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Two receptor binding regions of human FSH show sense-antisense similarity to the human FSH receptor

Jerry W. Slootstra[1] and Eric W. Roubos[2]

[1]Department of Histology, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands

[2]Department of Animal Physiology, Katholieke Universiteit Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands

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The sequences of two receptor binding regions of the ß-subunit of the human follicle-stimulating hormone (hFSH-ß) were compared with the DNA-derived antisense peptide sequence of the hFSH receptor. A striking sense-antisense similarity was established between these receptor binding regions and the hFSH receptor. Based on this sense-antisense similarity four putative hormone binding regions on the N-terminal extracellular region of the hFSH receptor are identified.

According to the molecular recognition theory (MRT) peptides derived from complementary DNA strands interact specifically (1, 2). MRT arose from the observation that corresponding amino acids encoded by sense and antisense RNA show a hydropathic complementarity (3). In various instances it has been confirmed that peptides can bind specifically to their complementary peptides; examples are ACTH (2), insulin (4) and arginine vasopressin (5).

In the spirit of MRT, interacting binding regions of proteins such as receptors and hormones could also consist of complementary sequences. This hypothesis is supported by the finding that in many cases antibodies raised against antisense peptide hormones recognize the respective hormone receptors (5-7). Furthermore, extensive sense-antisense similarities have been found between some hormone and receptor interaction sites (8-11). Unfortunately, for many other proteins–in particular hormones and their receptors–the interaction sites and/or the cDNA structures are unknown and consequently it is impossible to study sense-antisense similarity of these proteins.

Recently, the human follicle-stimulating hormone receptor (hFSH receptor) cDNA was cloned and sequenced (12). Since receptor binding regions of hFSH were previously characterized, it is possible now to study sense-antisense similarity between these binding regions of hFSH and the receptor.

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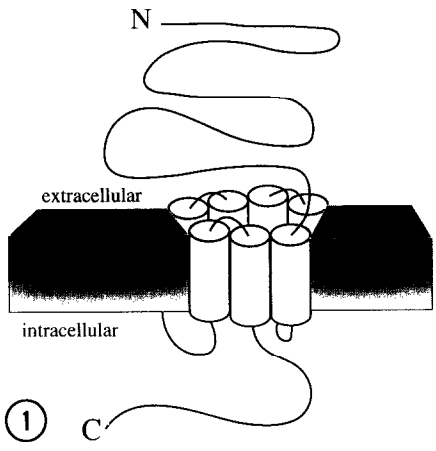

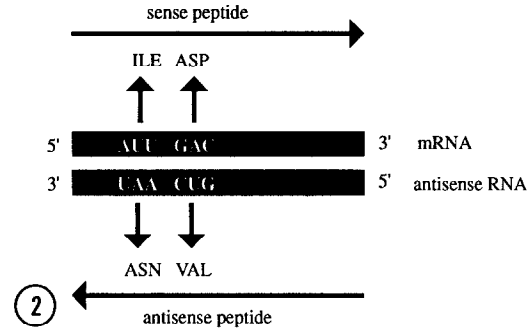

Fig 1. Schematic putative structure of the hFSH receptor. The receptor consists of seven transmembrane domains and a long N-terminal extracellular domain (19).

Fig 2. Deduction of the 5' to 3' antisense amino acid sequences from the mRNA sequences of the binding sites of peptide hormones and their corresponding receptors.

The hFSH receptor is a G-protein coupled receptor. G-protein coupled receptors supposedly consist of seven transmembrane regions that are interconnected by three extracellular and three intracellular loops (13). The hFSH receptor is characterized by a large extracellular N-terminal domain which is presumably important for hormone interaction (14)(Fig. 1). The hFSH hormone consists of an α- and a ß-subunit (15). The two regions identified as receptor binding regions are located on the ß-subunit of hFSH; sequence 33-53 and 81-95 (14-19). The first region contains the tetrapeptide sequences TRDL (sequence 34-37) and KTCT (sequence 49-52). The synthetic peptides TRDL and KTCT inhibit binding of [^{125}I]hFSH to the testis FSH receptor, as does synthetic hFSH-ß 33-53; the latter with increased potency (16-18). Moreover, synthetic hFSH-ß 33-53 behaves as a partial agonist of hFSH (18). The second receptor binding region of hFSH, sequence 81-95, also inhibits binding of [^{125}I]hFSH to the testis FSH receptor and is also a partial agonist of hFSH. We investigated whether hFSH-ß 33-53 and 81-95 (with special attention to the tetrapeptide sequences TRDL and KTCT) show sense-antisense similarity to the hFSH receptor.

