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Is lGnRH-III the most potent GnRH analog containing only natural amino acids that specifically inhibits the growth of human breast cancer cells?

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Abstract: Analogs of the decapeptide, gonadotropin-releasing hormone (GnRH), used in the treatment of hormone-dependent tumors, contain numerous unnatural amino acids, giving rise to many adverse effects. lGnRH-III, a natural isoform of GnRH isolated from the sea lamprey, is a weak agonist of GnRH in the pituitary, but inhibits the growth of human cancer cells in micromolar concentrations. As lGnRH-III is not a natural ligand in humans, it is possible that a more potent peptide, also containing only natural amino acids, can be synthesized. A positional scanning peptide library, focused on the variable region of the GnRH family of peptides, residues 5–8, was synthesized. The synthesized peptides were analyzed in competitive binding experiments and six new analogs were designed on the basis of the results. Their biological activities were evaluated in cell growth experiments. The only natural sequence selected was chicken GnRH-II. The synthetic library did not yield a more potent peptide than lGnRH-III. Copyright © 2006 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: lGnRH-III; peptide library; breast cancer; inhibition of growth

INTRODUCTION

The decapeptide, gonadotropin-releasing hormone (GnRH, pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) plays a major role in the regulation of reproductive competence. Released from the hypothalamus in a pulsatory manner, it is responsible for the regulation of FSH and LH synthesis and, thus, concurrently regulates sex-steroid synthesis. Several analogs of GnRH are used in the treatment of hormone-related disorders [1–3] as well as sex-steroid-dependent tumors [4–8]. The mechanism of action of these analogs is indirect, suppressing sex-steroid production by acting on the type-I GnRH receptors (GnRHRs) in the pituitary. Most of the 3000 synthetic peptide analogs of GnRH contain unnatural amino acids, which enhance the biological activity and half-life of the peptides [5]. These modifications, however, have been associated with numerous adverse effects, such as anaphylactic reactions in the case of antagonists containing D-Arg or related basic residues at position 8 [9]. Unnatural amino acids can also accumulate in the liver and other organs.

lGnRH-III (pGlu-His-Trp-Ser-His-Asp-Trp-Lys-Pro-Gly-NH_2), the third isoform from the sea lamprey [10], was shown to directly and specifically inhibit the growth of cancer cells expressing GnRHRs in micromolar concentrations, as this peptide acts only as a weak agonist in the pituitary and does not interfere with FSH and LH release [11,12]. In an attempt to increase the half-life of lGnRH-III in the circulation, the peptide was conjugated to a biodegradable polymer through the side chain of Lys^8 [13,14]. This resulted in an increased half-life of the peptide as well as an increased antiproliferative effect on human cancer cells. This approach could be used with other natural peptides as well, as the polymer was not found to be toxic for humans.

So far, 14 known isoforms in the GnRH family of peptides have been isolated from vertebrates [15,16]. Most of these differ only in their central region, from residues 5 to 8. This region determines the species-specificity of each isoform. It has been shown that isoforms having bioactive conformations, other than β-turn, bind to the human type-I GnRHR with low affinities [17]. However, the requirements for binding to type-I GnRHRs in the pituitary and on human cancer cells might be different, as several analogs acting as antagonists at the pituitary site act as agonists at receptors in the periphery [18–20].

As lGnRH-III is not a natural ligand of mammalian GnRHR but is biologically active, it is possible that a more potent analog, containing only L-amino acids, could be developed. It has not yet been attempted to synthesize and screen a library consisting of all the possible analogs of the GnRH family containing only natural amino acids. Because the variable region of this family of peptides is residues 5–8, a positional scanning library focusing on this region was designed (Figure 1). This study provides an insight for the requirements to binding and activation of GnRHRs on human cancer cells.

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pGlu-His-Trp-Ser-**Ooo-Xxx$_a$-Xxx$_b$-Xxx$_c$**-Pro-Gly-NH_2

pGlu-His-Trp-Ser-**Xxx$_a$-Ooo-Xxx$_b$-Xxx$_c$**-Pro-Gly-NH_2

pGlu-His-Trp-Ser-**Xxx$_a$-Xxx$_b$-Ooo-Xxx$_c$**-Pro-Gly-NH_2

pGlu-His-Trp-Ser-**Xxx$_a$-Xxx$_b$-Xxx$_c$-Ooo**-Pro-Gly-NH_2

Figure 1 GnRH positional scanning library. **Ooo** is the variable position, while **Xxx$_a$**, **Xxx$_b$**, and **Xxx$_c$** are the mix positions.

