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The Computer Modelling of Human TRH Receptor, TRH and TRH-like Peptides

R. BÍLEK, L. STÁRKA

Institute of Endocrinology, Prague, Czech Republic

This article is dedicated to Prof. MUDr. Vratislav Schreiber, DrSc. on the occasion of his 80th birthday

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Summary

The aim of this work was to verify the possibility of interactions between the human TRH receptor (an integral membrane protein which belongs to family 1 of G-protein coupled receptors) and TRH-like peptides presented in the prostate gland. These peptides are characterized by substitution of basic amino acid histidine (related to authentic TRH) for neutral or acidic amino acid, such as glutamic acid, phenylalanine, glutamine or tyrosine. The physiological function of TRH-like peptides in peripheral tissues is not precisely known. However, according to our recent experiments, we assume the existence of a local hormonal network formed by TRH-like peptides and TSH in the prostate gland. The network can be associated with circulating thyroid and steroid hormones, and may represent a new regulatory mechanism influencing the proliferative ability of prostatic tissue. A similar network of authentic TRH and TSH was already found in the gastrointestinal tract. The experimentally determined 3D-structures of human TRH receptor (hTRHr) and TRH-like peptides are not available. From this point of view we used *de novo* modeling procedures of G-protein coupled receptors on an automated protein modeling server used at the Glaxo Wellcome Experimental Research (Geneva, Switzerland). 3D-structures of TRH-like peptides were determined with a computer program CORINA (written by the team of J. Gasteiger, Computer-Chemie-Centrum and Institute for Organic Chemistry, University of Erlangen-Nurenberg, Germany). The generated PDB files with 3D-coordinates were visualized with Swiss-Pdb Viewer Release 3.51 (Glaxo Wellcome). From recent results it is evident that polar amino acids belonging to the extracellular terminus of hTRHr transmembrane regions can participate in interactions between TRH and hTRHr. There is no direct evidence that TRH-like peptides interact with the presented hTRHr model. On the contrary, with respect to the similar 3D-shape and the identity of terminal amino acids, it appears that these interactions are highly probable as well as the nearly 100 % cross-reactions between TRH or TRH-like peptides and antibody specific against authentic TRH. Closed terminal amino acids (pyroglutamic acid and proline-amide) of TRH or TRH-like peptides are important for these interactions. Desamido-TRH or glutamyl metabolites will be repelled by the negative potential of ASP195 (E: D93) and GLU298 (G: E137).

Key words

Throtropin-releasing hormone receptor • TRH • TRH-like peptides • Computer modelling

Fax +420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

Introduction

The hypothalamic thyrotropin-releasing hormone (TRH) was the first chemically defined hypophyseotropic hormone (Reichlin 1992). Its precursor, human prepro-TRH, consists of 242 amino acid residues, and contains six separate copies of the TRH progenitor sequence (Satoh and Mori 1994), which determine the primary structure of TRH as a tripeptide pyroglutamyl-histidinyl-proline amide. The transcriptional unit of prepro-TRH is localized on chromosome 3 in humans (three exons interrupted by two introns) (Yamada *et al.* 1990). The presence of TRH-immunoreactive peptides was reported not only in the hypothalamus, but also in peripheral tissues including the prostate gland, retina, placenta, gastrointestinal tract, neural tissues, male reproductive system and certain endocrine tissues (Bílek 2000). It can be supposed that TRH immunoreactivity can partially originate from TRH-homologous peptides, and that these peptides have significant cross-reactions with an antibody specific against authentic TRH. Up to now, four TRH-like peptides were identified and characterized by having their central amino acid histidine (related to authentic TRH) exchanged for glutamic acid (Cockle *et al.* 1989), phenylalanine (Khan *et al.* 1992, Gkonos *et al.* 1994), glutamine (Khan *et al.* 1992), or tyrosine (Lackey 1992). It emerges from these papers that the authentic TRH and extrahypothalamic TRH-like peptides have primary structures pyroGLU-HIS-PRO.NH_2 (TRH), pyroGLU-GLU-PRO.NH_2 (pEEPam), pyroGLU-PHE-PRO.NH_2 (pEFPam), pyroGLU-GLN-PRO.NH_2 (pEQPam), and pyroGLU-TYR-PRO.NH_2 (pEYPam), respectively. The actual research is targeted mainly on pEEPam and pEFPam, which may be most important members of TRH-like peptides.

