The mammalian gonadotropin-releasing hormone receptor (GnRH-R) is the only G-protein-coupled receptor (GPCR) in which the intracellular C-terminal tail is completely absent. In contrast to other GPCRs, the GnRH-R does not show rapid desensitization of total inositol phosphate (IP) production, and the rates of internalization are exceptionally slow. We investigated whether the incorporation of a cytoplasmic tail into the C terminus of the GnRH-R affects desensitization events and receptor internalization rates. A GnRH-R/TRH-R chimera was created where the intracellular tail of the rat thyrotropin-releasing hormone receptor (TRH-R) was engineered into the C terminus of the rat GnRH-R. Three different rat GnRH-R cDNA stop codon mutations (one for each reading frame) were also made. The GnRH-stimulated IP production of the wild-type rat GnRH-R expressed in either COS-7 or HEK 293 cells did not desensitize even after prolonged stimulation with GnRH. In contrast, the catfish GnRH-R (which does possess an intracellular tail) and the TRH-R rapidly (<10 min) desensitized following agonist stimulation. The GnRH-R/TRH-R chimera also desensitized following treatment with GnRH, resembling the pattern shown by the TRH-R and the catfish GnRH-R. Two of the stop coton mutants did not show desensitization of IP production, and the third mutant with the longest tail was not functional. Internalization experiments showed that the rat GnRH-R had the slowest endocytosis and recycling rates compared with the TRH-R, the catfish GnRH-R, and the chimeric GnRH/TRH-R. This study demonstrates that the addition of a functional intracellular C-terminal tail to the GnRH-R produces rapid desensitization of IP production and significantly increases internalization rates.

Gonadotropin-releasing hormone (GnRH)1 is a decapetide released by the hypothalamus which acts on specific receptors in the anterior pituitary. GnRH and its receptor (GnRH-R) are key mediators of the regulation of the reproductive axis controlling the synthesis and release of the gonadotropins, lutetinizing hormone, and follicle-stimulating hormone. The GnRH-R is a member of the large family of G-protein-coupled receptors (GPCRs) (1) and is preferentially coupled to phosphoinositidase C via the Gq/G11 family of G-proteins. Following stimulation by GnRH, the production of inositol phosphates (IPs) and diacylglycerol is induced resulting in the elevation of cytosolic calcium and the activation of protein kinase C (2–4). A number of GPCRs exhibit homologous desensitization upon prolonged stimulation with agonist. This involves uncoupling of G-proteins, rapid down-regulation of the receptor’s effector systems such as total inositol phosphate (IP) production followed by receptor internalization, and presumably subsequent proteolytic degradation. In particular this has been demonstrated for the m3 muscarinic acetylcholine receptor (5) and the thyrotropin-releasing hormone receptor (TRH-R) (6–9). The phosphorylation of specific amino acids in the cytoplasmic part of the C-terminal tail of several GPCRs has been implicated in desensitization events (3, 10, 11). This phosphorylation event is rapid, occurring within the first few minutes of agonist addition. Of all the GPCRs cloned to date, the mammalian GnRH-R is the only receptor in which the functionally important intracellular cytoplasmic C-terminal tail is completely absent, as it is truncated at the cell membrane immediately after the seventh transmembrane domain (1). Studies by our group and by others (7, 12, 13) have shown that in contrast to other GPCRs, the GnRH-R does not show rapid desensitization of IP production. It is possible that during evolution the mammalian GnRH-R has lost its intracellular cytoplasmic C-terminal tail as a result of a mutation creating a stop codon, particularly since GnRH receptors in non-mammalian species such as African catfish (14), goldfish, frog, and chicken (15) still possess their intracellular C-terminal tails. Here we have examined the rapid desensitization events of a non-mammalian (catfish) GnRH receptor. We have also created a GnRH-R/TRH-R chimera where the cytoplasmic tail of the rat TRH-R has been engineered into the C-terminal region of the rat GnRH-R. Furthermore, to investigate the possibility that a functional “ghost tail” (the amino acids translated from the natural stop codon to the next stop codon in the untranslated region) exists in mammalian species, we have created three different stop codon mutations (one for each reading frame) within the rat GnRH-R cDNA. The aim of this study has been to investigate whether the missing cytoplasmic C-terminal tail could be implicated in the inability of the rat’s GnRH-R to show rapid desensitization.

