A Primate-dominant Third Glycosylation Site of the $\beta_2$-Adrenergic Receptor Routes Receptors to Degradation during Agonist Regulation*

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$\beta_2$-adrenergic receptors ($\beta_2$AR) of all species are $N$-linked glycosylated at amino terminus residues $6$ and $15$. However, the human $\beta_2$AR has a potential third $N$-glycosylation site at ECL2 residue 187. To determine whether this residue is glycosylated and to ascertain function, all possible single/multiple Asn $\rightarrow$ Gln mutations were made in the human $\beta_2$AR at positions 6, 15, and 187 and were expressed in Chinese hamster fibroblast cells. Substitution of Asn-187 alone or with Asn-6 or Asn-15 decreased the apparent molecular mass of the receptor on SDS-PAGE in a manner consistent with Asn-187 glycosylation. All receptors bound the agonist isoproterenol and functionally coupled to adenyl cyclase. However, receptors without 187 glycosylation failed to display long term agonist-promoted down-regulation. In contrast, loss of Asn-6/Asn-15 glycosylation did not alter down-regulation. Cell surface distribution and agonist-promoted internalization of receptors and recruitment of $\beta$-arrestin 2 were unaffected by the loss of 187 glycosylation. Furthermore, acutely internalized wild-type and Gln-187 receptors were both localized by confocal microscopy to early endosomes. During prolonged agonist exposure, wild-type $\beta_2$AR co-localized with lysosomes, consistent with trafficking to a degradative compartment. However, Gln-187 $\beta_2$AR failed to co-localize with lysosomes despite agonist treatments up to 18 h. Phylogenetic analysis revealed that this third glycosylation site is found in humans and other higher order primates but not in lower order primates such as the monkey. Nor is this third site found in rodents, which are frequently utilized as animal models. These data thus reveal a previously unrecognized $\beta_2$AR regulatory motif that appeared late in primate evolution and serves to direct internalized receptors to lysosomal degradation during long term agonist exposure.

Like many other G-protein-coupled receptors the $\beta_2$-adrenergic receptor ($\beta_2$AR) undergoes a loss of cellular signaling during prolonged agonist exposure. This phenomenon, termed desensitization, serves to integrate $\beta_2$AR function within the context of a cell that is receiving many signals (1). In pathologic conditions, this adaptive response can also contribute to the pathophysiology of the disease and can limit therapeutic effectiveness of agonists administered over prolonged periods of time (1). Early molecular events during short term agonist activation include phosphorylation of the $\beta_2$AR by G-protein-coupled receptor kinases and protein kinase A, which act to rapidly regulate receptor function along a minute-by-minute time frame. With long term agonist exposure, an additional series of events leads to a net loss of cellular receptors, termed down-regulation, which markedly reduces the cellular response (such as cAMP production). The basis of receptor down-regulation includes degradation of the $\beta_2$AR protein, which is linked to early desensitization events, because G-protein-coupled receptor kinase-mediated phosphorylation of $\beta_2$AR leads to recruitment and binding of $\beta$-arrestin, which, via its scaffolding functions, facilitates internalization (2). A sorting of internalized receptors can result in reinsertion into the cell membrane or movement of a receptor into a degradative pathway with an ultimate loss of the intact receptor (3). The structural features of the $\beta_2$AR that appear to be involved in internalization, recycling, and/or down-regulation, include G-protein-coupled receptor kinase phosphorylation sites in the intracellular tail (4), the third intracellular loop protein kinase A phosphorylation site (5), the NPXXY motif of the seventh transmembrane spanning domain (6), and the PDZ-binding domain in the distal carboxyl-terminal tail of the receptor (7). Expression or trafficking of some G-protein-coupled receptors, such as the $\beta_2$AR, gastrin-releasing peptide receptor, and the V2-vasopressin receptor is dependent on post-translational modifications including $N$- and $O$-linked glycosylation (8–10). All $\beta_2$AR genes identified to date from multiple species have two sites for $N$-linked glycosylation within the amino terminus of the receptor at amino acid positions $6$ and $15$. Removal of these sites in the hamster $\beta_2$AR by mutagenesis results in receptors that have an impaired capacity to insert into the membrane. Nevertheless, $\sim50\%$ of the total receptor complement is ultimately expressed on the cell surface (8).