TRH-like peptides cannot be expressed from the TRH gene and, up to now, it is not known which gene is responsible for their expression. The physiological role of TRH-like peptides is also not precisely known. Our assumption, which is based on our recent results concerning experiments with the prostate gland, supposes that there might be an important connection of TRH-like peptides to the prostatic local autocrine/paracrine network mediated by extrahypothalamic TRH immunoreactivity corresponding to TRH-like peptides, and extrapituitary thyrotropin (TSH) immunoreactivity also found in the prostatic tissue. A similar system of intraepithelial lymphocyte hormonal regulation due to the local paracrine network of TRH/TSH has been described in the gastrointestinal tract (Shanahan 1997, Wang *et al.* 1997), where the extrahypothalamic TRH reacts with receptors on enterocytes, which is followed by thyrotropin (TSH) expression. TSH is bound to specific receptors on intraepithelial lymphocytes and has an influence on their maturation. It may be suggested that this role of TRH, which is not present in the prostate gland (Bílek *et al.* 1992), could be overtaken by prostatic TRH-like peptides, particularly when the endocrine cells of the prostate also produce TSH in addition to TRH-like peptides (Abrahamsson and Lilja 1989). TRH receptor specific mRNA was detected in most of the peripheral tissues tested, and it may be suggested that TRH receptor has specific functions in these tissues (Fukusumi *et al.* 1995).

Human thyrotropin-releasing hormone receptor (hTRHr) is a 45 kDa, 398 amino acids, 7 helices transmembrane G-protein coupled glycoprotein receptor, which activates the phosphatidylinositol-calcium-protein kinase C transduction pathway after the interaction with TRH. The gene responsible for its expression is localized in the long arm of human chromosome 8 (cytogenetic band 8q23) (Morrison *et al.* 1994). hTRHr belongs to an extensive group 1 (rhodopsin-like) of G-protein coupled receptors (GPCR) with structure (Swiss-Prot entry, accession number P34981, Swiss Institute of Bioinformatic and European Bioinformatics Institute, 30-May-2000) which consists of seven transmembrane regions linked with three extracellular and three intracellular loops. The glycosylated N-terminus is placed on the extracellular side of the membrane and a potentionally phospohorylated C-terminal end is found in a cytoplasmatic membrane site. Transmembrane regions of hTRHr are located in the amino acid residue 29-51 (chain A), 62-83 (chain B), 100-121 (chain C), 145-168 (chain D), 194-215 (chain E), 267-288 (chain F), and 297-319 (chain G). The primary structure of hTRHr is represented in Figure 1.

The first step in evaluation of the hypothesis concerning the prostatic local network composed of TRH-like peptides/TSH is to determine whether TRH-like peptides can interact with hTRHr. Because the experimentally determined three-dimensional (3D) structures of human TRH receptor, TRH and TRH-like peptides in XYZ (Carthesian) coordinates were not available, the freely accessible software running on Internet was applied for computing peptide or protein 3D-structures. A comparative protein modelling procedure

developed for G-protein coupled receptors (GPCR) was used for generating the hTRHr 3D-structure. The procedure is running on Swiss Model server in Geneva under the support of Glaxo-Wellcome (Peitsch 1995, 1996, Guex and Peitsch 1997). Program CORINA was used for generating 3D-structures of TRH and TRH-like peptides. The program was developed by Gasteiger *et al.* (1996). The 3D-structures of human TRH receptor, TRH and TRH-like peptides in the form of PDB files were visualized by freely available programs of Guex and Peitsch (1997) (Swiss-Pdb Viewer Ver 3.51) or Sayle (RasWin Molecular Graphics Ver 2.6.4, www.OpenRasMol.org).

The prostatic gland is most commonly affected by benign prostatic hyperplasia or prostatic cancer. They pose a difficult medical and social-economical problem regarding their high prevalence mainly among men over fifty years old. The expectant local prostatic network of TRH-like peptides/TSH may bring new possibilities of regulation mechanisms in proliferation of the prostate gland.