1 The abbreviations used are: GnRH, gonadotropin-releasing hormone; GnRH-R, GnRH receptor; GPCR, G-protein-coupled receptor; IP, inositol phosphate; TRH-R, thyrotropin-releasing hormone receptor; WT, wild type; BSA, bovine serum albumin.

Received for publication, November 24, 1997, and in revised form, March 3, 1998

Anders Heding, Milka Vrecl, Jan Bogerd‡, Alison McGregor, Robin Sellar, Philip L. Taylor, and Karin A. Eidne§

From the MRC Reproductive Biology Unit, Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh EH3 9EW, United Kingdom and ‡Department of Experimental Zoology, Universiteit Utrecht, Utrecht 3584 CH, The Netherlands

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1794 solely to indicate this fact.

§ To whom correspondence should be sent: MRC, Reproductive Biology Unit, Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh EH3 9EW, UK. Tel.: 44 131 228 5571; Fax: 44 131 229 2575; E-mail: keidne@hgmp.mrc.ac.uk.

2575; Fax: 44 131 228 5571; E-mail: keidne@hgmp.mrc.ac.uk.

© 1998 by The American Society for Biochemistry and Molecular Biology, Inc. Printed in U.S.A.
of IP production and its exceptionally slow internalization kinetics.

**EXPERIMENTAL PROCEDURES**

**Materials**—Insolubil-free Dulbecco’s modified Eagle’s medium, penicillin, and streptomycin were obtained from Life Technologies, Inc., Paisley, UK. Transfectam was obtained from Promega, Southampton, UK. All other tissue culture reagents and media were supplied by Sigma Cell Culture, Dorset, UK. Dowex resin was obtained from Bio-Rad. [H]{\textsuperscript{3}}HMyoinositol was from Amersham Pharmacia Biotech, Buckinghamshire, UK. TRH-(3-Me-His\textsuperscript{2})-[\textsuperscript{3}H] were from NEN Life Science Products, Hertfordshire; TRH-(3-Me-His\textsuperscript{2}) and chicken II GnRH were from Peninsula Laboratories, Merseyside, UK. All other compounds and reagents were obtained from Sigma, Dorset, UK. HEK 293 and COS-7 cells were obtained from the European Collection of Animal Cell Cultures, Center for Applied Microbiology and Research, Salisbury, UK.

**GnRH Receptor Constructs**—The full-length rat GnRH-R DNA in the vector pcDNA3 (Invitrogen) was subjected to oligonucleotide site-directed mutagenesis using standard methods to generate three stop codon mutations, one for each reading frame. The natural stop codon (TAA) was changed to GCCA for the +1 base mutant and GCA for the in-frame mutant. The −1 base mutant was created by changing the stop codon to GCA and deleting the naturally occurring T following the stop codon. In all three cases the stop codon was changed to a codon for alanine (Fig. 1A). To create the GnRH-R/TRH-R chimera, a ClaI restriction enzyme site was introduced in the stop codon of the rat GnRH-R cDNA, and ClaI sites were introduced at the N- and C-terminal ends of the rat TRH-R cytoplasmic C-terminal tail. The untranslated base sequence from nucleotides 981 to 990 in the rat GnRH-R was changed from TAA TTG GGA to CAA ATC GAT introducing the ClaI site and changing the stop codon to glutamine. The base sequence from nucleotides 997 to 1003 at the N terminus of the cytoplasmic C-terminal tail of the TRH-R was changed from AAG CTC to ATC GAT, and the untranslated base sequence from nucleotides 1238 to 1243 in the TRH-R was changed from ACT TCC to ATC GAT introducing two ClaI sites in the terminal cytoplasmic tail of the TRH-R. The amplified region was inserted into the ClaI site of the GnRH-R DNA in the vector pcDNA3. The cDNA clones were sequenced several times using an Applied Biosystems (Cheshire, UK) 373A automated sequencer. Sequence analysis was performed by means of the program Genejockey II (Biosoft, Cambridge, UK).

