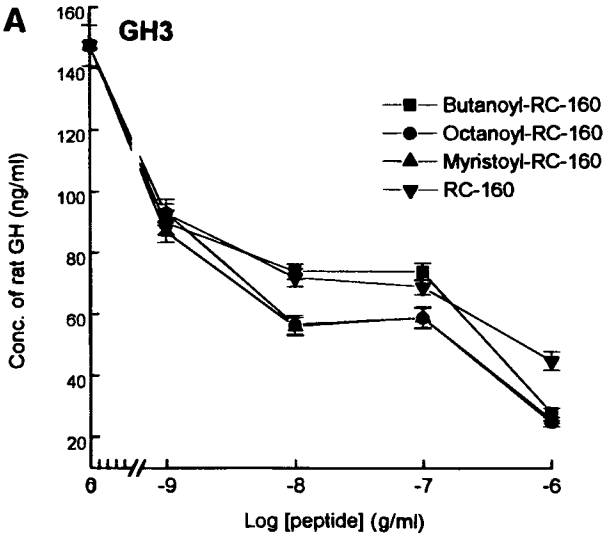
SNo.	Lipopeptides	Fatty acyl group	Calculated mass	Observed mass
1)	RC-160	NONE	1131.41	1130.40
2)	Butanoyl-RC-160	CH_3-$(CH_2)_2$-COOH	1201.51	1200.60
3)	Octanoyl-RC-160	CH_3-$(CH_2)_6$-COOH	1257.60	1256.73
4)	Myristoyl-RC-160	CH_3-$(CH_2)_{12}$-COOH	1341.76	1340.40
5)	Pamitoyl-RC-160	CH_3-$(CH_2)_{14}$-COOH	1369.82	1370.61
6)	Stearoyl-RC-160	CH_3-$(CH_2)_{16}$-COOH	1397.87	1396.81

Note: The peptides were purified by RP-HPLC. The parent peptide RC-160 was sequenced. The lipophilized peptides were characterized by ES-mass spectrometry.

Table 2A. Displacement of [^{125}I]-Tyr^1-Somatostatin Binding by Lipopeptides and RC-160, in the Rat Pituitary Adenoma Cell Line GH3

SNo.	Lipopeptides	IC_{50} (μg/ml) Cell line:GH3	Standard Error
1)	RC-160	0.089	±0.0055
2)	Butanoyl RC-160	0.62	±0.063
3)	Octanoyl RC-160	0.042	±0.0085
4)	Myristoyl-RC-160	0.048	±0.0035
5)	Pamitoyl-RC-160	0.061	±0.0052
6)	Stearoyl-RC-160	2.91	±0.28

Note: The cells were incubated with 0.5 nM of radiolabelled somatostatin, in the presence of the above peptides, for 2 hours at 4°C. The table above shows the IC_{50} values for the peptides.



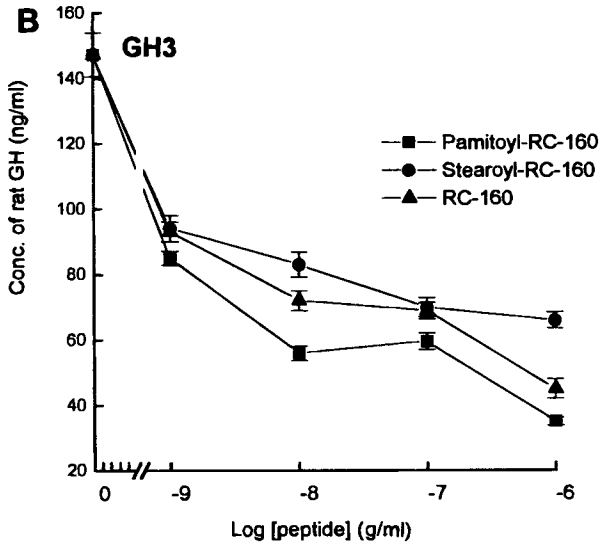


Fig. 2. Inhibition of GH secretion from GH3 cells by RC-160 and its lipophilized derivatives. Cells were plated in 6-well plates and incubated with varying concentrations of RC-160 or the lipopeptides, for 72 hours. The peptides were added twice daily during the 72 hour incubation. Subsequently, the culture supernatent was collected, centrifuged at 600 × g for 5 minutes. The concentration of rGH content in the culture supernatant was measured by RIA. The RIA was performed as detailed in "Materials and Methods."

somatostatin receptors on rat pituitary adenoma cells. Unlike pertussis toxin, treatment of cells with 100 μM GTPγS significantly abrogates the binding of ^{125}I-[Tyr^1]-somatostatin to its receptors expressed on GH3 cells ($p < 0.001$). This indicates that somatostatin receptors are coupled to pertussis toxin insensitive GTP binding regulatory proteins on GH3 cells (Fig. 3).