Interestingly, the human $\beta_2$AR has an additional potential $N$-glycosylation site, localized to the second extracellular loop, at amino acid 187; this consensus sequence is not found in a wide variety of non-primate species reported to date (see Fig. 1). This prompted us to examine the role of Asn-187 of the human $\beta_2$AR with the idea that this modification may represent a specific mechanism for receptor trafficking that occurred late in mammalian evolution and serves a unique function necessary for primate homeostasis.

MATERIALS AND METHODS

Constructs and Transfections—Site-directed mutagenesis of the human $\beta_2$AR cDNA was carried out so as to substitute Gln for Asn at $\beta_2$AR by G-protein-coupled receptor kinase phosphorylation sites in the intracellular tail (4), the third intracellular loop protein kinase A phosphorylation site (5), the NPXXY motif of the seventh transmembrane spanning domain (6), and the PDZ-binding domain in the distal carboxyl-terminal tail of the receptor (7). Expression or trafficking of some G-protein-coupled receptors, such as the $\beta_2$AR, gastrin-releasing peptide receptor, and the V2-vasopressin receptor is dependent on post-translational modifications including $N$- and $O$-linked glycosylation (8–10). All $\beta_2$AR genes identified to date from multiple species have two sites for $N$-linked glycosylation within the amino terminus of the receptor at amino acid positions $6$ and $15$. Removal of these sites in the hamster $\beta_2$AR by mutagenesis results in receptors that have an impaired capacity to insert into the membrane. Nevertheless, $\sim50\%$ of the total receptor complement is ultimately expressed on the cell surface (8).

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β2AR Thrid Glycosylation Site

FIG. 1. Amino acid sequence alignment of the second extracellular loops of β2ARs from various species. Amino acid 187 (human sequence) is indicated (arrow) and as shown is not present in a wide variety of non-primate species.

FIG. 2. Glycosylation of the human β2AR at Asn-187. A representative Western blot of mutated human β2ARs expressed in Chinese hamster fibroblast cells with the eight possible combinations of Asn → Glu substitution at positions 6, 15, and 187 is shown (inf; non–transfected Chinese hamster fibroblast cell lysates, see “Materials and Methods” for other abbreviations). The wild-type β2AR appears as a multiply glycosylated receptor with the major high molecular mass form at ~60 kDa. Each substitution reduced the apparent molecular mass proportionally, including those with the Asn-187 → Glu substitution in isolation or with other substitutions.

RESULTS AND DISCUSSION

Western blots of membranes from transfected cells expressing the wild-type and seven mutated β2ARs are shown in Fig. 2. As expected, an individual removal of position 6 or 15 glycosylation sites resulted in decreases in apparent molecular mass of the major high molecular mass form (from ~60 to ~56 kDa), and the loss of both sites reduced the molecular mass proportionally. Consistent with position 187 also being glycosylated, the NNQ receptor also had a lower apparent molecular mass like that of the QNN and NNQ receptors. Removal of the position 187 site, in combination with those at 6 and/or 15, had the expected additive decrease in apparent molecular mass of the major receptor species as shown.

Each of the expressed receptors had similar binding affinities for isoproterenol, and functionally stimulated adenyl cyclase (Table I). The consequences of long term (18 h) exposure of cells expressing each of the eight receptors on down-regulation are shown in Fig. 3. A striking and consistent effect imparted by the removal of the glycosylation site at position 187 was observed. As shown, wild-type β2AR underwent a 34 ± 3.4% down-regulation. A similar extent of down-regulation was also observed for the single and doubly substituted QNN, NNQ, and QNQ receptors. In contrast, single removal of the 187-glycosylation site (NNQ receptor) resulted in a complete loss of agonist-promoted down-regulation, and in fact an increase in expression was noted (15 ± 6.7%, p < 0.01 versus wild-type). This same phenotype was observed with all mutant β2ARs.
treated for 12 h with 0.25 μM H9262, then for an additional 12 h with 10 μM H9262.