MATERIALS AND METHODS

Materials

HOBt, HBTU, DIEA, Piperidine, DMSO, DMF, and THF were from Applied Biosystems (Foster City, CA). Fmoc-Ala-OH, Fmoc-Asp(But)-OH, Fmoc-Gly-OH, Fmoc-His(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Pro-OH, pGlu-OH, Fmoc-Ser(But)-OH, Fmoc-Trp-OH, and Rink amide AM resin were from Novabiochem (San Diego, CA). Diethyl ether, acetic acid, hydrochloric acid, ammonium hydroxide, acetonitrile, methanol, Tris-HCl, $MgCl_2$, and sucrose were from Fisher Scientific (Rockford, IL). Cell culture grade DMSO, EGTA, EDTA, thioanisole, EDT, $NaHCO_3$, and NaCl were from SIGMA Aldrich (Milwaukee, WI).

Library Design

In the four positional scanning libraries (Figure 1) **Ooo** is the variable region with 19 natural L-α-amino acids excluding Cys. **Xxx$_{a-c}$** are equimolar mixtures of the same 19 natural L-α-amino acids. Thus, the position 5 library, for example, consists of 19 different decapeptide mixtures and each mixture contains 6859 ($19 \times 19 \times 19$) separate molecules.

Synthesis and Cleavage of Positional Scanning Library

Rink amide MBHA resin (substitution: 0.45 mmol/g) containing 0.323 mmol Fmoc groups was swollen in DMF for 60 min. The *N*-α-Fmoc protecting groups were removed by treatment with 20% piperidine in DMF for 10 min twice. The resin was washed with DMF seven times. Four molar excess of the amino acids was mixed with 4 eq HATU (Anaspec, San Jose, CA) in 20% DMSO in NMP and added to the resin along with DIEA (8 eq). The mixture was shaken for 60 min. After each coupling step, the resin was washed seven times with DMF. Residues of the mix positions were coupled as an equimolar mixture of the 19 natural L-α-amino acids, excluding Cys, following the same coupling method. Then the peptide-resin was divided into 19 equal portions and placed into separate reaction vessels on the LabMate synthesizer (Advanced Chemtech, Louisville, KY). The amino acids of the variable position were coupled in these vessels using the same method, except that the scale of the synthesis was 0.017 mmol. The synthesis was completed by coupling the remaining fixed residues.

The cleavage procedure was also performed in the LabMate synthesizer. The resins containing each sublibrary were treated with the cleavage-cocktail TFA : thioanisole : water : EDT (90 : 5 : 2.5 : 2.5, v/v/v/v) for 2 h while shaking. Then the released peptides were precipitated with cold diethyl ether and washed twice with diethyl ether. The precipitate was dissolved in 80% (v/v) ACN in 50 mM ammonium-acetate (pH 4.5) and collected into separate glass vials. The solutions were lyophilized at least twice to evaporate scavengers.

Synthesis of cGnRH-II and Selected Sequences

All peptides were synthesized in a 0.025 mmol scale by solid-phase peptide synthesis on the Synergy peptide synthesizer (Applied Biosystems, Foster City, CA) using Rink amide AM resin from Applied Biosystems and *N*-α-Fmoc protected amino acids. The following side chain protecting groups were used: Ser, *t*Bu; His, Trt; Glu, *t*Bu, Tyr, *t*Bu and Lys, Boc. The amino acids were coupled with a 1 : 1 molar mixture of HBTU/HOBt, following the protocol provided by Applied Biosystems.

The peptides were cleaved from the resin and the side chain protecting groups were removed with TFA : thioanisole : EDT : water (90 : 5 : 2.5 : 2.5, v/v/v/v) while the resin was stirred for 15 min at 0 °C and for 2 h at room temperature. Then the released peptides were precipitated with ice-cold diethyl ether, collected by filtration, separated from the resin by dissolving in TFA, precipitated again in diethyl ether, dissolved in 10% acetic acid, and freeze-dried.