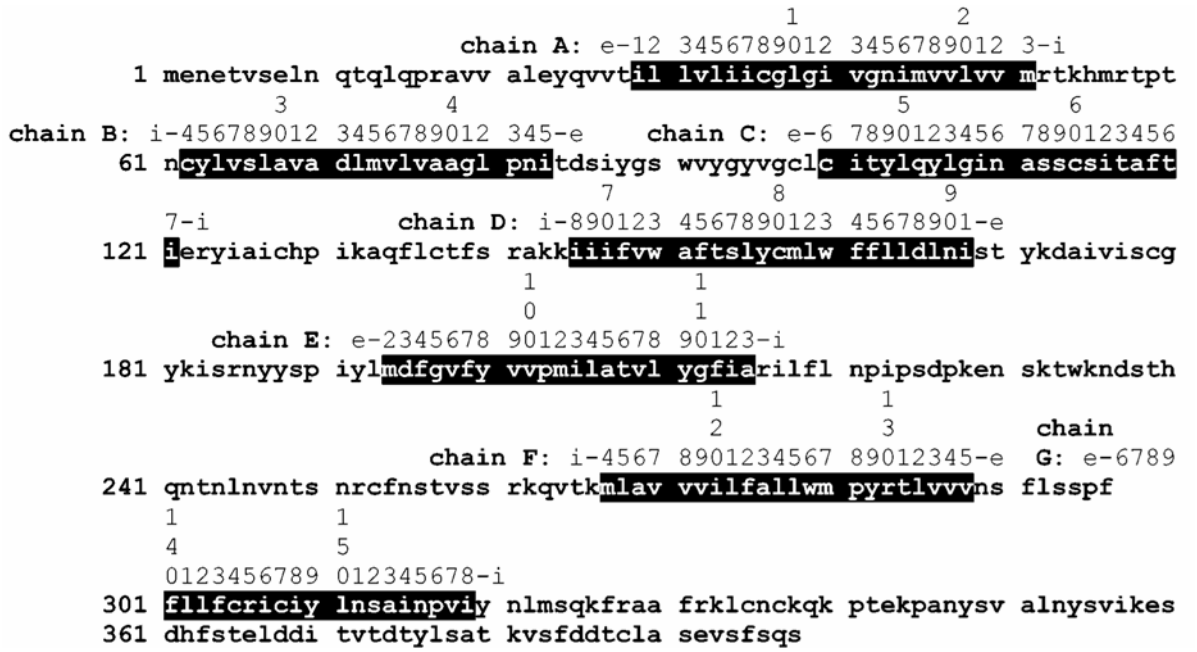

Fig. 1. Primary structure of human TRH receptor (white characters, black background) with transmembrane regions (chains A – G, red characters). Amino acids of these regions were used for generation of hTRHr computer model with numbering according to the line above the primary structure of hTRHr, e- extracellular end of chains, i- intracellular end of chains.

Computer modeling

Ligand-receptor interactions depend on the molecular surface and the volume of ligand and receptor, respectively. These parameters are derived from Van der Waals radius, which is the lowest distance between two atoms, which are not covalently bound. Molecular surfaces are based on the Van der Waals radius and they are composed of spheres for individual atoms with their intersecting sections removed. The second factor influencing the interactions is that the surfaces of interacting molecules need to be of complementary character in terms of hydrophobicity, polarity, and electrostatic forces.

Previous results of molecular mechanics and dynamics simulations concerning the prediction of actions joined with binding of the ligand suggest that TRH initially interacts with residues in the extracellular loops and subsequently moves into the surface and then into the transmembrane binding pocket (Colson *et al.* 1998a), where TRH is fully buried (Laakkonen *et al.* 1996). The interaction between TRH and surface recognition site of hTRHr extracellular domains can induce the change of conformation in residues that block access to the transmembrane binding pocket. The recently described computer simulation of hTRHr surface curvature with superposed electrostatic potential (Colson *et al.* 1998a) together with mutation experiments resulted in prediction of the extracellular surface binding cavity, which consists of TYR181, LYS 182, ARG185 near the extracellular end of chain E, and ASN289, PHE296 located close to the extracellular side of chain F and G. GLU298 (in the presented model chain G: GLU(E) in

position 137, Fig. 1) in the extracellular end of transmembrane chain G produce a negative potential, which is surrounded by the positive potential generated primarily by LYS182 in the extracellular loop. Negatively charged carbonyl groups of TRH backbone could interact with the positive charge of LYS182, whereas the positive charge generated by HIS of TRH can interact with negative charges of the short extracellular loop between chains F and G. TYR181, LYS182, SER290 and GLU298 (chain G: E137) exert low mobility due to hydrogen bond interactions among these residues, and they can be classified as a static component responsible for TRH recognition on the hTRHr surface. The binding of TRH can increase the anticorrelated vibrational motions of the loops and provide a way of inducing conformational changes to open a pathway into the transmembrane pocket (Colson *et al*. 1998a), which is formed by four direct binding contacts between pyroGLU ring carbonyl of TRH and hydroxyl group of TYR106 (chain C: Y52) (Perlman *et al.* 1994a), ring N-H of pyroGLU and ASN110 (chain C: N56) (Perlman *et al.* 1994b), HIS and aromatic ring of TYR282 (chain F: Y129), and terminal carboxamide of PRO (TRH) and ARG306 (chain G: R145) (Perlman *et al.* 1996). The transmembrane binding pocket was predicted and refined using mutation of hTRHr or derivation of TRH together with computer modelling based on Monte Carlo/stochastic dynamics stimulations (Laakkonen *et al.* 1996). TRH binding in the transmembrane pocket results in disruption of the hydrophobic cluster, which holds the hTRHr in inactive conformation. The active conformation of hTRHr may, together with subsequent interaction of agonists lead to signal transmission. The hydrophobic cluster (Colson *et al.* 1998b) consists of highly conserved aromatic TYR282 (in the presented model chain F: Y192) linked by a hydrogen bond to TYR192 on the extracellular loop between chains D and E in the unoccupied receptor. Also other members of the hydrophobic cluster are conserved aromatic residues TRP279 (F: W126) and PHE199 (E: F97). TRH binding results in replacing of hydrogen-bond between TYR192 and TYR282 (F: Y192) by an interaction of HIS side chain from TRH with aromatic ring of TYR282 (F: Y192) (Colson *et al.* 1998b). It was mentioned (Perlman *et al.* 1997) that interactions among conserved residues ASP71 (B: D33), ASN43 (A: N15) and ASN316 (G: N155) (ASP71 bridges ASN43 and ASN 316) may be structurally important for the function of hTRHr. The results concerning the transmembrane and polar component or hydrophobic cluster are presented as 2D-structures in Figure 2 or as 3D-pictures in Figures 3A and 3B. The surface curvature of hTRHr model with a superposed electrostatic potential is presented in Figures 3C and 3D.