**Tissue Culture**—Monolayer cultures of COS-7 cells (3 × 10\textsuperscript{5} cells in 75-cm\textsuperscript{2} flasks) were transiently transfected with WT rat GnRH-R, rat TRH-R, rat GnRH receptor constructs, or the catfish GnRH-R in the eukaryotic expression vector pcDNA3 using a standard DEAE-dextran method. Cells were then grown for 48 h in Dulbecco’s modified Eagle’s medium containing 10% heat-inactivated fetal calf serum, glutamine (0.3 mg/ml), penicillin (100 units/ml), and streptomycin (100 units/ml) at 37 °C in a humidified atmosphere of 5% CO\textsubscript{2} in air before use. HEK 293 cells were stably transfected with the appropriate DNA using Transfectam. Receptor expressing clones were selected with Geneticin (1 mg/ml) and identified with a functional receptor binding assay. The cell lines expanded from these clones were then used for further study.

**Iodination of GnRH Agonist**—Iodinated radiolabeled GnRH analog was prepared using the glucose oxidase/lactoperoxidase method and purified by chromatography on a Sephadex G-25 column in 0.01 M NaOH and 1% SDS (NaOH/SDS) solution. Nonspecific binding for each time point was determined under the same conditions in the presence of 1 μM unlabeled agonist. For control experiments, untransfected HEK 293 cells were assayed under identical conditions. After subtraction of nonspecific binding, the internalized radioactivity was expressed as a percentage of the total binding at that time interval. All time points were performed in triplicate in at least three separate experiments.

**Receptor Binding Assay**—Ligand binding assays were carried out on cell membranes from receptor expressing cells as described previously (17). Briefly, purified membranes were incubated for 120 min in assay buffer (40 mM Tris-HCl, 2 mM MgCl\textsubscript{2}, 0.1% BSA, pH 7.4) at 4 °C with [\textsuperscript{125}I]-labeled GnRH agonist, [\textsuperscript{125}I]-labeled GnRH antagonist, or [\textsuperscript{125}I]-labeled GnRH agonist/antagonist (\textsuperscript{125}I) for 30 min at 37 °C. The membranes were then filtered, and the radioactivity was counted. All assays were performed in triplicate. Binding parameters were determined from displacement curves. Protein concentrations were measured using a Bio-Rad protein assay kit (Bio-Rad) with a BSA standard.

**Total Inositol Phosphates**—Total IPs were extracted and separated as described previously (12, 18). Briefly, 24-well plates containing 1 × 10\textsuperscript{5} cells per well were incubated with insoluble free Dulbecco’s modified Eagle’s medium containing dialyzed heat-inactivated fetal calf serum and [\textsuperscript{3}H]Myoinositol (2 μCi/well) for 48 h. Medium was then removed, and cells were washed with buffer containing 25 mM HEPES (pH 7.4) followed by addition of the appropriate ligand at a range of time points. The reaction was stopped by adding cold perchloric acid or boiling water. Neutralization of supernatants from extracted cells was then transferred to tubes containing AG 1-X8 anion exchange resin. Total IPs were then eluted, and the radioactivity was counted.