Materials and Methods

The 5' to 3' antisense sequence of the complementary non-coding DNA strand of the hFSH receptor was translated into an antisense hFSH receptor protein (as in Fig. 2). The 3' to 5' antisense translational reading was not used because 3' to 5' translational reading has not been observed in nature and therefore the relevance of any observed sense-antisense similarity is not clear. Using the "search and find" command of the application Word 4.0 (Microsoft Corp.) hFSH-ß was compared with the antisense receptor sequence. The search and find command serve to identify two or more consecutive amino acid matches. From the data obtained, regions of high sense-antisense similarity were identified. These regions were aligned with the receptor binding regions of hFSH-ß. These antisense receptor regions were also compared with protein sequences in a protein data base (Swiss-Prot, release number 18.0, 1991) using the application Fasta 1.3 (20).

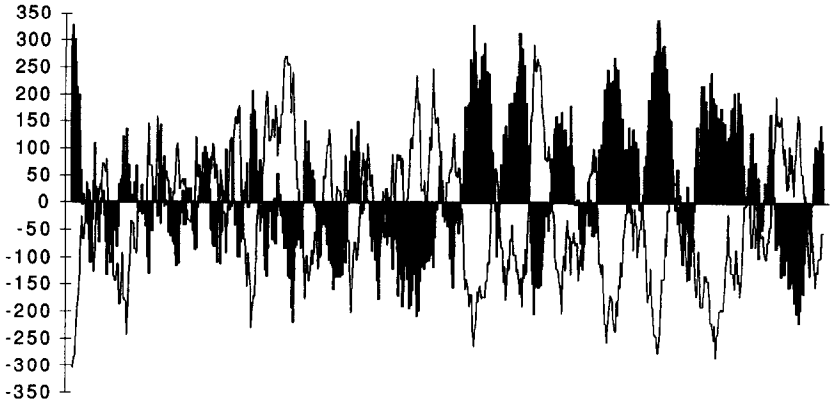

Fig 3. Kyte and Doolittle hydropathic profiles of the sense and antisense hFSH receptor. Grey profile: the sense receptor sequence; white profile: the antisense receptor sequence; I to VII correspond to the seven putative transmembrane domains; N, N terminal start; C, C terminal end.

Hydropathic profiles of the sense and antisense hFSH receptor and of the receptor binding regions of hFSH-ß were generated using the Kyte and Doolittle method (with an averaging window of seven amino acids) using the application Geneworks 1.0 (IntelliGenetics, Inc., Mountain View, CA 94040, USA). The hydropathic profiles of the regions of high sense-antisense similarity between hFSH-ß and the receptor were compared. According to MRT the hydropathic profile of an antisense peptide should present a mirror image of its complementary (sense) peptide (*cf.* Fig. 3). By comparing the slopes of the lines in both profiles the presence or absence of hydropathic complementarity was expressed. A "+" was assigned when the slopes had opposite sign which indicates hydropathic complementarity, or when one of them (or both) was (were) zero. A "-" was assigned when slopes had the same sign.

Results and Discussion

The receptor binding regions of hFSH, hFSH-ß 33-53 and 81-95, exhibit similarity to the antisense sequence of the hFSH receptor. This sense-antisense similarity is located in four regions; receptor sequences sequences 57-86 (I), 165-197 (II), 262-294 (III) and 179-211 (IV) (Table 1). The hFSH receptor regions I-IV are all located in the extracellular N-terminal domain, the putative hormone binding region. Antisense receptor sequences I-III show similarity to hFSH-ß 33-53, especially to the tetrapeptide sequences TRDL and KTCT. Antisense receptor sequence IV shows similarity to hFSH-ß 81-95. The complementary antisense receptor regions show no obvious homology with any other protein of the Swiss-Prot database.