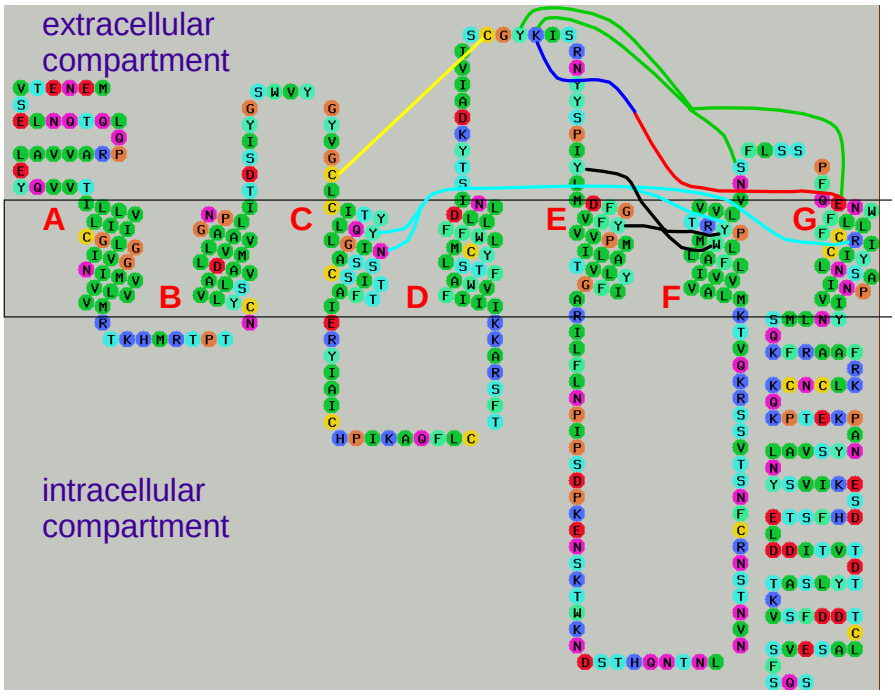

Fig. 2. 2D-structure of human TRH receptor generated by F. Campagne, B. Maigret, Laboratoire de Chimie Theorique de Nancy, Vandoeuvre-les-Nancy, France; and J.M. Bernassau, Sanofi Recherche, Montpellier, France, on Viseur program, release 2.35. The seven transmembrane regions (chains A – G) in the oblong were used for the construction of 3D hTRHr computer model. Extracellular disulphide bond important for high affinity interaction between TRH and hTRHr is described as a yellow line (Perlman *et al.* 1995, Cook *et al.* 1996), the static component of hTRHr responsible for TRH recognition on hTRHr surface (Colson *et al.* 1998a) as a green line, the potential directed access of TRH to hTRHr (Colson *et al.* 1998a) as red(-)/blue(+) line, the transmembrane pocket for binding TRH within transmembrane bundle (Perlman *et al.* 1996, 1994a, 1994b) as light blue line, and hydrophobic cluster switching between active and inactive state of hTRHr (Perlman *et al.* 1994b) as black line.