Long term agonist-promoted down-regulation of the human β2AR (control) for 18 h in culture and then washed, and membranes were prepared. Receptor expression was determined with [125I-CYP saturation binding. See "Materials and Methods" for details. Any substitution that removed the position 187-glycosylation site (NNQ, NQQ, QNQ, QQQ) resulted in no loss of receptor expression (down-regulation), and indeed increases in expression were observed as shown. *, p = 0.02 or less, n = 4–5 experiments.

(NNQ, QNN, NQQ, and QQQ) where the position 187 was mutated, regardless of the presence or absence of the other glycosylation sites (Fig. 3). The QQQ receptor displayed the greatest increase in expression during agonist exposure. This may be because of the fact that the QQQ receptor has somewhat less down-regulation than wild-type and thus less impediment to the up-regulation events imposed by QQQ, as compared with NNQ. Additional studies were also performed using the glycosylation inhibitor tunicamycin. Chinese hamster fibroblast cells expressing wild-type β2AR at 50% confluency were treated for 12 h with 0.25 μg/ml tunicamycin in the media and then for an additional 12 h with 10 μM isoproterenol. Tunicamycin-treated cells underwent only 46 ± 1.3% of the isoproterenol-promoted down-regulation observed with non-tunicamycin-treated cells. These results further support the notion that it is the loss of glycosylation imposed by the mutation at 187 that leads to the altered down-regulation phenotype of the NNQ receptor.

The basis for the marked phenotype imposed by the glycosylation deficiency at position 187 was further explored with the NNQ mutant, thereby maintaining wild-type glycosylation at positions 6 and 15. At baseline (i.e. absence of agonist exposure) both wild-type and NNQ β2AR were predominantly expressed at the cell surface, as qualitatively shown by confocal microscopy (Fig. 4) and quantitatively determined by whole-cell radioligand binding with hydrophilic and hydrophobic ligands (95 ± 8.3 versus 90 ± 2.4% of the total receptor complement were at the cell surface for wild-type versus NNQ, respectively, n = 4). One of the earliest events in internalization of β2AR upon agonist binding is the recruitment of β-arrestin from the cytosol to the cell-surface-expressed receptors. β-arrestin-green fluorescent protein translocation was ascertained by confocal microscopy in live cells (Fig. 5). Agonist (10 μM isoproterenol) exposure resulted in a time-dependent recruitment of β-arrestin in cells expressing either receptor. As shown, in wild-type (NNN)-expressing cells, the predominantly intracellular distribution of β-arrestin becomes mostly cell surface by 5 min of exposure to isoproterenol. The translocation of NNQ was qualitatively indistinguishable from the wild-type β2AR. Consistent with these observations, agonist-promoted receptor internalization as determined by quantitative hydrophilic radioligand binding with intact cells revealed identical kinetics both in terms of the maximal response (45 ± 4.4 versus 49 ± 6.2% internalized) and the rate constant (k = 0.16 ± 0.029/min versus 0.12 ± 0.022/min, n = 4) for the NNQ and NNQ receptors, respectively (Fig. 6). Taken together, these data indicate that the initial events in agonist-promoted down-regulation, which leads to the redistribution of cell surface β2AR to the cell interior, are not perturbed by the absence of glycosylation at Asn-187 of the receptor.

We considered then that the agonist-promoted up-regulation observed with the glycosylation site 187-deficient NNQ β2AR could be the result of an altered (enhanced) synthesis of new receptors during agonist exposure, or internalized receptors that are not targeted in a wild-type manner to the degradation pathway. New receptor synthesis in the presence of agonist was quantitated by radioligand binding after the exposure of the cells to an irreversible receptor-alkylating agent (Pindobind, Sigma). Cells were treated with 10 μM Pindobind for 2 h (which alkylated ~70% of the receptors), washed, and placed back in