**RESULTS**

Fig. 1A shows sequence alignments of the C-terminal end of known sequences of cloned WT GnRH receptors, whereas Fig. 1B shows the chimeric (GnRH-R/TRH-R) and stop codon (+1 base, −1 base and in frame) receptor constructs. Putative phosphorylation sites for protein kinase C and casein kinase II are highlighted. The +1 base mutant and the −1 base mutant both exhibited reduced (30–40%) [\textsuperscript{125}I]-GnRH agonist binding and GnRH-stimulated IP production (50–60%) when compared with the WT GnRH-R (Fig. 2). The in-frame stop codon mutant with the longest extension (52 amino acids) demonstrated no GnRH agonist binding nor IP production. The GnRH-R/TRH-R chimera was able to bind GnRH agonist and stimulate IP production even though its tail extension was the longest (79 amino acids) of all these constructs.

In COS-7 cells transiently expressing the rat WT GnRH-R, we observed that the GnRH-stimulated IP production did not desensitize even after stimulation with GnRH for up to 45 min (the rate of IP production did not slow down) (Fig. 3A). In contrast, the IP production of the WT rat TRH-R did undergo desensitization after the first 5 min of TRH stimulation (Fig. 3B) following which a decrease in IP production was observed. The GnRH-R/TRH-R chimera was also able to desensitize after 10 min stimulation with GnRH (Fig. 3C). Desensitization of IP production seen for the chimera resembled that observed for the TRH-R, demonstrating the importance of the functional C-terminal tail. Unlike the mammalian GnRH-R, the catfish GnRH-R desensitized rapidly (within the first 5 min) following stimulation with GnRH (in this case, chicken II GnRH, the preferred endogenous ligand for the catfish GnRH-R (14)) (Fig. 3D). From the data shown in Fig. 3 the initial rate of IP production (the first 10 min of stimulation) and the rate after 10 min cells were calculated as described by Perlman and Greisen (8). As seen in Table I the rat WT GnRH-R did not show significant desensitization in contrast to the rat WT TRH-R, the GnRH-R/TRH-R chimera, and the catfish GnRH-R. The +1 base and −1 base GnRH-R stop codon mutants stimulated IP production; however, they did not undergo IP desensitization thereby resembling the WT GnRH-R (data not shown).

In order to establish whether desensitization of IP produc-
tion was cell type-specific, we carried out similar experiments with HEK 293 cells stably expressing WT rat GnRH-R, WT catfish GnRH-R, WT TRH-R, and the GnRH-R/TRH-R chimera. We again observed that the TRH-R, catfish GnRH-R, and the chimera desensitized, whereas the rat GnRH-R did not (Fig. 4). We did, however, observe a difference between the two sets of experiments. In the 293 cells expressing the TRH-R, catfish GnRH-R, and the chimera, the IP response desensitized after approximately 100 s which is considerably faster than the COS-7 cells that desensitized after 5–10 min. From the data shown in Fig. 4 the initial rate of IP production (the first 120 s of stimulation) and the rate after 120 s was calculated as described by Perlman and Gershengorn (8). As seen in Table II the rat WT GnRH-R did not show significant desensitization in contrast to the rat WT TRH-R, the GnRH-R/TRH-R chimera, and the catfish GnRH-R when stably expressed in HEK 293 cells. Equilibrium dissociation constants (Kd) and receptor numbers (B_max) were 0.2 nM and 3.1 pmol/mg protein for the rat GnRH-R expressing HEK 293 cells, Kd = 0.7 nM and B_max = 3.7 pmol/mg protein for the catfish expressing HEK 293 cells, and Kd = 1.6 nM and B_max = 9.5 pmol/mg protein for the GnRH-R/TRH-R expressing HEK 293 cells. We have previously published Kd = 4 nM and B_max = 13.5 pmol/mg protein values for the TRH-R expressing HEK 293 cells (13). In GH_3 cells which