Solubility Properties of Lipopeptides

RC-160 was dissolved in 50 mM phosphate buffered saline. All the lipopeptides were dissolved in 50 mM phosphate buffered saline containing 10% DMSO. The solubility of myristoyl-RC-160 was measured by the procedure described by Lipinski *et al.*, (1997). The solubility of myristoyl-RC-160 at the precipitation point was found to be 5 μg/ml in aqueous buffer containing 0.01% DMSO.

Table 2B. Effect of RC-160 and Its Lipophilized Derivatives on the Inhibition of Rat GH, in the Rat Pituitary Adenoma Cell Line GH3 *In Vitro*

SNo.	Lipopeptides	IC_{50} (ng/ml) Cell line: GH3	Standard Error
1)	RC-160	7.03	±0.51
2)	Butanoyl RC-160	12.16	±0.58
3)	Octanoyl RC-160	3.68	±0.30
4)	Myristoyl-RC-160	2.55	±0.23
5)	Pamitoyl-RC-160	2.73	±0.25
6)	Stearoyl-RC-160	61.72	±2.58

Note: The cells were incubated with peptides for 72 hours, and the rat GH levels were measured in the culture supernatent by RIA, as detailed in "Materials and Methods." The table above shows the IC_{50} values for each of the peptides.

Protease Sensitivity of Lipopeptides Versus RC-160

Lipophilization of peptides is designed to protect the peptide against proteolytic degradation in serum. The stability of myristoyl-RC-160, as compared to RC-160 was assessed by evaluating their susceptibility towards crude papaya protease

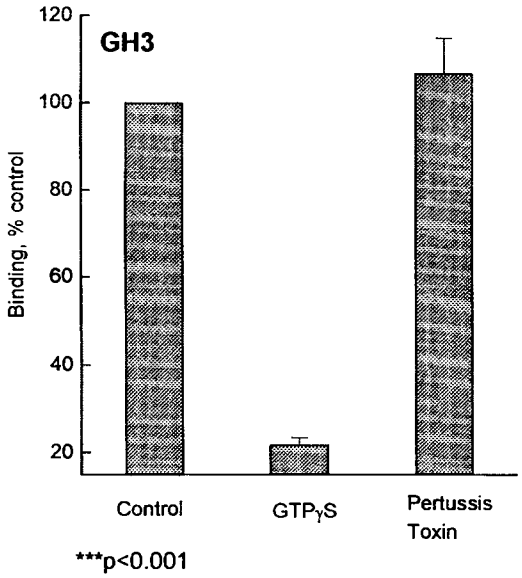


Fig. 3. Abrogation of the binding of ^{125}I-[Tyr]^1-somatostatin in the presence of 100 μM of GTPγS in the rat pituitary adenoma cell line GH3. However, pertussis toxin (100 ng/ml) does not abolish the binding of ^{125}I-[Tyr]^1-somatostatin to its receptors on GH3 cells. The cells were incubated with 0.5nM ^{125}I-[Tyr]^1-somatostatin in the presence of GTPγS for two hours at 4°C. The cells were incubated with 100ng/ml pertussis toxin for 18 hours prior to the receptor binding assay. The receptor binding assay was done as specified in "Materials and Methods." The excess radioactivity was washed with binding buffer, the cells were subsequently lysed and counted. ***$p < 0.001$.

(Fig. 4). Myristoyl-RC-160 exhibited greater stability to protease digestion, even after 24 hours, as compared to butanoyl-RC-160 and RC-160 itself.

DISCUSSION

Acromegaly is a chronic disease caused by the tumoral hypersecretion of GH. Although the disease progresses slowly, increased mortality in acromegalic patients resulting from cardiovascular and respiratory diseases, justify early and effective therapy for patients (1). The use of somatostatin analogs like octreotide has revolutionized the management of acromegalic patients, especially in whom surgery and radiotherapy have not proved curative (2).