All peptides were purified by RP-HPLC on a semipreparative C18 Luna column (Phenomenex, Torrance, CA) with a flow rate of 4 ml/min. Eluent A was 50 mM NH_4OAc (pH 4.5) and eluent B was 60% (v/v) ACN in 50 mM NH_4OAc. The purity of the peptides were >98%, as proven by analytical RP-HPLC on a C18 column. The peptides were characterized using ESI-MS on a Perkin-Elmer quadrupole mass spectrometer.

Cell Lines and Culturing

MDA-MB 231 breast adenocarcinoma cells (ATCC, Manassas, VA) were maintained in DMEM (Atlanta Biologicals Inc., Lawrenceville, GA) supplied with 10% FCS (Atlanta Biologicals Inc.) in a humidified atmosphere at 37 °C in the presence of 5% CO_2. All experiments were done using cells with passage numbers that were not more than 10 higher than the ATCC passage number.

Radioligand Binding Experiments

The protocol for the binding experiments was based on that of Segal-Abramson and associates [21]. 10^{-7} M of the synthesized peptides was incubated in the presence of 25 pM ^{125}I-GnRH (Peninsula Laboratories Inc., San Carlos, CA) with 2×10^5 attached cells in binding buffer (40 mM Tris-HCl, 0.25 M sucrose, mammalian protease inhibitor cocktail from SIGMA (P8340), pH 7.4) for 1 h at room temperature. The cells were washed with cold D-PBS and lyzed in 0.4 M NaOH and the radioactivity was counted in a Beckmann gamma counter. Total binding was measured in the absence of cold ligand, while nonspecific binding was determined in the presence of 10^{-5} M cold lGnRH-III. Each experiment was repeated three times, with analysis in duplicate.

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The binding curve for cGnRH-II was obtained following the above protocol, using a series of cGnRH-II concentrations from 10^{-5} M to 10^{-11} M.

Residue Selection

The data from the competition binding experiments were analyzed using one-way ANOVA followed by Dunnett's posttest to choose the amino acids at the variable positions, which caused significant decrease in radioactivity, indicating competitive displacement of the radioligand. The radioactivity of the selected samples also had to be lower than that of the nonspecific tube containing 10^{-5} M lGnRH-III.

(^{3}H)Thymidine Incorporation

10^4 cells were seeded in 48-well plates (Nalge NUNC USA, Rochester, NY) avoiding the outer wells. After the cells had reached 60–70% confluency, they were treated with 10^{-4}–10^{-9} M analogs for three days in a serum-free medium. Both the peptides and the medium were changed daily. On the fourth day, 0.2 μCi [methyl-^{3}H]thymidine (Amersham Biosciences UK Limited, Buckinghamshire, England) was added to each well. Following incubation for 6 h, the medium was discarded and the attached cells were washed with D-PBS twice and then with 10% TCA for 30 min at 4 °C. The TCA was removed and the cells were washed with D-PBS and solubilized in 0.4 M NaOH. The solution was mixed with scintillation cocktail (Optiphase Polysafe, Perkin-Elmer, Wellesley, MA) and the radioactivity was counted in a Beckmann scintillation counter.

RESULTS

Synthesis of Peptide Libraries

The yields from the syntheses are shown in Table 1. Theoretical yield was calculated by multiplying the average molecular weight (MW) of a library (the average of the lowest and highest MWs in the library) by 0.017, which was the scale of the synthesis. This estimate was used for the ease of calculation, as average MW of the lightest (Gly) and heaviest (Trp) amino acids is 121.61, while that of all 19 natural L-α-amino acids is 119.71.

The libraries were analyzed using ESI-MS (Figure 2). A random position was analyzed from each synthesis to ensure that the peaks are in the desired range of mass.

When synthesizing library 7, some resin was lost, resulting in low overall yields (Table 1).

During the cleavage of the position 5 library, no precipitate was formed after the addition of diethyl ether, probably due to the incomplete drying of the peptide-resin. This resulted in low overall yields. Still, enough of each peptide preparation was obtained for the binding studies.