FIG. 1. Sequence alignment of GnRH receptors. A, sequence alignment from amino acid 320 to the stop codon of the C-terminal region of cloned GnRH-Rs from different species. Note that the non-mammalian catfish GnRH-R has an extended cytoplasmic C-terminal tail. B, sequence alignment of the C-terminal region of rat stop codon mutants and chimera GnRH receptor constructs. In the rat GnRH-R stop codon mutant constructs, amino acids from the natural stop codon in the rat GnRH-R have been mutated in each of the three reading frames thereby extending the C-terminal tail in the 3' untranslated region to the next occurring stop codon. Three different rat GnRH-R stop codon mutants, rat GnRH-R + 1 base, −1 base, and in frame, were created. The GnRH-R chimera was constructed by inserting the rat TRH-R cytoplasmic tail in frame into the rat GnRH-R stop codon. Putative phosphorylation sites for protein kinase C (PKC) are shown in bold, and casein kinase II phosphorylation sites are in bold and underlined.
endogenously express TRH-Rs, we observed that the desensitization of IP production occurred after 10 min stimulation (data not shown), and we have previously shown that pituitary gonadotrope alpha T3-1 cells endogenously expressing GnRH-Rs do not undergo rapid desensitization of IP production (19). Untransfected cells and cells transfected with receptors not matching the stimulating agonist did not respond to stimulation (data not shown).

endogenously express TRH-Rs, we observed that the desensitization of IP production occurred after 10 min stimulation (data not shown), and we have previously shown that pituitary gonadotrope alpha T3-1 cells endogenously expressing GnRH-Rs do not undergo rapid desensitization of IP production (19). Untransfected cells and cells transfected with receptors not matching the stimulating agonist did not respond to stimulation (data not shown).

Rates of agonist-stimulated total IP production in COS-7 cells
Experiments were performed as described in the legend to Fig. 3. Rates are given as percent of total IP produced per minute.

| Receptor expressed in COS-7 cells | Initial rate 0–10 min | Rate after 10 min | Desensitization |
|----------------------------------|-----------------------|-------------------|-----------------|
| Rat GnRH-R                       | 0.105 ± 0.006         | 0.094 ± 0.008     | 10 ± 2          |
| Rat TRH-R                        | 0.41 ± 0.003          | 0.044 ± 0.002     | 89 ± 1          |
| GnRH-R/TRH-R chimera             | 0.15 ± 0.009          | 0.009 ± 0.002     | 94 ± 2          |
| Catfish GnRH-R                   | 0.28 ± 0.001          | 0.058 ± 0.004     | 79 ± 1          |

To investigate the possible role of the added cytoplasmic tail on the ability of the rat GnRH-R to internalize, we performed a series of internalization experiments using the four stably transfected HEK 293 cell lines described above. Approximately 70% of the rat TRH-Rs were internalized after 15 min stimulation with TRH, whereas approximately 10% of the rat GnRH-Rs were internalized after 15 min stimulation with GnRH (Fig. 5). The rat GnRH-R/TRH-R chimera and the catfish GnRH-R internalized considerably faster than the rat GnRH-R but slower than the TRH-R. More than 20% of the GnRH-R/TRH-R chimera receptors were internalized after 15 min stimulation with GnRH, and approximately 50% of the catfish GnRH-Rs were internalized after 15 min stimulation with chicken II GnRH (Fig. 5). By using a two-component model described by Koenig and Edwardson (20), we calculated the rate constant for endocytosis (k_e) and the rate constant for recycling (k_r) (Table III). The rate of endocytosis for the TRH-R was faster than for the other receptors, and the rate of recycling was similar to that of the chimera and the catfish GnRH-R but twice that of the rat GnRH-R. The rate constants for the GnRH-R/TRH-R chimera were approximately double the rate constants for the rat GnRH-R. Internalization experiments were also performed with GH3 and alpha T3-1 cells, and internalization rates were comparable to those observed for the HEK 293 cells expressing TRH or GnRH receptors (data not shown).
Desensitization and Internalization of the GnRH-R