**Table I**

| Receptor | Kᵢ, ISO | ISO stimulation of adenyl cyclase | B<sub>max</sub> |
|----------|---------|----------------------------------|------------|
| NNN      | 253 ± 42 | 33.7 ± 6                         | 207 ± 55   |
| QNN      | 374 ± 77 | 37.2 ± 5                         | 203 ± 33   |
| NQQ      | 257 ± 76 | 42.3 ± 5                         | 133 ± 14   |
| QNN      | 247 ± 29 | 24.1 ± 1                         | 304 ± 48   |
| QQN      | 371 ± 136| 38.4 ± 1                         | 226 ± 15   |
| NQQ      | 183 ± 70 | 27.2 ± 2                         | 133 ± 17   |
| NNN      | 111 ± 14 | 49.1 ± 5                         | 381 ± 70   |
| QQQ      | 296 ± 113| 35.4 ± 4                         | 368 ± 62   |
the incubator. At various time points up to 12 h, cells were harvested, and $^{125}$I-CYP saturation binding was undertaken. Newly synthesized receptors appear as an increase in $^{125}$I-CYP binding over time. (Of note, pretreatment of cells with the protein synthesis inhibitor cycloheximide resulted in no increase in $^{125}$I-CYP binding, consistent with the technique identifying newly synthesized receptors.) The rate of new receptor synthesis in the presence of $10^{-6}$/M isoproterenol, which is the condition relevant to the agonist-promoted up-regulation of NNN, was not significantly different, and, if anything, the trend was toward being less for NNQ compared with NNN ($k = 0.07 \pm 0.02$/min versus $0.12 \pm 0.04$/min, $n = 4$). It thus appears that the accumulation of NNQ receptors evoked by the agonist is not based on decreased receptor internalization or increased de novo receptor synthesis, suggesting that the NNQ receptor has altered entry into the degradation pathway(s) after internalization.

The fate of internalized receptors was assessed by confocal microscopy of fixed cells, with an emphasis on co-localization of NNN or NNQ with early endosomes (a short term event) and lysosomes (a long term event). Results of studies with the EEA1 antibody, which identifies early endosomes, are shown in Fig. 7. For both NNN and NNQ receptors, a 15-min exposure to isoproterenol resulted in internalization (Fig. 7, d and h) and co-localization to early endosomes (f and i). Similar findings were observed with incubations up to 4 h (data not shown). Taken together with the other short term agonist data shown in Figs. 5 and 6, it appears that NNQ internalizes and is localized to the early endosomes in a wild-type manner. However, as shown in Fig. 8, NNQ fails to co-localize with lysosomes during prolonged agonist incubation. For the NNN wild-type $\beta_2$AR, one can clearly observe a loss of cell surface and total receptor expression (a versus d). Most internalized receptors co-localized with lysosomes as indicated by the merged image with cathepsin-D (f). In contrast, there is no apparent net loss of the NNQ receptor because of agonist exposure (g versus j), and the intracellular NNQ receptor does not co-localize with the lysosomal marker (l). Shown are representative images from 4 to 5 experiments.

Fig. 7. Agonist-promoted co-localization of NNQ-$\beta_2$AR with early endosomes is unimpaired. Confocal images from cells visualizing receptor (FLAG), early endosomes (EEA1), and the merged image are shown. Agonist exposure resulted in the internalization of NNN and NNQ (d and h) receptors, as well as the co-localization to early endosomes (f and i). Shown are representative images from 4 to 5 experiments.

The NNQ-$\beta_2$AR fails to enter the lysosomal degradation pathway. For wild-type (NNN) $\beta_2$AR, agonist exposure resulted in a net loss of $\beta_2$AR and a redistribution of the receptor to the cell interior (a versus d). Most internalized receptors co-localized with lysosomes as indicated by the merged image with cathepsin-D (f). In contrast, there is no apparent net loss of the NNQ receptor because of agonist exposure (g versus j), and the intracellular NNQ receptor does not co-localize with the lysosomal marker (l). Shown are representative images from 4 to 5 experiments.

Fig. 8. The NNQ-$\beta_2$AR fails to enter the lysosomal degradation pathway. For wild-type (NNN) $\beta_2$AR, agonist exposure resulted in a net loss of $\beta_2$AR and a redistribution of the receptor to the cell interior (a versus d). Most internalized receptors co-localized with lysosomes as indicated by the merged image with cathepsin-D (f). In contrast, there is no apparent net loss of the NNQ receptor because of agonist exposure (g versus j), and the intracellular NNQ receptor does not co-localize with the lysosomal marker (l). Shown are representative images from 4 to 5 experiments.