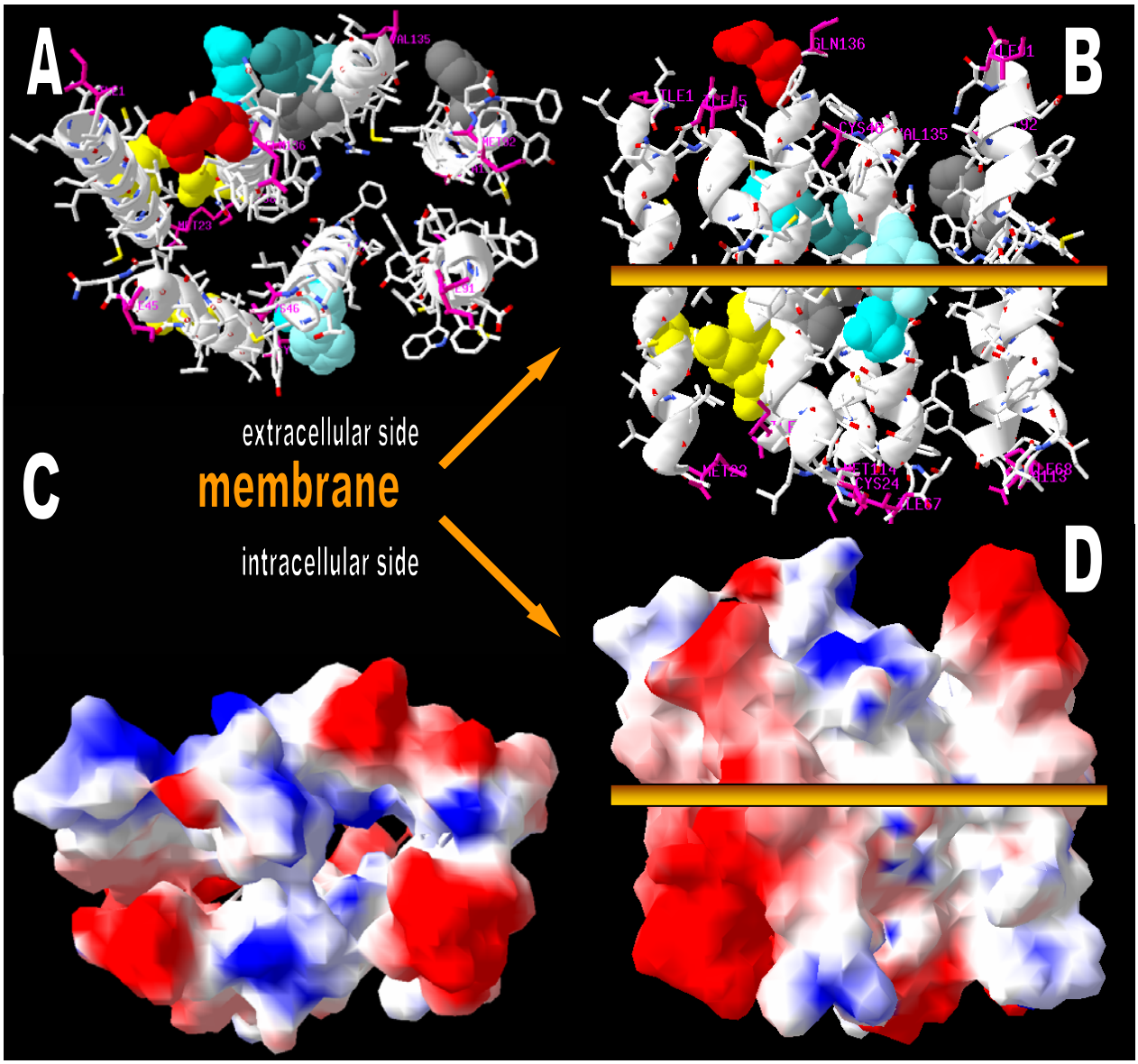

Fig. 3. Front and side view of 3D-model of human TRH receptor with coordinates generated by SwissMod server. Chains flagging as A-G (Fig. 1, 2) are in counter clockwise direction (Fig. 3A), and terminal amino acids of individual chains with their labels are colored in violet (Fig. 3A, 3B). In Figures 3A and 3B the negative potential of GLU298 is presented (chain G: E137) which appears to direct the access of TRH or TRH-like peptides to hTRHr (Colson *et al.* 1998a) in red color; expectant transmembrane binding pocket (Perlman *et al.* 1996, 1994a, 1994b) consisting of TYR106 (C: Y52), ASN110 (C: N56), TYR282 (F: Y129, dark green color), ARG306 (G: R145) in light blue color; the hydrophobic cluster (Perlman *et al.* 1994b) constructed of TYR282 (F: Y129, dark green color), TRP279 (F: W126), PHE199 (E: F97) in grey color; and highly conserved polar component (Perlman *et al.* 1997) of ASN43 (A: N15), ASP71 (B: D33), ASN316 (G: N155) important for structure of hTRHr in yellow color. The surface curvature of hTRHr model with superposed electrostatic potential (negative charge in red, positive in blue color) is presented in Figures 3C and 3D.

The primary structure of the whole molecule of hTRHr and the parts of hTRHr used for computer modeling including the different numbering of amino acid residues in an authentic molecule or computer model is shown in Figure 1. The applied computing method cannot generate the 3D-structure of long connecting peptide loops in the extracellular or intracellular compartment. The number of possible conformations of loops is extremely high, and probably a well running algorithm solving 3D-structures of these long connecting loops in GPCRs is not yet available. The presented computer model of the 3D-structure of hTRHr consists only of seven transmembrane helices of hTRHr (Fig. 2), but it seems to be adequate for the interactions between hTRHr and TRH or TRH-like peptides, which are small peptides. It is commonly accepted that G-protein coupled receptors appear to bind large glycoprotein hormones predominantly within their extracellular domains, small

ligands (as TRH and TRH-like peptides) within the transmembrane helical bundle, and peptide ligands within the extracellular domains and the transmembrane bundle (Perlman *et al.* 1996). However, the situation appears to be more complicated because the topology of transmembrane regions may be substantially influenced by extracellular or intracellular loops. The conserved extracellular cysteines (CYS98, CYS179) form a disulphide bond, which is important for maintaining the high affinity conformation of TRH receptor (Perlman *et al.* 1995, Cook *et al.* 1996). Han and Tashjian (1995) described that the deletion or substitution mutations in extracellular loops of hTRHr resulted in a decrease or loss of TRH binding.

