*Hypothesis*

# Identification of a conserved protein motif in a group of growth factor receptors

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Residues 370–383 (helix C) of the human nerve growth factor receptor (NGF-R) are highly similar to the sequence of the 14 residue wasp toxin, mastoparan. Both regions are predicted to form amphiphilic α-helices, as is the amino-terminal region of the third intracytoplasmic loop (i3) of the $\beta_2$-adrenergic receptor ($\beta_2$AR). As both mastoparan and the $\beta_2$AR i3 interact with G-proteins, it is suggested that helix C of the NGF-R may facilitate interactions with a cytoplasmic protein. A similar structural motif was identified in the cytoplasmic domains of a number of other growth factor receptors, suggesting an important role for this motif in signal transduction mechanisms.

Mastoparan; Nerve growth factor; G-protein; Signal transduction

## 1. INTRODUCTION

The actions of nerve growth factor (NGF) mediated by binding to its receptor (NGF-R) are crucial to the survival and maintenance of sympathetic and sensory neurons (reviewed in [1]). Following binding of NGF to the high affinity form of NGF-R, activation of a number of second messenger systems has been described, including that of phosphatidyl inositol hydrolysis and elevation of intracellular $Ca^+$ levels [2]. It has also been suggested that the conversion from a low to high affinity type of receptor is dependent upon interactions with a second protein [4]. These results have led to the speculation that NGF exerts its actions through the NGF-R via coupling to some type of G-protein. The inhibition of NGF-induced neurite extension in PC12 cells by antibodies directed against the ras p21 protein is consistent with this hypothesis [3]. However, in contrast to the well-characterized family of G-protein-linked receptors which contain 7 transmembrane domains, the NGF-R contains only a single intracytoplasmic domain. In addition, direct comparisons of the primary sequences of NGF-R [5–7] with the family of G-protein-linked receptors does not reveal any highly conserved regions.

Recently, a region of similarity was shown to exist between the sequence of the hamster $\beta_2$-adrenergic receptor ($\beta_2$AR) and the 14 amino acid residue wasp toxin, mastoparan [11]. Both deletion mutagenesis of the hamster $\beta_2$AR [9] and proteolysis experiments of rhodopsin [8] have led to the conclusion that intracytoplasmic domain 3 of the $\beta_2$AR (i3, residues 221–273) is crucial for coupling of this receptor to $G_s$, and the subsequent activation of adenylate cyclase. In particular, the region from residues 222–229 was determined to be essential for cyclase activation, while deletion of residues 258–270 resulted in severe attenuation of cyclase stimulation. These regions are predicted to form amphiphilic α-helical structures, similar to that determined for mastoparan, a potent activator of $G_o$ [10]. It has therefore been suggested that the secondary structure of these regions is responsible for the $\beta_2$AR-$G_s$ interactions [11]. We have therefore examined the amino acid sequence of the human NGF-R for regions of secondary structure similar to those predicted to be present both in the $\beta_2$AR as well as in the peptide mastoparan.

## 2. METHODS

Secondary structure predictions of amino acid domains were carried out using both the Chou-Fasman [18] and Garnier et al. [12] procedures to detect potential areas of α-helicity. Selected regions were then evaluated to determine the hydrophobic moment according to the method of Eisenberg et al. [13]. Protein analysis was carried out with the Sequence Analysis Software Package Version 6.0, Genetics Computer Group, University of Wisconsin Biotechnology Center.

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## 3. RESULTS AND DISCUSSION

Comparison of the amino acid sequence of the cytoplasmic domain of human NGF-R (residues 245–399) to the hamster $\beta_2$AR i3 using either the Bestfit or Gap algorithms [16] did not reveal any regions of high similarity. Secondary structure determinations predict that the NGF-R cytoplasmic domain contains 3 regions of potential $\alpha$-helical structure, residues (A) 316–325, (B) 354–365, and (C) 370–383. Calculation of hydrophobic moments suggested that regions A and C could form amphiphilic $\alpha$-helices. Graphical representation of these regions using the helical wheel procedure [14] shows that helix C possesses a distinct arc of hydrophobicity and one of charged residues (Fig. 1). This arrangement is similar to that of the N-terminal region of the $\beta_2$AR i3, as well as to that of mastoparan. Furthermore, the relative spacing (in degrees of arc going in a clockwise direction) between 3 of the 4 charged residues of NGF-R helix C (Arg-379, 0°; Asp-371, 80°; and Arg-378, 100°) are identical to the distances predicted between the 3 charged residues of mastoparan, but differ from those of the $\beta_2$AR (Arg-1, 0°; Arg-8, 20°; and Lys-7, 120°). In fact, residues 370–380 of NGF-R and residues 3–13 of mastoparan show 100% conservation of the relative positions of both the charged and hydrophobic residues. This similarity can more readily be seen in a helical net diagram [15], in which the distributions of both the hydrophobic and charged residues is virtually