Analysis of Peptide Libraries Using Radioligand Binding

Figure 3 shows the results of the radioligand binding experiments. In the position 5 library, the peptides with

Table 1 Yields from positional scanning libraries of the variable region of lGnRH-III

Amino acid	Theor. yield[a]/mg	Variable position (library)			
		5	6	7	8
		yield/mg (%)			
Ala	19.19	3.0 (15.63)	10.8 (56.28)	5.8 (30.22)	11.3 (58.88)
Arg	20.65	6.3 (30.51)	10.9 (52.78)	9.9 (47.94)	15.2 (73.61)
Asn	19.93	2.3 (11.54)	11.0 (55.19)	5.9 (29.6)	12.5 (62.72)
Asp	19.95	3.8 (19.05)	9.9 (49.62)	6.0 (30.08)	9.7 (48.62)
Gln	20.17	4.3 (21.32)	9.2 (45.61)	5.7 (28.26)	10.6 (52.55)
Glu	20.19	2.6 (12.88)	10.6 (52.5)	6.0 (29.72)	14.6 (72.31)
Gly	18.96	1.4 (7.38)	13.2 (69.62)	3.8 (20.04)	10.4 (54.85)
His	20.32	1.1 (5.41)	12.5 (61.52)	5.2 (25.59)	14.4 (70.87)
Ile	19.91	1.1 (5.52)	11.3 (56.76)	4.6 (23.10)	7.5 (37.67)
Leu	19.91	4.6 (23.10)	14.9 (74.84)	6.1 (30.64)	12.8 (64.29)
Lys	20.17	5.5 (27.27)	14.0 (69.41)	6.8 (33.71)	12.9 (63.96)
Met	20.22	4.7 (23.24)	10.1 (49.95)	5.5 (27.20)	9.1 (45.00)
Phe	20.49	1.5 (7.32)	13.6 (66.37)	4.8 (23.43)	10.0 (48.80)
Pro	19.68	1.3 (6.61)	9.6 (48.78)	6.4 (32.52)	12.3 (62.50)
Ser	19.47	1.8 (9.24)	13.7 (70.36)	3.7 (19.00)	14.4 (73.96)
Thr	19.71	2.7 (13.70)	10.6 (53.78)	5.2 (26.38)	14.6 (74.07)
Trp	21.15	1.0 (4.73)	7.2 (34.04)	4.5 (21.28)	8.8 (41.61)
Tyr	20.76	4.5 (21.68)	15.0 (72.25)	4.7 (22.64)	10.1 (48.65)
Val	19.68	3.3 (16.77)	13.9 (70.63)	6.1 (31.00)	5.5 (27.95)

[a] Theoretical yield was calculated as indicated in the text.

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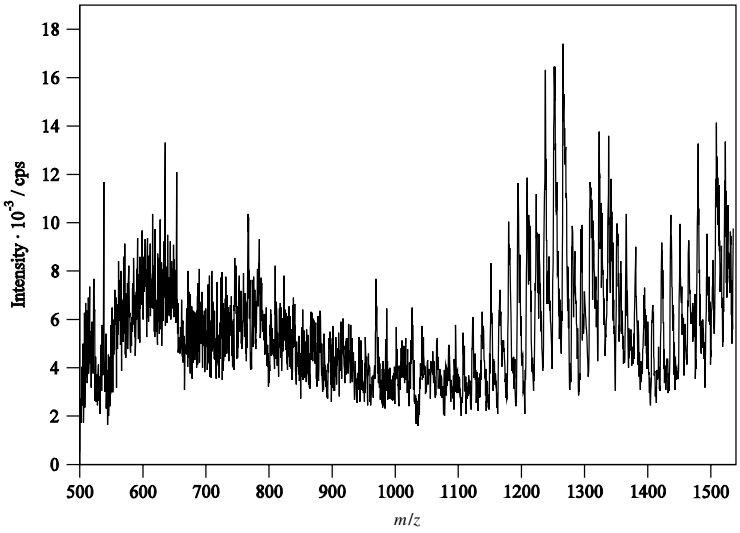

Figure 2 Mass spectrum of peptide library 8 with met as the variable position.

Tyr, His, and Phe substitutions competed with ^{125}I-GnRH; the Tyr substitution resulted in the greatest (42.7%) displacement.

In the position 6 library, only Gly and Trp substitutions resulted in significant displacement of the radioligand; peptides with Trp substitution caused 44.75% displacement (Figure 3).

In the position 7 library, only Met and Trp substitutions resulted in significant displacement; peptides with Met substitution caused 42.5% displacement (Figure 3).