Materials and Methods

The primary structures of transmembrane regions (chains A, B, C, D, E, F, G) were placed *via* E-mail to the input of the Swiss Model server. Regions were automatically compared using heuristic search algorithm BLAST tailored for sequence similarity searching with group specific templates having a generated 3D-structure. Templates were computed with Herzyk´s and Hubbard´s simulated annealing Monte Carlo procedure (Herzyk and Hubbard 1995), which orients rigid helices to satisfy structural restraints. The restraints were derived from theoretical considerations of the protein structure, from analysis of two dimensional rhodopsin projection maps, from experimental information on native and mutant proteins, and from sequences of related proteins. The Swiss Model offers rhodopsin, human substance K receptor, human β_2-adrenergic receptor, human neuropeptide Y1 receptor and bacteriorhodopsin as templates of the family of G-protein coupled receptors. Based on the BLAST algorithm, the human neuropeptide Y1 receptor (highest score 84, lowest Poisson unlikelihood probability (P-value) $1.8e^{-10}$) was used as the template for modelling of the human TRH receptor. Swiss Model protein modelling server has generated the PDB file containing the Carthesian coordinates of atoms in the TRH receptor molecule using the ProMod II software. The details of these procedures of protein modeling and visualizing are described in the literature (Peitsch 1995, 1996, Guex and Peitsch 1997). The energy minimization was computed by program Gromos 96 in the system of ProMod II (van Gunsteren and Berendsen 1990)

Problems with generating of 3D-structures of TRH and TRH-like peptides were based on primary structures of these peptides, which have both terminal amino acids blocked (pyroglutamic acid, proline amide). These blocked amino acids do not belong to the standard amino acids library of Swiss Model server and Swiss-Pdb Viewer with computed topology files. The 3D-structures of TRH and TRH-like peptides were generated *de novo*, again *via* the Internet, by program CORINA on server running in Computer-Chemie-Centrum, University of Erlangen-Nurnberg, Germany. The program was developed by Gasteiger *et al.* (1996), and it is a rule- and data-based system, that automatically generates 3D atomic coordinates from the constitution of a molecule as expressed by a connection table or linear code.

Results and Discussion

Swiss-Pdb Viewer can compute the molecular surfaces from PDB files together with an electrostatic potential occurring on the surface using Coulomb´s law and atomic partial charges with proteins taken to be at pH 7.0 and with default protonation state for all residues. The model of human TRH receptor is demonstrated as a ribbon (Figs 3A and 3B) or surface drawings (Figs 3C and 3D) (front and side view), where amino acids representing the transmembrane binding pocket, hydrophobic cluster and polar component are visualized in the form of Van der Waals radii (Figs 3A and 3B). The estimation of stereochemical quality of the hTRHr model was performed by program Procheck v.3.0 (Laskowski *et al.* 1993, Rullmann 1996). The results in the form of the Ramachandran plot of hTRHr chains are demostrated in Figure 4. From Ramachandran plot statistics it is evident that 86 % of residues are in most favored regions A, B, L, 9 % in additional allowed regions, 3 % in generously allowed regions and only 2 % of residues are in the disallowed regions. Overall average of G-factors makes up –0.12. G-factors provide a measure of how unusual the properties are (bond lenghts, bond angles, dihedral angles). Ideally, value should be above –0.5. The energy minimization was computed by Gromos96 (200 cycles of steepest descent, 300 cycles of conjugate gradient), and the final total energy was –666.89 KJ/mol.

TRH and TRH-like peptides (Fig. 5) are represented in contrast to hTRHr either as a surface model without a superposed potential field (Swiss-Pdb Viewer do not contain a library for non-standard blocked terminal amino acids) or as a stick model. In Figure 5 probable structural interactions are presented between

TRH or TRH-like peptides and transmembrane domains of hTRHr situated on the extracellular side. From this model of hTRHr the terminal proline amide of TRH or TRH-like peptides seem to be involved in interactions with amino acids belonging to chains C, D and E of hTRHr, while central amino acids or terminal pyroglutamic acid of these peptides mainly interact with chains F and G, respectively. Possible interactions can be better investigated using the model of hTRHr where the amino acids are drawn as balls derived from Van der Waal´s radii in color dependent on their type, i.e. acidic amino acids are in red color, basic are blue, polar are yellow and non-polar amino acids are colored in gray (Fig. 6). The interactions can be attributed to polar GLN105 (chain C: E51), SER122 (C: S58), ASN167 (D: N90), acidic ASP195 (E: D93), basic ARG283 (F: R130), polar GLN297 (G: Q136) and acidic GLU298 (G: E137).