TABLE III
Rate constants of receptor endocytosis and recycling in HEK 293 cells

| Receptor stably expressed in HEK 293 cells | Endocytosis rate constant $k_e$ | Recycling rate constant $k_r$ |
|-------------------------------------------|--------------------------------|-----------------------------|
| Rat GnRH-R                                | 0.012 ± 0.001                  | 0.033 ± 0.003               |
| Rat TRH-R                                 | 0.19 ± 0.001                   | 0.06 ± 0.001                |
| GnRH-R/TRH-R chimera                     | 0.03 ± 0.002                   | 0.063 ± 0.001               |
| Catfish GnRH-R                            | 0.099 ± 0.006                  | 0.065 ± 0.004               |

codon to the next stop codon in the 3'-untranslated region) for millions of years. We also demonstrated that cells transfected with the stop codon mutants bound GnRH agonist less well than cells transfected with WT GnRH-R, possibly due to reduced expression. The binding was observed to decrease with increasing length of the tail, and the stop codon mutant with the longest tail (52 amino acids) showed no binding at all, indicating that the cells might not be able to process a GnRH-R with a long unnatural C-terminal tail. In support of this, COS-7 cells transfected with the GnRH-R/TRH-R chimera bound GnRH agonist about 40% as well as cells transfected with WT GnRH-R, even though the added TRH-R C-terminal tail (79 amino acids) is longer than any of the ghost tails.

To investigate whether the addition of a known C-terminal tail belonging to another GPCR would affect the function of the rat GnRH-R, we created a GnRH-R/TRH-R chimera where the cytoplasmic tail of the rat TRH-R was engineered into the C terminus of the rat GnRH-R. It could be argued that the creation of a rat GnRH-R/catfish GnRH-R chimera where the C-terminal tail of the catfish GnRH-R had been added to the C-terminal of the rat GnRH-R would be more relevant. However, we chose to use the C-terminal tail of the rat TRH-R as this receptor is also a pituitary GPCR and is from the same species as the GnRH-R. Moreover, the C-terminal tail of the TRH-R is of functional importance as has been shown in several studies where mutated TRH receptors with increasingly truncated C-terminal tails were studied (6, 10). The TRH-R has also been shown by several groups to undergo desensitization at the level of IP production (7, 8). Similar data were not available for the catfish GnRH-R before this study. In addition, the amino acid sequence homology between the mammalian and the catfish GnRH-R is only 38% (14) and also the catfish GnRH-R does not recognize mammalian GnRH as its primary agonist.

We observed that GnRH stimulated total IP production in cells expressing WT rat GnRH-R did not desensitize even after prolonged stimulation with GnRH. This is in agreement with several other reports (7, 12, 19) describing the lack of desensitization of GnRH induced IP production in various cell lines (HEK 293, GH₃, and alpha T3-1 cells) expressing the receptor. The rapid desensitization observed for the TRH-R is also supported by the findings of other groups (7, 8) who studied the rapid desensitization events of TRH-R-stimulated IP production in GH₃ cells. The onset of desensitization in these cells varied considerably (from approximately 1 min to 20 min) in these studies, perhaps a result of different methods used.

The GnRH-R/TRH-R chimera desensitized in a manner similar to that observed for WT TRH-R and catfish GnRH-R, indicating that the inability of the rat’s GnRH-R to desensitize is probably caused by the receptor’s lack of a cytoplasmic C-terminal tail. It has been established that upon agonist stimulation of GPCRs the receptors become phosphorylated on different intracellular amino acids, many of which are situated on the C-terminal tail. This phosphorylation is mediated by protein kinases such as protein kinase A, protein kinase C, β-adrenoreceptor kinase, or other members of the family of G-protein receptor kinases (3, 5, 20–22). The fact that the GnRH-R lacks a cytoplasmic C-terminal tail and thus important phosphorylation sites may explain its inability to undergo rapid desensitization. The time course of desensitization of total IP production was cell line-dependent as desensitization was faster in HEK 293 cells (100 s) than in COS-7 cells (5–10 min). The reason for this is not clear but could possibly be due to differences in the intracellular mechanisms of the two cell types.