Fig. 6. Agonist-promoted internalization of the position 187 glycosylation-deficient $\beta_2$AR is unimpaired. Cells were exposed to $10^{-6}$/M isoproterenol for the indicated times and then washed, and whole-cell radioligand binding with the hydrophilic radioligand $^3$H-CGP12177 was carried out as described under "Materials and Methods." Results are from 4 experiments.
TABLE II
Sequence comparisons of the second extracellular loops of primate β2-ARs

Amino acid reference numbers are from the human sequence. The higher order primates (human and greater and lesser apes) all have the N-linked glycosylation site at position 187. In contrast, none of the lower order primates sequenced to date have this site.

| Species       | Sequence Comparison |
|---------------|---------------------|
| human         | RATHGEACINYYETCDDFT |
| chimpanzee    | RATHGEACINYYETCDDFT |
| orangutan     | RATHGEACINYYETCDDFT |
| gorilla       | RATHGEACINYYETCDDFT |
| rhesus monkey | RATHGEACINYYETCDDFT |
| gibbon        | RATHGEACINYYETCDDFT |
| spider monkey | RATHGEACINYYETCDDFT |
| galago        | RATHGEACINYYETCDDFT |
| tamarin       | RATHGEACINYYETCDDFT |
| lemur         | RATHGEACINYYETCDDFT |

marker (1). Four hours of agonist exposure represents an optimal time point for co-localization of wild-type receptor with lysosomes, as longer exposures result in degradation and a decreased signal. Nevertheless, even with more prolonged agonist exposure (up to 18 h), we never observed the co-localization of NNQ with lysosomes (data not shown). To further support the confocal findings, cells expressing NN or NNQ were treated with vehicle or isoproterenol for 4 h, whole-cell homogenates were prepared, and the proteins were fractionated on a Percoll gradient. Consistent with the findings of others (25), one of the six fractions (fraction 5) was enriched in lysosomes as determined by Western blots using the cathepsin-D antibody. 125I-CYP radioligand binding was performed on the proteins from this lysosomal fraction to ascertain whether agonist exposure increased receptors in this pool for NNQ-expressing cells and had no effect on the pool of NNQ receptors. Although this fraction undoubtedly contains other compartments/proteins in addition to the specific lysosomes identified by confocal microscopy, we indeed observed increased agonist-promoted receptors in the fraction from NNQ cells (from 123 ± 24 to 218 ± 42 fmol/mg, p = 0.03, n = 8), but not NNQ cells (from 65 ± 12 to 65 ± 17 fmol/mg). Although the magnitude of the agonist effect with the wild-type is modest in this assay, the lack of any change with the NNQ mutant is consistent with the confocal results.

The phenotype of the NNQ receptor, then, appears to be one in which the agonist-promoted down-regulation process is perturbed at the recycling step because of a lack of glycosylation at this third extracellular site. The net effect is a lack of down-regulation and, in fact, a net increase in cellular receptors due to the continued synthesis of new receptors in an environment of decreased degradation. Thus a previously unrecognized site necessary for agonist-promoted down-regulation has been identified. However, it is noted that the β2-ARs of other species that lack Asn-187 (e.g. hamster, guinea pig, rat β2-AR) (20–24) nevertheless have been reported to undergo agonist-promoted down-regulation in vivo, although the phenomenon has been minimally explored in recombinant cells expressing non-human β2-ARs (23). This may suggest that other sites/mechanisms are in place in these receptors that abrogate the necessity of Asn-187. As noted earlier, this site is absent in a wide variety of diverse species despite the high degree of surrounding sequence homology (Fig. 1). However, of the 15 β2-AR genomic sequences reported from primates, 5 have the Asn glycosylation site at the second intracellular loop residue, as is observed in the human receptor (Table II). These five Asn-187-containing primates are higher order primates (human, greater and lesser apes), whereas those without this glycosylation site are all lower order primates (monkey, lemur, tamarin, and galago). This suggests that perhaps Asn-187 provides a specific role for receptor regulation in the larger higher order primates. This mechanism may have evolved because of (i) pressure for this specific type of glycosylation-dependent trafficking or (ii) as a compensatory consequence to retain receptor down-regulation after the evolutionary loss of an alternate down-regulatory motif. β2-AR sequence comparisons of primate species that carry Asn-187 with those that do not and comparisons with non-primates are hampered by the lack of full-length sequences in primates. Thus scenario (ii) above cannot be adequately explored at this time. Nevertheless, the current study has identified a previously unrecognized site that is essential for long term agonist-promoted down-regulation and has apparently appeared late in primate evolution.

