# The extracellular domain of substance P (NK1) receptor comprises part of the ligand binding site

Tung Ming Fong, Hong Yu, and Catherine D. Strader
Department of Molecular Pharmacology and Biochemistry, Merck Sharp and Dohme Research Laboratories, Rahway, New Jersey, 07065 USA

## INTRODUCTION

The peptide neurotransmitter substance P (SP) binds preferentially to the NK1 subtype of neurokinin receptor (NK1R) to elicit various biological responses. SP also binds to two other subtypes (i.e., NK2 and NK3) of neurokinin receptors with lower affinities. Sequence analysis of the cloned rat NK1R indicates that it belongs to the heptahelical receptor family (Yokota et al., 1989). Elucidation of the molecular basis of subtype-specific interaction between SP and NK1R will require the localization of the binding domain. In the present report, we have used mutagenesis and heterologous expression approaches to show that part of the extracellular $NH_2$-terminal sequence is required for high affinity binding of peptides. The cDNA encoding the human NK1R was also cloned, and sequence comparison with the rat NK1R reveals 22 amino acid substitutions. Site-directed mutagenesis suggested that the 97th residue in the second extracellular segment interacts with some peptides but not others.

## RESULTS AND DISCUSSION

The cloned rat NK1R, human NK1R and their mutants were expressed in COS cells. To determine the binding affinity of peptide agonists SP, substance K (SK) and neurokinin B (NKB), competition binding experiments were performed in the presence of 0.2 nM $^{125}$I-Bolton Hunter labeled SP and various concentrations of peptides using intact cells. Free $^{125}$I-SP was separated from bound $^{125}$I-SP by glass fiber filters. The equilibrium dissociation constant $K_d$ was calculated from the $IC_{50}$ value (Fig. 1).

Based on the amino acid sequence analysis, the residues 1–31 are predicted to be on the extracellular side. Two substitution mutants of rat NK1R were constructed. With the S(1–13) mutant where the $NH_2$-terminal residues 1–13 of rat NK1R were substituted by the sequence MDYKDDDDKPW, a threefold decrease in ligand binding affinity was observed (Table 1). With the S(1–27) mutant where the $NH_2$-terminal residues 1–27 of rat NK1R were substituted by the same sequence, the specific $^{125}$I-SP binding was further decreased ($K_d$ > 100 nM). These data suggest that the residues 14–27 are required for high affinity binding of peptides to the NK1R.

Functional expression of both the rat and human NK1R in COS cells indicated all three peptide agonists have higher binding affinities at the human receptor than the rat receptor (Table 1). This functional difference must be attributable to the sequence divergence because the human NK1R has 22 amino acid residues that are different from rat NK1R (Fong et al., 1992). The largest divergence is at position 97 where rat NK1R contains a Val while human NK1R contains a Glu. The

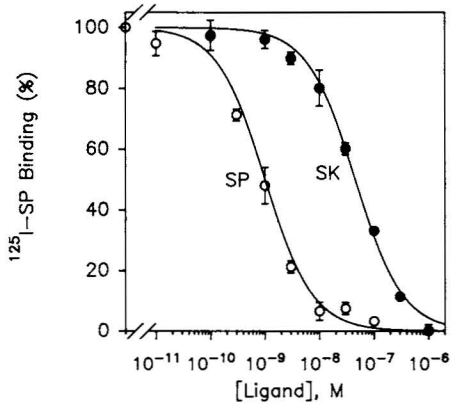


FIGURE 1 Inhibition of $^{125}$I-SP binding to the human NK1R by unlabeled peptide. The curves were calculated according to the equation [CPM(L) − CPM(1 μM SP)]/[CPM(0) − CPM(1 μM SP)] = $IC_{50}/(L + IC_{50})$ in which CPM(L) and CPM(0) represent bound $^{125}$I-SP in the presence and the absence of unlabeled peptide, respectively; L represents the concentration of unlabeled peptide; and $IC_{50}$ represents the concentration of unlabeled peptide that causes 50% of inhibition. For SP, $K_d$ was calculated according to $K_{d(SP)} = IC_{50} - [^{125}I\text{-SP}]$. For other peptides, $K_d$ was calculated according to $K_d = IC_{50}/(1 + [^{125}I\text{-SP}]/K_{d(SP)})$.

Address correspondence to Dr. T. M. Fong, Mail Code 80M-213, Merck Sharp and Dohme Research Laboratories, PO BOX 2000, Rahway, NJ 07065.

TABLE 1 **Agonist binding affinities of two substitution mutants of rat NK1R, wild type rat NK1R, a single amino acid substitution mutant of rat NK1R, and human NK1R**

| Receptor | Kd (nM), mean ± SEM (*n*) | | |
|---|---|---|---|
| | SP | SK | NKB |
| Rat NK1R S(1-13) | 10.7 ± 2.6 (2) | 157 ± 30 (2) | — |
| Rat NK1R S(1-27) | > 100 (3) | — | — |
| Rat NK1R | 3.2 ± 0.7 (5) | 55 ± 26 (3) | 179 ± 11 (3) |
| Rat NK1R V97E | 2.9 ± 0.7 (4) | 58 ± 6 (3) | 77 ± 21 (2) |
| Human NK1R | 0.7 ± 0.2 (4) | 25 ± 6 (4) | 57 ± 9 (4) |

97th residue is predicted to be within the second extracellular segment. A single amino acid substitution of rat NK1R was constructed to place Glu at the Val position by site-directed mutagenesis. Measurements of binding affinities indicated that the affinity of NKB to the mutant rat NK1R is similar to that for the human NK1R, whereas the affinities of SP and SK are not changed by the V97E substitution (Table 1). The rat NK3 receptor (Shigemoto et al., 1990), for which NKB has a high affinity (NKB > SK > SP), also contains a Glu residue at the analogous position. These data suggest that the Glu97 residue in the human NK1R is involved in interaction with NKB but not SP or SK.

The present results indicate that the extracellular domain of NK1R comprises part of the peptide binding domain. Previous studies on adrenergic and muscarinic receptors have demonstrated that the binding domain for these small molecules is in the bilayer domain (O'Dowd et al., 1989; Strader et al., 1989; Wheatley et al., 1988). Because neurokinin peptides are about 10 times larger than acetylcholine and catecholamines, it is not surprising that the peptide-receptor interaction involves residues from the extracellular domain. Other studies using lectin or antibody are consistent with the present conclusion. For example, concanavalin A reduces the affinity of SP binding (Takamatsu et al., 1991). Antibodies against the second and fourth extracellular segments of NK2 receptor inhibit the binding of SP to NK1R by cross-reaction (Parnet et al., 1991). Further mutagenesis experiments are required to fully understand the basis of peptide-receptor interaction.

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