The following observations further demonstrate sense-antisense similarity between hFSH and its receptor. The antisense hFSH receptor sequence and hFSH-ß have six short fragments in common; AHH, CTF, CTV, RDL (twice) and TFK (Fig. 4). In hFSH-ß four of these are present in the receptor binding region 33-53 (CTF, RDL (twice) and TFK) and one is present in the receptor binding region 81-95 (CTV). Only AHH is absent from the receptor binding regions hFSH-ß 33-53 and 81-95. In the hFSH antisense receptor five of the short fragments are present in the extracellular N-terminal domain of the hFSH receptor and only one is not (again AHH) which lies in the antisense

TABLE 1. Sense-antisense similarity of the receptor binding regions of hFSH and the extracellular N-terminal segment of the hFSH receptor

hFSH-ß 27-59			N	WCAGYCYTRDLVYKDPARPKIQKTCTFKELVYE	C
I	as receptor	86- 57	N	YHLQDIILRDL YFLQV PKS-K CTFLDDSKL	C
II	as receptor	197-165	N	CI-LGSIECTVVYFLNPIL IQP-NHTFKAQPHE	C
III	as receptor	294-262	N	FVANWMKLRDLPSPVCKGTAMAGIGEAGFHEGD	C
I	s receptor	86- 57	C	IVELVDNQSIE IKELD GFGSF AGKQIVRLK	N
II	s receptor	197-165	C	ADLQTGNFACNHIEQIGNK NLWLIVSEFSLGVF	N
III	s receptor	294-262	C	KNCIPHLESIQRRWNAFACCHSPYTLSAEMLAV	N
hFSH-ß 73-105			N	SLYTYPVATQCHCGKCDSDSTDCTVRGLGPSYC	C
IV	as receptor	211-179	N	IRQFF-IIIIA-IHCI-LGSIECTVVYFLNPIL	C
IV	s receptor	211-179	C	NPLEELNNNDSLNVADLQTGNFACNHIEQIGNK	N

s: 5' to 3' sense protein sequence; as: 5' to 3' antisense protein sequence; N: N-terminal end; C: C-terminal end; -: stop codon; blank space: gap. The residues identical to the receptor binding region of hFSH are boxed. The location of the TRDL and KTCT tetrapeptide sequences and hFSH-ß 81-95 is indicated by shaded boxes. Note that the antisense receptor sequences show additional sequence similarity; I and II share the sequence YFL (also boxed); III and IV share the sequence HXCAF. Also note that the overlapping receptor sequences II and IV contain glycosylation motifs (NGT and NLS).

sequence of the fourth transmembrane region of the hFSH receptor. Although AHH might appear not to be involved in hormone-receptor interaction, some evidence points to the contrary (21). Receptor region II overlaps the receptor region IV (Table 1); thus the CTV fragment is present in receptor regions II and IV. Remarkable but enigmatic is the

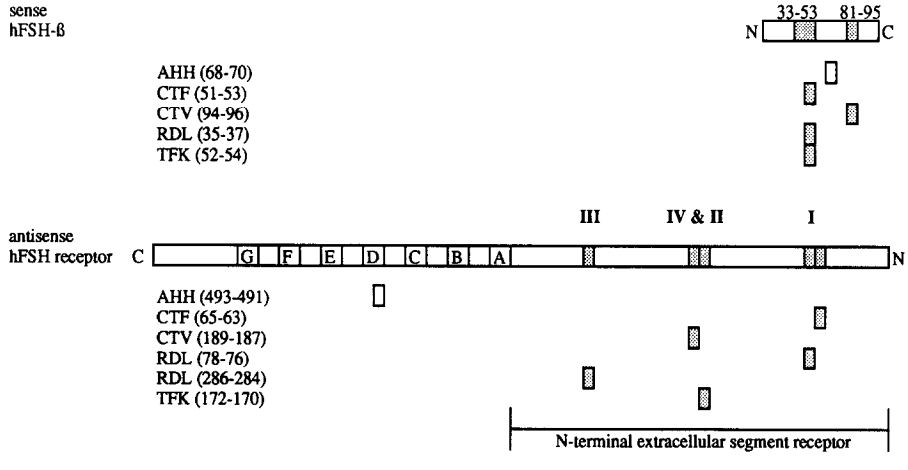

Fig 4. Schematic representation of the location of short fragments shared by hFSH-ß and the antisense hFSH receptor sequence. The location of the short fragments is indicated by boxes that are shaded when present either in the receptor binding sites of hFSH-ß (sequences 33-53 and 81-95) or in the N-terminal extracellular region of the receptor. The putative seven transmembrane regions of the receptor are indicated as A to G. Antisense receptor regions I - IV correspond to I - IV as indicated in Table 1. Note that the short fragments CTV, CTF and TFK show some homology.