Phosphorylation of receptors has also been reported to be important for desensitization, for example via phosphorylation by β-adrenoreceptor kinase followed by recruitment of the cytosolic protein β-arrestin (20, 23) or by phosphorylation of amino acids on the C-terminal tail (11). Further studies will investigate possible phosphorylation and the functional importance of specific amino acids in the C-terminal tails of the GnRH-R/TRH-R chimera and the catfish GnRH-R. Because truncation of the C-terminal tail of the TRH-R impairs endocytosis (6), it is perhaps not surprising that the rat GnRH-R internalizes with a lower rate of endocytosis than the catfish GnRH-R and the TRH-R. The finding that the GnRH-R/TRH-R chimera also internalizes faster than the WT GnRH-R suggests that a cytoplasmic C-terminal tail is necessary for rapid internalization. We used a two-component model to calculate endocytosis and recycling rate constants $k_e$ and $k_r$, respectively. This model does not take into account synthesis of new receptors or receptor degradation. However, the model was found to be acceptable as receptor degradation is not believed to take place during the time course of an internalization experiment (21). Furthermore, we have found that internalization experiments performed in the presence of cycloheximide (10 μg/ml), a protein synthesis inhibitor, did not affect the results (data not shown).

The receptors gave similar k-values, with the exception of the rat GnRH-R which recycled at about half the rate of the others. The $k_e$ for the GnRH-R/TRH-R chimera was approximately 2-fold higher than the corresponding value for the WT GnRH-R but still lower than the values for the catfish GnRH-R and the TRH-R. Internalization rates of the GnRH-R/TRH-R are not the same as the internalization rates for the TRH-R, thus demonstrating that factors other than the C-terminal tail might also be involved in the rate of internalization. In support of this, recent evidence has suggested that sites within the third intracellular loop of the m2 muscarinic acetylcholine receptor and in the second intracellular loop of the GnRH-R are involved in receptor internalization (24, 25).

The physiological relevance of the slow internalization and lack of rapid desensitization of the mammalian GnRH-R are not understood. It is, however, possible that the receptor’s slow internalization and lack of rapid desensitization is important for keeping a large number of active receptors at the gonadotropin surge level, in particular during the GnRH surge that precedes the preovulatory luteinizing hormone surge (26). It can be speculated that in more primitive ancestral species the GnRH surge is not as profound as in mammals. In summary, we have found evidence that shows the missing cytoplasmic C-terminal tail of the rat GnRH-R to be at least partly responsible for the inability of this receptor to desensitize at the level of total IP production. We also found that the introduction of the cytoplasmic C-terminal tail of the rat TRH-R into the C-terminal.
terminus of the rat GnRH-R increased the internalization rates.

Acknowledgments—We are grateful to Dr. T. A. Bramley for the $^{125}$I-chicken II tracer; to Dr. B. Byrne, Dr. E. Faccenda, and Dr. H. Rahe for critical evaluation of the manuscript; and to Dr. G. B. Willars for helpful advice.