The biological activity of somatostatin is mediated by membrane associated G-protein coupled receptors (SSTRx, x = 1–5) (4). *In vitro* and *in vivo* studies suggest a major role for SSTR2 and SSTR5 in the inhibition of GH secretion by somatostatin and its analogs, both in humans and rats (20–22). Moreover, previous studies have reported that SSTR2 mRNA appears to be the most prevalent subtype in human pituitary adenomas (21). The clinically available somatostatin analogs, like octreotide and RC-160, which are used to control GH in acromegalics, bind efficiently to SSTR2 but with lower affinity to SSTR5.

The role of SSTR2 in the negative control of endocrine and exocrine secretion is further strengthened by the presence of this subtype in various hypersecretory conditions like pituitary adenoma, prolactinomas, gastrinomas, carcinoid tumors, which respond *in vitro* and *in vivo* to the effect of somatostatin analogs (23). Hence, understanding the molecular interactions between RC-160 and SSTR2 is crucial, for designing analogs with better biological activity and stability profile than RC-160.

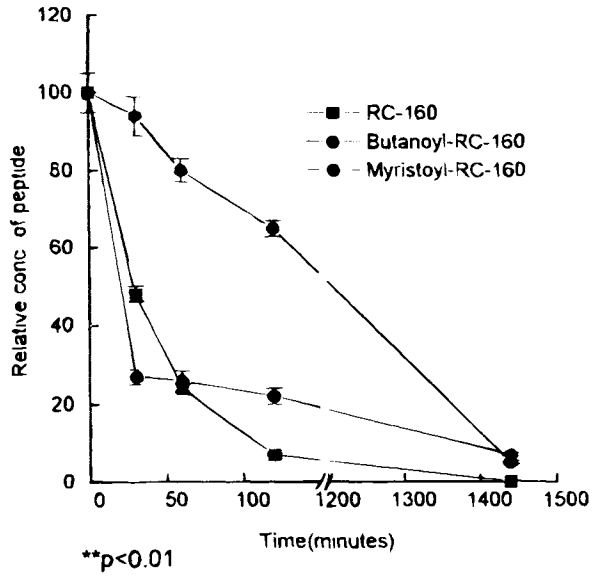


Fig. 4. Susceptibility of myristoyl-RC-160 and RC-160 towards crude papaya protease. The peptides were incubated with crude papaya protease, in digestion buffer pH=8 at 37°C. The digestion reaction was monitored at different time points, by injecting aliquots of the reaction mixture into an HPLC system, equipped with a C_{18} reverse phase column. **$p < 0.01$.

Molecular modeling has been done to elucidate the critical amino acid residues mediating the interaction of peptide analogs with SSTR2 (24,25). The ligand binding site of SSTR2 was demonstrated to be located in a pocket formed by hydrophobic regions. The putative binding pocket of SSTR2 is lined with hydrophobic residues which are involved in lipophilic interactions with the aromatic residues of octreotide. The interaction of the SSTR2 with somatostatin agonists appear to be primarily hydrophobic in character. Hence, increasing the hydrophobicity of RC-160 should increase the receptor affinity of RC-160, which should improve its biological activity, as well as confer it with improved stability and increased resistance towards proteolytic degradation in serum.

Lipophilization of RC-160 seems to enhance the binding of RC-160 to somatostatin receptors (indicated by the IC_{50} values) on GH3 cells. The receptor affinity of the lipopeptides seems to increase with increasing hydrophobicity and reaches a maximum at myristoyl-RC-160 (carbon chain length = 14) ($p < 0.001$). However, a further increase in peptide hydrophobicity seems to reduce the receptor affinity. Pamitoyl-RC-160 (carbon chain length = 16) has a slightly lower receptor affinity as compared to myristoyl-RC-160 ($p < 0.05$) whereas stearoyl-RC-160 (carbon chain length = 18) binds to somatostatin receptors on GH3 cells with the least affinity amongst the lipopeptides ($p < 0.001$). This leads to two very important conclusions, firstly increasing peptide hydrophobicity, increases the receptor affinity of RC-160 and secondly, this improvement in receptor affinity occurs only within a certain range of hydrophobicity, after which increasing the lipophilicity of the peptide does not correlate with an increase in receptor binding. An exception to this trend was the lower receptor affinity of butanoyl-RC-160. The coupling of RC-160 to butanoic acid possibly causes a perturbation in the conformation of RC-160, which reduces its receptor affinity.