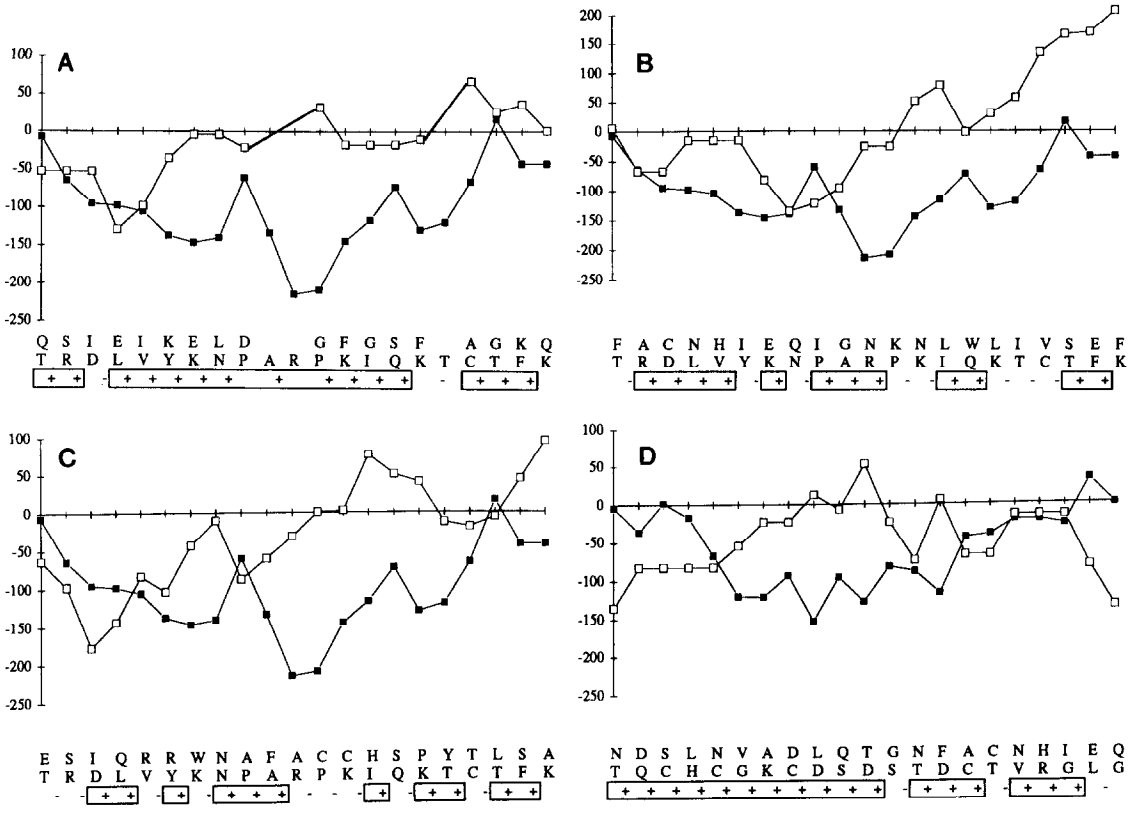

Fig 5. Hydropathic complementarity of the receptor binding regions of hFSH and the putative hormone binding regions of the hFSH receptor; x-axis : first row is receptor sequence (receptor sequences I-IV in A-D, respectively, indicated by open squares), second row are hormone sequences (hFSH-ß 33-53 in A-C and hFSH-ß 81-95 in D indicated by closed squares) and third row is hydropathic complementarity between hormone and receptor sequence (present: +; absent: -).

presence of CTV in antisense receptor sequence II at a position where antisense receptor sequences I and III contain an RDL (Table 1).