REFERENCES

1. Eidne, K. A., Sellar, R. E., Couper, G., Anderson, L., and Taylor, P. L. (1992) Mol. Cell. Endocrinol. 90, R5–R9
2. Morgan, R. O., Chang, J. P., and Catt, K. J. (1987) J. Biol. Chem. 262, 1166–1171
3. Lefkowitz, R. J., Hausdorff, W. P., and Caron, M. G. (1990) Trends Pharmacol. Sci. 11, 190–194
4. Anderson, L. (1996) Rev. Reprod. 1, 193–202
5. Tobin, A. B., Willars, G. B., Burford, N. T., and Nahorski, S. R. (1995) Br. J. Pharmacol. 116, 1723–1728
6. Nussenzveig, D. R., Heinflink, M., and Gershengorn, M. C. (1993) J. Biol. Chem. 268, 2389–2392
7. Davidson, J. S., Wakefield, I. K., and Millar, R. P. (1994) Biochem. J. 300, 299–302
8. Perlman, J. H., and Gershengorn, M. C. (1991) Endocrinology 129, 2679–2686
9. Falck-Pedersen, E., Heinflink, M., Alvira, M., Nussenzveig, D. R., and Gershengorn, M. C. (1994) Mol. Pharmacol. 45, 684–689
10. Matus-Leibovitch, N., Nussenzveig, D. R., Gershengorn, M. C., and Oron, Y. (1995) J. Biol. Chem. 270, 1041–1047
11. Wang, Z., Hipkin, R. W., and Ascoli, M. (1996) Mol. Endocrinol. 10, 748–759
12. McArdle, C. A., Forrest-Owen, W., Willars, G., Davidson, J., Poch, A., and Kratzaizer, M. (1995) Endocrinology 136, 4864–4871
13. Anderson, L., Alexander, C. L., Faccenda, E., and Eidne, K. A. (1995) Biochem. J. 311, 385–392
14. Tensen, C., Okuzawa, K., Blommeheer, M., Rebers, F., Leurs, R., Bogerd, J., Schulz, H., and Geor, H. (1997) Eur. J. Biochem. 243, 134–140
15. Troskie, B. E., Sun, Y., Happen, J., Seaford, S. C., Illing, N., and Millar, R. P. (1997) Program of the Endocrine Society 79th Annual Meeting, Minneapolis, MN, abstr.
16. Bramley, T. A., McPhee, C. A., and Menzies, G. S. (1992) Placenta 13, 555–581
17. Cook, J. V., Faccenda, E., Anderson, L., Cooper, G. C., Eidne, K. A., and Taylor, P. L. (1993) J. Endocrinol. 139, R1–R4
18. Anderson, L., Milligan, G., and Eidne, K. A. (1993) J. Endocrinol. 136, 51–58
19. Anderson, L., McGregor, A., Cook, J. V. F., Chilvers, E., and Eidne, K. A. (1995) Endocrinology 136, 5228–5231
20. Koenig, J. A., and Edwardson, J. M. (1997) Trends Pharmacol. Sci. 18, 276–287
21. Kohlbka, B. (1992) Annu. Rev. Neurosci. 15, 87–114
22. Richardson, R. M., Kim, C., Benovic, J. L., and Hosey, M. M. (1993) J. Biol. Chem. 268, 13650–13656
23. Menard, L., Ferguson, S. S. G., Zhang, J., Lin, F. T., Lefkowitz, R. J., Caron, M. G., and Barak, L. S. (1997) Mol. Pharmacol. 51, 800–808
24. Arora, K. K., Cheng, Z., and Catt, K. J. (1997) Mol. Endocrinol. 11, 1203–1212
25. Goldman, P. S., Schlaf, M. L., Shapiro, R. A., and Nathanson, N. M. (1996) J. Biol. Chem. 271, 4215–4222
26. Moenter, S. M., Brand, R. C., and Karsch, F. J. (1992) Endocrinology 130, 2978–2984
Gonadotropin-releasing Hormone Receptors with Intracellular Carboxyl-terminal Tails Undergo Acute Desensitization of Total Inositol Phosphate Production and Exhibit Accelerated Internalization Kinetics
Anders Heding, Milka Vrecl, Jan Bogerd, Alison McGregor, Robin Sellar, Philip L. Taylor and Karin A. Eidne

*J. Biol. Chem.* 1998, 273:11472-11477.
doi: 10.1074/jbc.273.19.11472

Access the most updated version of this article at [http://www.jbc.org/content/273/19/11472](http://www.jbc.org/content/273/19/11472)

Alerts:
- When this article is cited
- When a correction for this article is posted

[Click here](http://www.jbc.org/content/273/19/11472.full.html#ref-list-1) to choose from all of JBC's e-mail alerts

This article cites 25 references, 12 of which can be accessed free at [http://www.jbc.org/content/273/19/11472.full.html#ref-list-1](http://www.jbc.org/content/273/19/11472.full.html#ref-list-1)