The GH-inhibitory activity of lipophilized RC-160 seems to vary as a function of the hydrophobicity of lipophilized RC-160. The long chain lipopeptides, are far more potent in inhibiting GH release as compared to RC-160 and the short chain lipopeptides ($p < 0.001$). The anti-secretory activity of RC-160 seems to increase with increasing hydrophobicity of the lipopeptide. However, this improvement in bioactivity, seems to saturate around myristoyl-RC-160 and pamitoyl-RC-160. A further increase in hydrophobicity did not produce better inhibition of GH release. Stearoyl-RC-160 (carbon chain length = 18) was far less potent in inhibiting GH secretion, as compared to its pamitoylated or myristoylated counterpart ($p < 0.001$). The anti-secretory activity of RC-160 and the lipopeptides parallels their receptor affinity. Accordingly, butanoyl-RC-160 and stearoyl-RC-160 (whose receptor affinities are the lowest) were the least potent in inhibiting GH, whereas myristoyl-RC-160 seems to be most potent in inhibiting GH secretion *in vitro*.

The anti-secretory activity of RC-160 and its lipophilized derivatives is mediated by high affinity somatostatin receptors on pituitary adenoma cells. The addition of a non-hydrolyzable GTP analog GTPγS (100 μM), blocked the binding of ^{125}I-[Tyr^{1}]-somatostatin to its receptor whereas pertussis toxin (100 ng/ml) did not. Therefore, RC-160 and its lipophilized derivatives seem to interact with somatostatin receptors on GH3 cells via pertussis toxin insensitive GTP binding proteins.

The aqueous solubility/dissolution rate is one of the physicochemical properties that has been used to predict the membrane permeability and oral bioavailability of drug molecules (26). Numerous *in vitro* drug dissolution methodologies have been described in literature. These include the determination of the apparent partition coefficient of the drug between an organic solvent like octanol (or chloroform) and an aqueous buffer (27–28). However, comprehensive models for predicting oral absorption based on drug dissolution tests have been limited. This is due, in part to the complexity of processes occurring in the gastrointestinal tract and in part to the complex pharmacokinetics of drugs making it difficult to obtain accurate absorption estimates from systemic availability (26). Since, myristoyl-RC-160 was found to be insoluble in octanol and in chloroform the method of Lipinski *et al.*, (1997) was used to estimate the solubility of myristoyl-RC-160. This method has the advantage that the drug solubility is ascertained in a manner as close to possible to the actual solubilization procedure used in the biological assays detailed in "Materials and Methods". The solubility of myristoyl-RC-160 is determined by detecting the onset of precipitation, when the peptide solution (in PBS containing 10% DMSO) is added to the aqueous buffer. The absorption maxima of peptides is below 400nm, hence the increased UV absorbance from light scattering was measured between 600–800 nm.

The relative stability of these lipopeptides versus RC-160 was assessed by treating them with crude papaya protease. It was observed that RC-160 and butanoyl-RC-160 were degraded at a much faster rate than myristoyl-RC-160. A similar trend is observed regarding the serum half life of both these peptides (data not shown). RC-160 is degraded in normal mouse serum, within two hours, whereas myristoyl RC-160 is detectable in serum even after 24 hours. The myristoyl moiety of the lipopeptide, may be shielding the peptide against enzymatic degradation in serum. This may partially explain the improved activity of myristoyl-RC-160, as compared to RC-160 discussed above.

The improved anti-secretory activity of long chain lipopeptides like myristoyl-RC-160 can be primarily attributed to their improved ability to bind to the somatostatin receptor on pituitary adenoma cells, compared to RC-160. Furthermore, myristoyl-RC-160 does not get degraded by proteases in the serum, so is present in the cellular microenvironment for a greater length of time as compared to RC-160. The enhanced biological stability of myristoyl-RC-160, makes it possible for it to display improved GH-inhibitory activity at lower concentrations, compared to RC-160.

The above data seems to suggest that long chain lipopeptides like myristoyl-RC-160 may be of potential importance in the control of GH hypersecretion in acromegaly. Another important observation made in the above study, is that lipophization of RC-160 is a good strategy to enhance its biological activity, increase its receptor affinity and stability, thereby lowering its toxicity profile and improving its therapeutic index. However, this enhancement of biological activity occurs only within a certain narrow range of peptide hydrophobicity. Hence, optimizing the peptide hydrophobicity should be a key consideration in the rational design and synthesis of potent and selective lipopeptides.

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