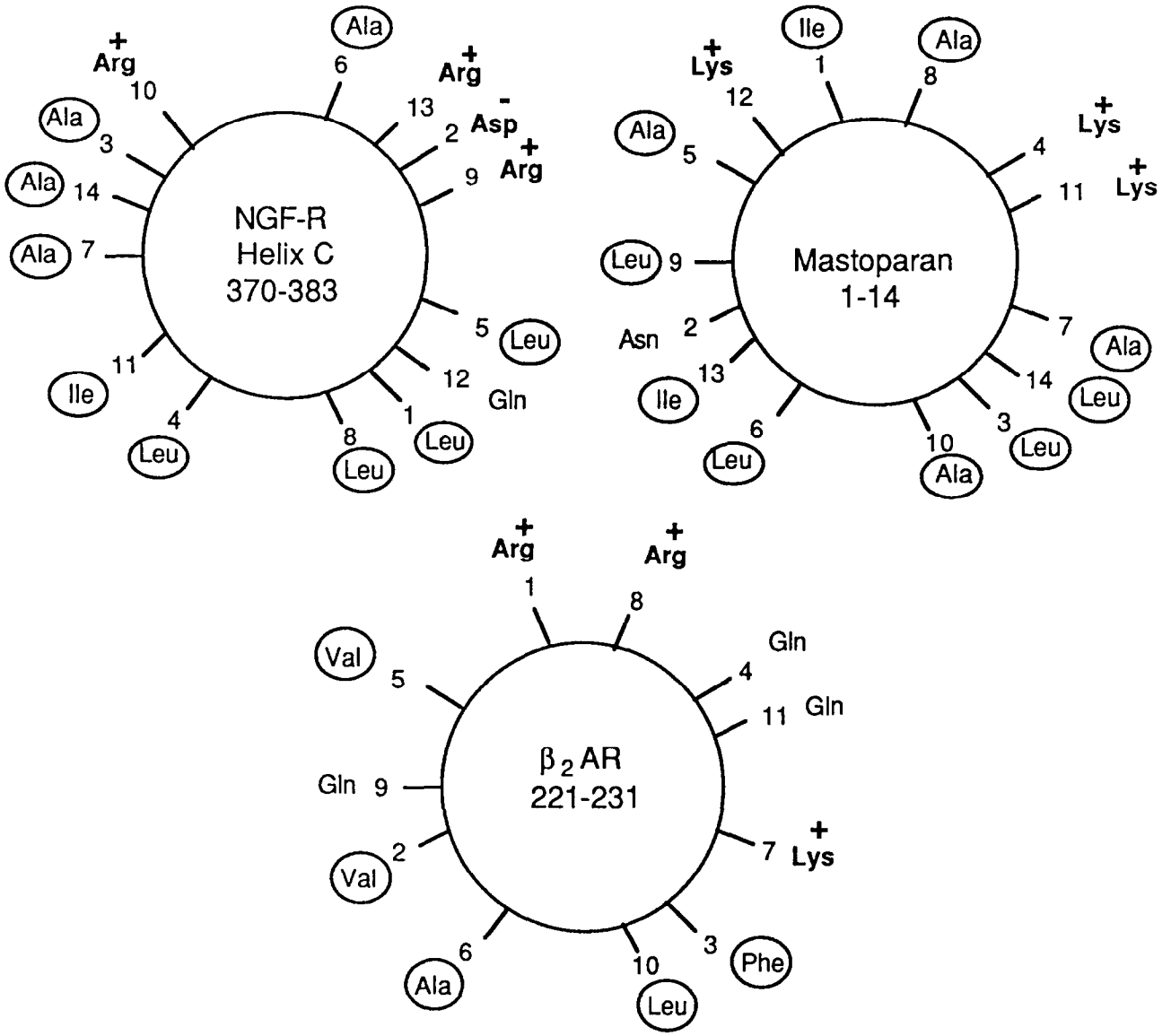


Fig. 1. Helical wheel representations of potential $\alpha$-helical regions. The amino acid residues of the NGF-R helix C, mastoparan, and the N-terminal region of $\beta_2$AR i3 have been displayed using Edmundson helical wheels. Hydrophobic residues are encircled, charged residues are in bold face. Residues are separated from each other by 100°. Position 1 in each wheel corresponds to the first residue of the plotted sequence.