REFERENCES

1. Kohout, T. A., and Leffkowitz, R. J. (2003) Mol. Pharmacol. 63, 9–18
2. Claing, A., Laporte, S. A., Caron, M. G., and Leffkowitz, R. J. (2002) Prog. Neurobiol. 66, 61–79
3. Sorkin, A., and von Zastrow, M. (2002) Nat. Rev. Mol. Cell. Biol. 3, 600–614
4. Ferguson, S. S., Menard, L., Barak, L. S., Koch, W. J., Colapietro, A. M., and Caron, M. G. (1995) J. Biol. Chem. 270, 24782–24789
5. Bouvier, M., Collina, S., O’Dowd, B. F., Campbell, P. T., Deblasi, A., Koblika, B. K., MacGregor, C., Irons, G. P., Caron, M. G., and Leffkowitz, R. J. (1989) J. Biol. Chem. 264, 16786–16792
6. Barak, L. S., Tiberi, M., Freedman, N. J., Kwatra, M. M., Leffkowitz, R. J., and Caron, M. G. (1994) J. Biol. Chem. 269, 2790–2795
7. Xiang, Y., and Koblika, B. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 10776–10781
8. Rands, E., Candelore, M. R., Cheung, A. H., Hill, W. S., Strader, C. D., and Dixon, R. A. (1990) J. Biol. Chem. 265, 10759–10764
9. Benya, R. V., Kusui, T., Katsumo, T., Tauda, T., Martey, S. A., Battey, J. F., and Jensen, R. T. (2000) Mol. Pharmacol. 58, 1540–1551
10. Sagdehi, H., and Birnbaum, M. (1999) Glycobiology 9, 731–737
11. Ahn, S., Nelson, C. D., Garrison, T. R., Miller, W. E., and Leffkowitz, R. J. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 1740–1744
12. Green, S. A., Spassoff, A. P., Coleman, R. A., Johnson, M., and Liggett, S. B. (1996) J. Biol. Chem. 271, 24029–24035
13. Green, S., and Liggett, S. B. (1994) J. Biol. Chem. 269, 26215–26219
14. Green, S. A., Cole, G., Jacinto, M., Innis, M., and Liggett, S. B. (1993) J. Biol. Chem. 268, 23116–23121
15. Mason, D. A., Moore, J. D., Green, S. A., and Liggett, S. B. (1999) J. Biol. Chem. 274, 12670–12674
16. Moore, R. H., Tuffaha, A., Millan, E. E., Dai, W., Hall, H. S., Dickey, B. F., and Knoll, B. J. (1999) J. Cell Sci. 112, 329–338
17. Volpizzoli, L. A., Lah, J. J., and Levey, A. I. (2001) J. Biol. Chem. 276, 47590–47598
18. Green, S. A., Zimmer, K. P., Griffiths, G., and Mollman, I. (1987) J. Cell Biol. 105, 1227–1240
19. Smith, P. K., Krohn, R. I., Hermanson, G. T., Malins, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goekoe, N. M., Olson, B. J., and Klein, D. C. (1985) Anal. Biochem. 150, 76–85
20. Auman, J. T., Seidler, F. J., Tate, C. A., and Slotkin, T. A. (2001) Am. J. Physiol. 281, R1895–R1901
21. Effleth, M. S., and Redl, J. L. (1990) Eur. J. Pharmacol. 182, 387–392
22. Nanoff, C., Freusburg, M., Tsuchi, E., and Schutz, W. (1989) J. Cardiovasc. Pharmacol. 13, 198–203
23. Molenaar, P., Smolich, J. J., Russell, F. D., McMartin, L. R., and Summers, R. J. (1990) J. Pharmacol. Exp. Ther. 255, 393–400
24. Cheung, A. H., Dixon, R. A., Hill, W. S., Sigal, I. S., and Strader, C. D. (1990) Mol. Pharmacol. 37, 775–779
25. van der Spoel, A., Bonten, E., and d’Azn, A. (1998) EMBO J. 17, 1588–1597
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