According to MRT, interaction between sense and antisense peptides and hormones and receptors is based upon hydropathic complementarity (1). The hydropathic profiles of hFSH-ß 33-53 and 81-95 are hydrophilic (Fig. 5) and are part of the two regions of hFSH-ß that have the highest values for hydrophilicity (14). Receptor sequences I and IV show a clear hydropathic complementarity with the receptor binding regions of hFSH (Fig. 5A, D). Receptor sequences II and III show a less clear hydropathic complementarity with the receptor binding regions of hFSH (Fig. 5B, C).

We suggest that hFSH receptor sequences I, II, III and IV are involved in hormone binding. Receptor sequence I in particular exhibits strong sense-antisense similarity to one of the receptor binding regions of hFSH. We feel that the sense-antisense similarity between hFSH and its receptor reported here is yet another strong indication that specific noncovalent protein-protein interactions are mediated via sense-antisense peptide interaction, as asserted by MRT.

Acknowledgments

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References

1. Blalock, J.E. (1990). Trends Biotechnol. 8, 140-144.
2. Clarke, B.L. and Blalock, J.E. (1991) In: Antisense nucleic acids and proteins, fundamentals and applications (J.N.M. Mol and A.R. van der Krol, Eds.), pp. 169-185. Marcel Dekker, Inc., New York.
3. Blalock, J.E. and Smith, E.M. (1984). Biochem. Biophys. Res. Commun. 121, 203-207.
4. Knutson, V.P. (1988) J. Biol. Chem. 263, 14146-14151.
5. Johnson, H.M. and Torres, B.A. (1988) J. Immunol. 141, 2420-2423.
6. Abood, L.G., Michael, G.J., Xin, L., and Knigge, K.M. (1989) J. Recept. Res. 9, 19-25.
7. Clarke, B.L. and Bost, K.L. (1990) Biochem. Biophys. Res. Commun. 168, 1020- 1026.
8. Slootstra, J.W. and Roubos, E.W. (1990) Trends Biotechnol. 8, 279-281.
9. Slootstra, J.W. and Roubos, E.W. (1991) In: Antisense nucleic acids and proteins, fundamentals and applications (J.N.M. Mol and A.R. van der Krol, Eds.), pp. 205-228. Marcel Dekker, Inc., New York.
10. Ghiso, J., Saball, E., Leoni, J., Rostagno, A., and Frangioni, B. (1990). Proc. Natl. Acad. Sci. USA 87, 1288-1291.
11. Campbell, W. and Okada, H. (1991). Biochem. Biophys. Res. Commun. 175, 207-214.
12. Minegish, T., Nakamura, K., Takakura, Y., Ibuki, Y., and Igarashi, M. (1991). Biochem. Biophys. Res. Commun. 175, 1125-1130.
13. Lefkowitz, R.J. and Caron, M.G. (1988) J. Biol. Chem. 263, 4993-4996.
14. Reichert-Jr, L.E., Dattatreyamurty, B., Grasso, P., and Santa-Coloma, T.A. (1991). Trends Pharmacol. 12, 199-203.
15. Pierce, J.G. and Parsons, T.F. (1981) Ann. Rev. Biochem. 50, 465-495.
16. Sluss, P.M., Krystek-Jr., S.R., Andersen, T.T., Melson, B.E., Huston, J.S., Ridge, R., and Reichert-Jr, L.E. (1986). Biochemistry 25, 2644-2649.
17. Schneyer, A.L., Sluss, P.M., Huston, J.S., Ridge, R.J., and Reichert-Jr, L.E. (1988). Biochemistry 27, 666-671.
18. Santa Coloma, T.A., Dattatreyamurty, B., and Reichert-Jr, L.E. (1990). Biochemistry 29, 1194-1200.
19. Santa Coloma, T.A. and Reichert-Jr, L.E. (1990). J. Biol. Chem. 265, 5037-5042.
20. Pearson, W.R. and Lipman, D.J. (1988). Proc. Natl. Acad. Sci. USA 85, 2444-2448.
21. Willey, K.P. and Leidenberger, F. (1989). J. Biol. Chem. 264, 19716-19729.