In the position 8 library, eight amino acid replacements, Ala, Gln, Glu, Gly, Lys, Met, Ser, and Tyr, resulted in competitive displacement of the radioligand; greatest displacements were caused by peptides with Met (43.65%) and Glu (43.48%) substitutions (Figure 3).

Primary Structures of Most Effective Peptides

By screening the synthetic library, only one natural amino acid sequence corresponding to cGnRH-II (pGlu-His-Trp-Ser-His-Gly-Trp-Tyr-Pro-Gly-NH_2) was selected. This peptide recognizes two distinct binding sites with $IC_{50,1} = 75.19 \pm 8.05$ μM and $IC_{50,2} = 33.00 \pm 4.44$ nM, respectively, on MDA-MB 231 cells (Figure 4).

The combination of the amino acids producing the greatest displacement are included in the peptides

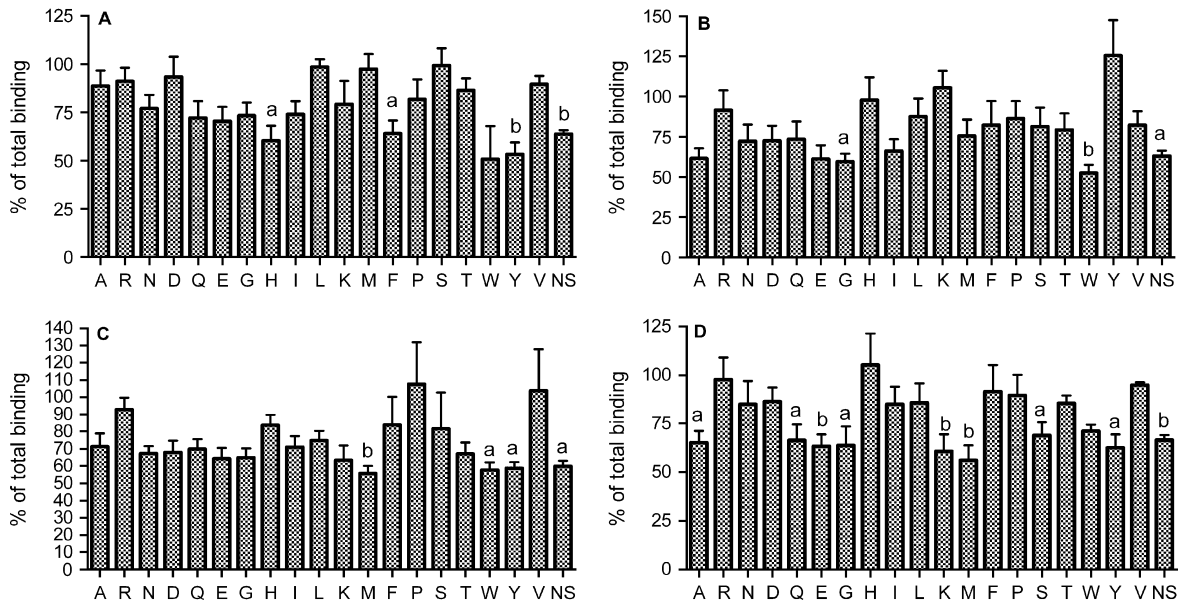

Figure 3 Competitive displacement of ^{125}I-GnRH by 10^{-7} M sublibraries at each variable position. (A) position 5, (B) position 6, (C) position 7, (D) position 8. The X-axes show one-letter codes for amino acids. NS stands for nonspecific binding, measured in the presence of 10^{-5} M lGnRH-III. a, $p < 0.05$; b, $p < 0.01$.

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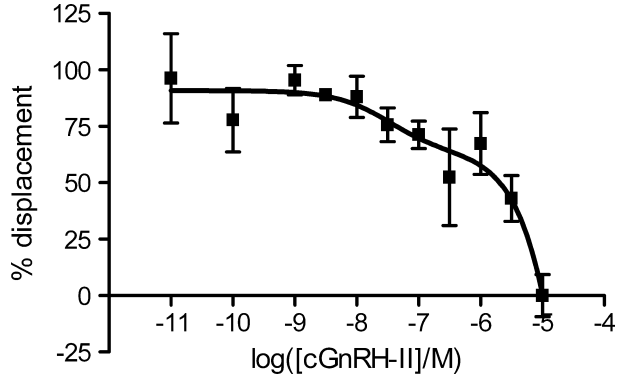

Figure 4 Competitive displacement of ^{125}I-GnRH by cGnRH-II from MDA-MB 231 cells. $IC_{50,1} = 75.19 \pm 8.05$ mM and $IC_{50,2} = 33.00 \pm 4.44$ nM, respectively.