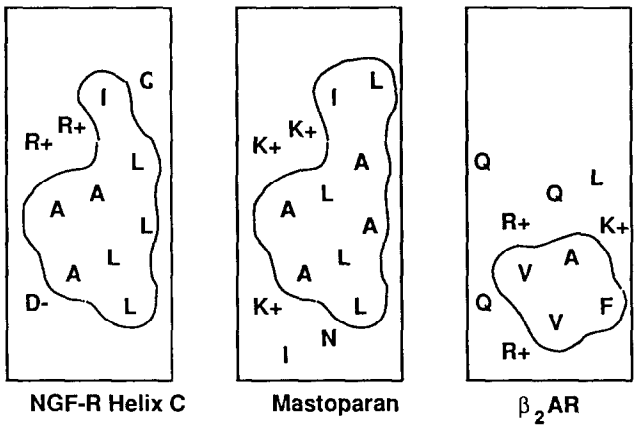


Fig. 2. Helical nets of potential amphiphilic $\alpha$-helical regions. The amino acid residue of the NGF-R helix C, mastoparan, and the $\beta_2$AR i3 N-terminal region are displayed. Hydrophobic residues are encircled.

Table I

Alignment of potential growth factor receptor amphiphilic helices

| Name | Residue | | | | | | | | | | | | | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mast | 3 | L | K | A | L | A | A | L | A | K | K | I | L | 10 |
| NGF-R | 370 | L | D | A | L | L | A | A | L | R | R | I | Q | R 5 |
| EGF-R | 937 | F | R | E | L | I | I | E | F | S | K | M | A | R 25 |
| PDGF-R | 919 | F | S | Q | L | V | L | L | L | E | R | L | L | G 26 |
| IGF1-R | 1229 | F | L | E | I | I | S | S | I | K | E | E | M | E 22 |
| I-R | 1244 | F | L | E | I | V | N | L | L | K | D | D | L | H 23,24 |
| | Cons. | H | X | X | H | H | X | X | H | C | C | X | H | C |

The consensus residues were derived by visual inspection. H, hydrophobic; C, charged; X, any. All sequences correspond to the human receptors. The position number of the first residue of each sequence is given. EGF, epidermal growth factor; PDGF, platelet derived growth factor; IGF1, insulin-like growth factor 1; I, insulin

identical in the 2 molecules (Fig. 2). The similarity of the $\beta_2$AR i3 helix to mastoparan is less obvious. The amino acid sequence of NGF-R helix C is highly conserved from human to chicken [7], having conservative substitutions at positions 375 (valine to alanine) and 379 (lysine to arginine).

The carboxy terminal portion of the $\beta_2$AR i3 was also shown to be necessary for optimum interaction with $G_s$ [11], which prompted comparison of its predicted secondary structure to the NGF-R helices. The helical wheel representations of both NGF-R helix A and the $\beta_2$AR i3 C-terminal region contained 5 charged residues, generally located in a distinct arc of the helix (not shown). However, the relative positioning of the charged residues was not well conserved.

The high degree of similarity that exists between the secondary structures of the NGF-R helix C and mastoparan prompted further examination of a number of other receptor molecules which contain only a single cytoplasmic domain (Table I). Some of these receptors (IR, IGF1-R, IGF2-R) have also been suggested to interact with G-proteins [19–21] or to undergo receptor dimerization upon agonist binding [22,23]. The sequences of these receptors were examined visually for the presence of an arc of hydrophobicity and the presence of charged residues in the opposite side of an $\alpha$-helix. A region containing at least 5 contiguous hydrophobic residues was found in the receptors for EGF, PDGF, IGF-1 and insulin but not for IGF-2 (Fig. 3). Interestingly, this region was located in all 4 receptors immediately following their respective tyrosine kinase domains. These regions are all predicted to form $\alpha$-helices by the Chou-Fasman rules, except for that of the IGF1-R, in which only the carboxyl portion has a high $\alpha$-helical tendency. Although the NGF-R contains a glutamine residue in one of the 5 conserved hydrophobic positions, its presence may be compensated for by the extended length of its hydrophobic arc towards the carboxy-terminus.

Comparison of the 5 receptor sequences and mastoparan indicates that positions 10, 13, and 9 (except for EGF-R) are always occupied by a charged residue. The overall alignment of the 6 regions is consistent with that expected of amphiphilic $\alpha$-helices [14], having a strong clustering of hydrophobic residues on one side of the helix, and more polar side groups (both charged and uncharged) on the opposite side.