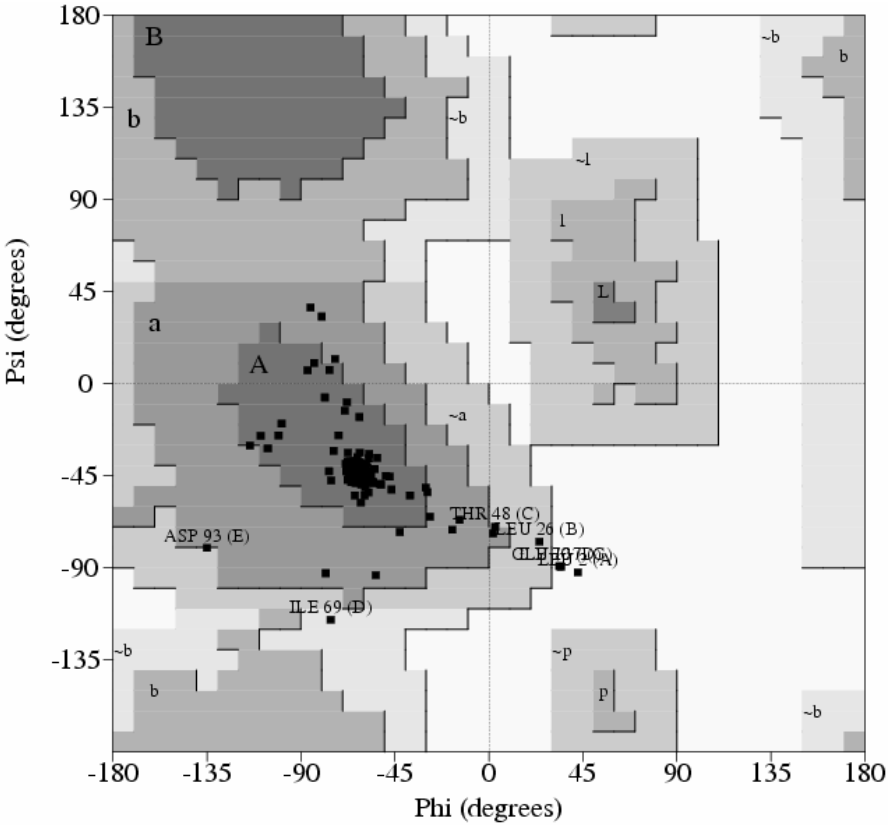

Fig. 4. Ramachandran plot of hTRHr model which reveals that more than 95 % of observed dihedral angles are clustered in the sterically allowed regions. Ramachandran plot statistics according Procheck: most favored regions [A,B,L] = 114 residues (85.7 %), additional allowed regions [a,b,l,p] = 12 residues (9.0 %), generously allowed regions [~a,~b,~l,~p] = 4 residues (3.0 %), and disallowed regions [XX] = 3 residues (2.3 %). 133 non-glycine and non-proline residues, 14 end-residues (excl. Gly and Pro), 7 glycine residues, 4 proline residues, and total number of residues = 158.

It can be assumed on the presented 3D-model of hTRHr, whose coordinates were generated on the Swiss Model server that polar amino acids belonging to the extracellular terminus of hTRHr transmembrane regions can participate in interactions between TRH or TRH-like peptides and hTRHr. There is no direct evidence that TRH-like peptides, whose coordinates were generated on the server using program CORINA, interact with the presented hTRHr model. On the contrary, with respect to the similar 3D-shape and the identity of terminal amino acids, it appears that these interactions are highly plausible as well as nearly 100 % of cross-reactions between TRH or TRH-like peptides and antibody specific against authentic TRH. It is also evident that there must be effective repulsive Coulombic forces, which obstruct the interactions between TRH or TRH-like peptides and hTRHr, if these peptides deblock their terminal amino acids. The desamido or glutamyl metabolites are acidic and they will be repulsed due to negative potential of ASP195 (E: D93) and GLU298 (G: E137).