Table 2 Primary structures of peptides selected from libraries

Analogs	Primary structure
1	pGlu-His-Trp-Ser-Tyr-Trp-Met-Met-Pro-Gly-NH_2
2	pGlu-His-Trp-Ser-Tyr-Trp-Met-Glu-Pro-Gly-NH_2
3	pGlu-His-Trp-Ser-Tyr-Trp-Met-Lys-Pro-Gly-NH_2
4	pGlu-His-Trp-Ser-Tyr-Trp-Trp-Met-Pro-Gly-NH_2
5	pGlu-His-Trp-Ser-Tyr-Trp-Trp-Glu-Pro-Gly-NH_2
6	pGlu-His-Trp-Ser-Tyr-Trp-Trp-Lys-Pro-Gly-NH_2

listed in Table 2. The best replacement candidates, at positions 5 and 6, were Tyr and Trp, respectively. Met and Trp substitutions at position 7 resulted in the best displacement. Met and Glu substitutions at position 8 had the greatest effect. Lys was also selected for this position.

Biological Activity of Selected Analogs

None of the selected peptides inhibited the growth of MDA-MB 231 cells significantly in a dose-dependent manner.

DISCUSSION

This study was aimed at identifying more potent peptides, containing only L-amino acids, than lGnRH-III. Therefore, the upper limit for binding affinity was 0.1 μM; consequently, the lower affinity analogs, including lGnRH-III, were not selected during screening.

Three of the four residues, which occur naturally at position 5, were selected by screening the synthetic library (Table 3). Leu may not have been selected because of the poor solubility of Leu-containing sublibraries, even at low concentrations.

Position 5 seems to be a conserved structure since no amino acid substitutions other than those found in natural sequences resulted in peptides that bound to receptors. Even though Ala-scan studies of lGnRH-III showed that His^5 can be substituted with Ala without the loss of binding affinity [22,] Ala substitution hardly produced any displacement. A possible explanation is that the peptides were present in 0.1 μM concentrations for screening the libraries, while the whole cell experiments showed that the affinity of [Ala^5]lGnRH-III for the receptor is 1.3 μM [22].

Table 3 Amino acids found in natural sequences of GnRH family of peptides and selected from the synthetic library

Amino acid position in GnRH							
5		6		7		8	
natural	library	natural	library	natural	library	natural	library
Tyr (7)	*Tyr*	*Gly (12)*	*Gly*	Leu (8)	Met	*Ser (3)*	*Ser*
His (5)	*His*	Glu (1)	Trp	*Trp (5)*	*Trp*	*Lys (2)*	*Lys*
Phe (1)	*Phe*	Asp (1)		Val (1)		Asn (2)	Ala
Leu (1)						Leu (2)	Gly
						Arg (2)	Glu
						Trp (1)	Met
						Gln (1)	*Gln*
						Tyr (1)	*Tyr*

The number in parentheses next to the natural amino acid indicates natural abundance among the isolated peptides. Amino acids in italics are found in natural sequences and were the best candidates in the libraries.

The most abundant residue at position 6 is Gly [23]. Otherwise, only acidic residues occupy this position in the peptide for the ancient lamprey, indicating that this mutation is highly conserved. Though the Trp is the most effective substitution at position 6, the residue is not found at this position in any of the isoforms. Asp was not effective, even though this residue is found at position 6 in lGnRH-III. As mentioned previously, the low concentration of individual peptides in sublibraries might prevent the detection of all peptides with micromolar affinity for the receptor. Glu substitution resulted in displacement, which was not significant.

In the natural isoforms, either Leu or Val or Trp occupies position 7. Only the natural amino acid chosen from the library screen, Trp, is found in lGnRH-III at position 7 (Table 3). Trp is also found at this position in cGnRH-II and salmon-GnRH. Neither Leu nor Val substitution resulted in any displacement of the radioligand, again possibly due to the poor solubility of these sublibraries. On the other hand, substitution with Met resulted in similar displacement to that by Trp.