The above considerations suggest that the NGF-R contains a region of secondary structure which could facilitate interactions with a second cytoplasmic protein. This region should therefore be considered a likely candidate site for mutagenesis studies of the NGF-R interactions with other proteins. The presence of similar amphiphilic $\alpha$-helical structures in the cytoplasmic domains of a number of other growth factor receptors further supports the concept that this motif plays a functionally important role in the mechanisms of signal transduction.

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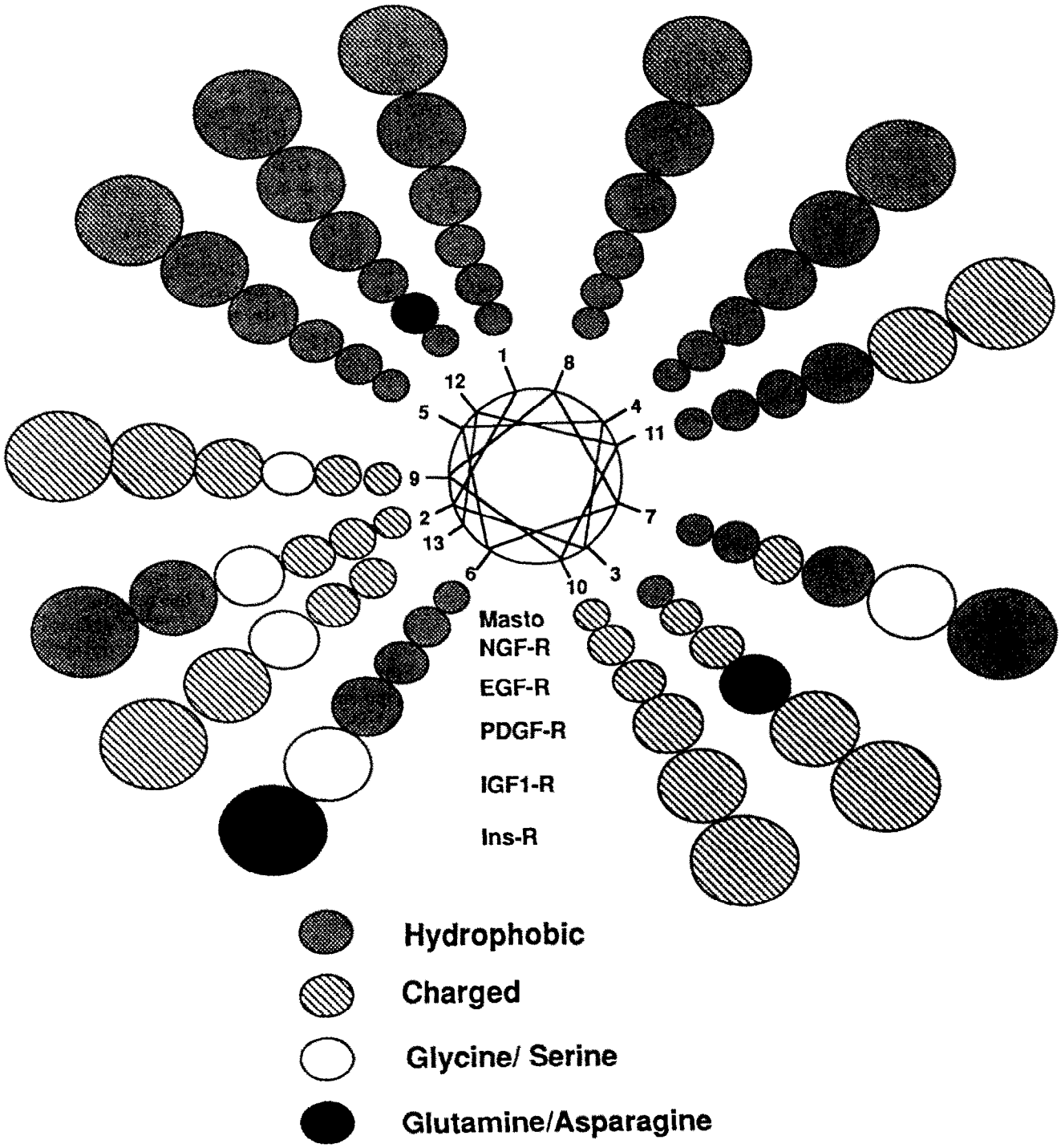


Fig. 3. Helical wheel representation of potential growth factor amphiphilic helices.

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