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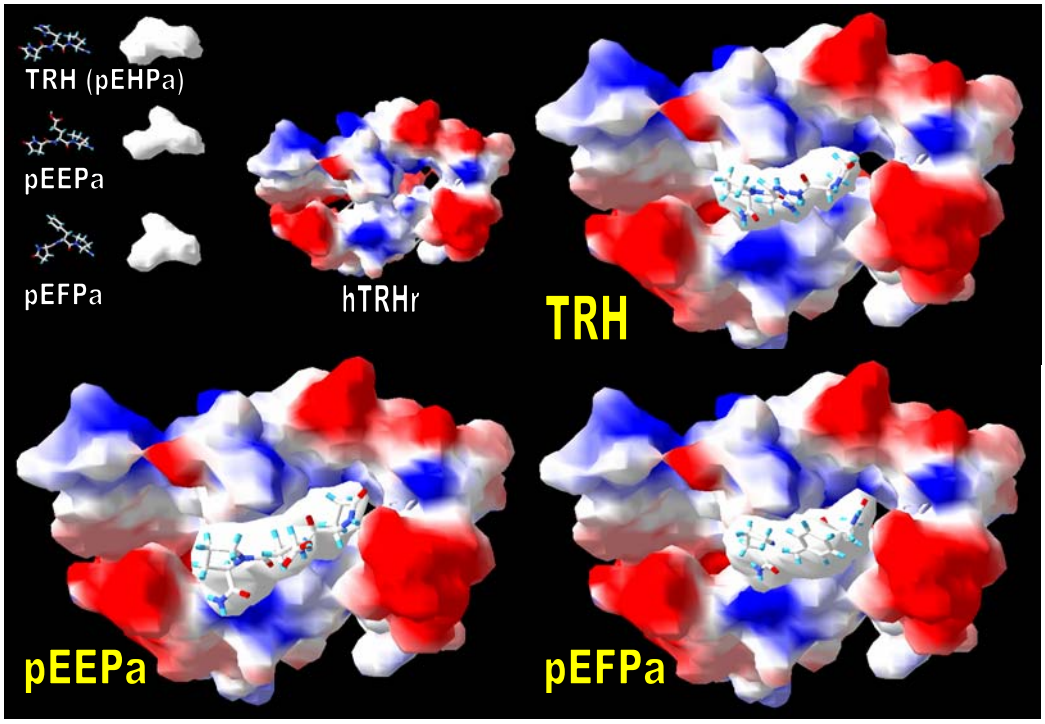

Fig. 5. The surface or stick model of TRH, TRH-like peptides, and surface curvature of hTRHr with superposed electrostatic potential. Here are also presented probable structural interactions between TRH or TRH-like peptides and extracellular domains (mainly chains C, D, and E for proline amide, and chains F, G for central amino acids or terminal pyroglutamic acid of TRH and TRH-like peptides) of hTRHr transmembrane regions occurring on extracellular side.

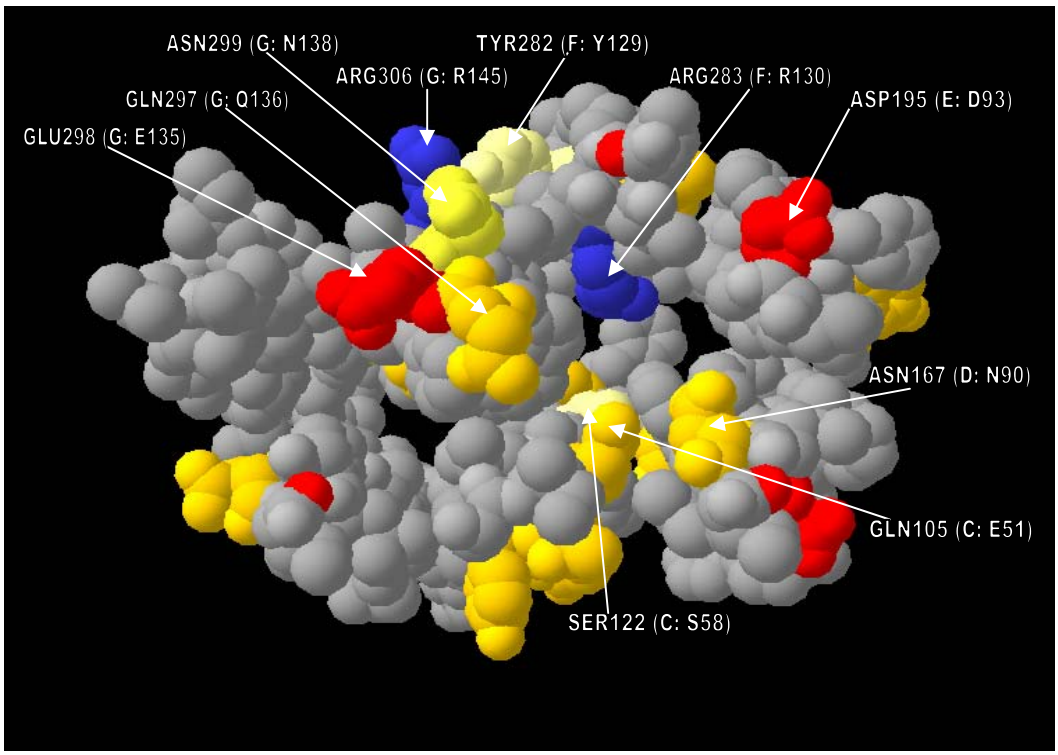

Fig. 6. Model of hTRHr where the amino acids are drawn as balls derived from Van der Waals radii in color dependent on their type, i.e. acidic amino acids have red color, basic are blue, polar are yellow, and non-polar amino acids are colored in grey.

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Reprint requests

R. Bílek, Institute of Endocrinology, Národní 8, 116 94 Prague 1, Czech Republic. Fax +420 2 2490 5325, e-mail: rbilek@endo.cz