The largest variety of amino acids in the natural GnRH peptides occurs at position 8 (Table 3). Eight different amino acids occupy this position in the variants from 14 species so far. Thus, the residue at this position seems to be the least conserved of

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those in the whole peptide. This is supported by the Ala-scan experiments, in which modification was tolerable only at position 8 [22]. Ala is also one of the substitutions that resulted in significant replacement of the radioligand in the receptor binding experiments (Table 3), which supports the Ala-scan results. In the natural isoforms, Ser is the most abundant residue at position 8; it is found in three fish species. Ser was also chosen by the library screening, though it did not cause the largest displacement. Lys at position 8 occurs exclusively in the lamprey (I and III) and was expected to cause significant displacement during analysis of the synthetic libraries. The other two naturally occurring amino acid residues that resulted in effective variants were Gln and Tyr. Gln is in cGnRH-I, while Tyr is in the highly conserved cGnRH-II.

The amino acid substitutions that resulted in the largest displacements at position 8 were Met and Glu, neither of which are found in natural GnRH isoforms.

The ionic interaction of Arg^8 of mammalian GnRH-I with $Asp^{7.32(302)}$ of type-I GnRHR is responsible for the high affinity of the peptide for the receptor [24]. In the binding experiments, however, Arg substitution did not result in any displacement, indicating that the requirements for binding to GnRH receptors on human cancer cells might be different from those for receptors in the pituitary. Furthermore, when a biodegradable polymer was attached to lGnRH-III through Lys^8, the half-life and biological activity of the peptide improved significantly [13,14].

Only one natural peptide, cGnRH-II, was chosen from the library screening. This peptide was shown to bind type-I GnRHRs with micromolar affinity [25]. Thus, it is possible that the receptor on MDA-MB 231 cells, mediating the effects of lGnRH-III, is a type-II rather than a type-I GnRHR. Type-II GnRHR is selective for cGnRH-II, to which it binds with high affinity. Competitive radioligand binding experiments indicate that cGnRH-II recognizes distinct high and low affinity binding sites on MDA-MB 231 cells (Figure 4). It was shown previously that cGnRH-II has high affinity for type-II GnRHRs [25,26].

It is also possible, though highly unlikely, that the binding requirements for type-I GnRHRs in the periphery are significantly different from those at the pituitary site. The most important residue in GnRH for high affinity binding to type-I GnRHR is Arg^8 and this substitution in the present study resulted in poor displacement. This is supported by the observations that antagonists of the pituitary type-I GnRHRs, including Cetrorelix, act as agonists on GnRHRs found on tumor cells [27,28]. This question seems to be solved by the evidence that the antiproliferative effect of Cetrorelix on ovarian and endometrial cancer cells is mediated through type-II GnRHRs [29]. The secondary messenger pathway involved was not investigated, though previously it had been shown that analogs of GnRH activate the G_i pathway in ovarian cancer cells [29,30].

Contradicting the previous findings, a report from Maiti *et al.* showed that both GnRH and cGnRH-II increase IC $[Ca^{2+}]$ in prostate cancer cells by activating the ryanodine receptor but not the inositol trisphosphate receptor [31]. Neither cGnRH-II nor Cetrorelix, however, was able to inhibit the growth of prostate cancer cells. Only a new cGnRH-II antagonist inhibited the growth of these cells in micromolar concentrations [31]. The investigation of the role of cGnRH-II and its putative receptor in cancer cell growth started in 2002 [32]. The paucity of results and many contradictions make the characterization of this system difficult.

On the basis of the competing binding results, six peptides with primary structures different from those of the natural isoforms were selected (Table 2). As they produced large displacements at 0.1 μM concentration, it was expected that they would be more potent than lGnRH-III itself. None of the peptides, however, was able to inhibit the growth of MDA-MB 231 cells in a dose-dependent manner after three days of treatment.

One of the disadvantages of the positional scanning library over the iterative approach is the difference between the selection of the most potent analogs. In case of the positional scanning library, at the end of the screening the best amino acid substitutions are combined, resulting in new sequences. In case of the iterative approach, the library is tested at each position and the best amino acid substitutions are kept as fixed residues. This method is more time-consuming but should give a more reliable result.

Of the peptides synthesized and tested in this study, lGnRH-III was the most potent. Only one peptide, cGnRH-II, bound to MDA-MB 231 cells with high affinity.

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