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Molecular and pharmacological characterization of the murine tachykinin NK_3 receptor

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Abstract

Starting with a partial sequence from Genbank, polymerase chain reaction (PCR) was utilized to isolate the full-length cDNA for NK_3 receptor from mouse brain. The murine NK_3 receptor has a predicted sequence of 452 amino acids, sharing 96% and 86% identity to the rat and human NK_3 receptors, respectively. Binding affinities and functional potencies of tachykinin receptor agonists were similar in HEK (human embryonic kidney) 293 cells expressing murine NK_3 receptor and human NK_3 receptor, although substance P and neurokinin A were more potent stimulators of Ca^{2+} mobilization in murine NK_3 receptor cells. NK_3 receptor-selective antagonists from two structural classes, had 10- to 100-fold lower binding affinities for murine NK_3 receptor compared to human NK_3 receptor, and about 5- to 10-fold reduced potency in the murine NK_3 receptor functional assay. The results demonstrate species differences in the potencies of tachykinin receptor antagonists in murine and human NK_3 receptors, and the lower potencies in the former should be taken into consideration when using murine disease models.

Keywords: Tachykinin NK_3 receptor, mouse; Tachykinin NK_3 receptor, human; Tachykinin; Tachykinin NK_3 receptor antagonist

1. Introduction

The mammalian tachykinins, or neurokinins, are a family of small peptides, including substance P, neurokinin A and neurokinin B, which are distributed in the central and peripheral nervous systems, in particular in capsaicin-sensitive primary afferent neurons (Maggio, 1988; Otsuka and Yoshioka, 1993; Maggi et al., 1993, 1995; Maggi, 1996). Three tachykinin receptor subtypes mediate the actions of the tachykinins: NK_1, NK_2, and NK_3 receptors, which belong to the superfamily of G protein-coupled, seven transmembrane spanning receptors (Maggio, 1988; Nakanishi, 1991; Maggi et al., 1993). The human variants of the three tachykinin receptors have been cloned and expressed (Gerard et al., 1990; Huang et al., 1992; Buell et al., 1992), and potent and selective, non-peptide antagonists for the NK_1, NK_2, and NK_3 have been identified (Snider et al., 1991; Emonds-Alt et al., 1992; Desai et al., 1992; McLean et al., 1993; Emonds-Alt et al., 1995; Giardina et al., 1996). These compounds have been extensively used to investigate preclinically the possible pathophysiological roles of the individual tachykinin receptors.

A potential conflicting factor in such investigations is the significant evidence for marked species differences in the potencies of tachykinin receptor antagonists (Snider et al., 1991; Beaujouan et al., 1993; Watling et al., 1994; Chung et al., 1995; Emonds-Alt et al., 1995; Maggi, 1995; Sarau et al., 1997). The mouse is widely used for evaluation of compounds in models of inflammatory diseases.

Abbreviations: NK_1, neurokinin 1; NK_2, neurokinin 2; NK_3, neurokinin 3; HEK, Human embryonic kidney; HEK 293-human NK_3 receptor, HEK 293 cells transiently expressing the human NK_3 receptor; HEK 293-murine NK_3 receptor, HEK 293 cells transiently expressing the murine NK_3 receptor; EC_{50}, concentration of agonist producing 50% of maximal response; IC_{50}, concentration of antagonist causing 50% inhibition of agonist response; K_i, apparent inhibition constant; PCR, polymerase chain reaction

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The murine NK_1 and NK_2 receptors have been cloned, expressed and characterized (Sundelin et al., 1992). Here we describe the first report on the cloning, expression and pharmacological evaluation of the murine NK_3 receptor. A comparison of the profiles of selective tachykinin ligands in murine NK_3 receptor- and human NK_3 receptor-expressing cells is presented.

2. Materials and methods

2.1. Materials

[^{125}I]-[MePhe^7] neurokinin B (specific activity, 2200 Ci/mmol) was obtained from New England Nuclear (Boston, MA, USA). Neurokinin A, neurokinin B, substance P, [MePhe^7]neurokinin B were purchased from Peninsula Laboratories (Belmont, CA, USA) and senktide (succinyl-[Asp^{9}MePhe^8]-SP(6-13)) from California Peptide Research (Napa, CA, USA). SB 222200 [(*S*)-(−)-*N*-(α-ethylbenzyl)-3-methyl-2-phenylquinoline-4-carboxamide], SB 223412 [(*S*)-(−)-*N*-(α-ethylbenzyl)-3-hydroxy-2-phenylquinoline-4-carboxamide], SR 142801 ((*S*)-(+)-*N*-{[3-[1-benzoyl-3-(3,4-dichlorophenyl)piperidine-3-yl]prop-1-yl}-4-phenylpiperidin-4-yl}-*N*-methylacetamide), SR 48964 ((*S*)-*N*-methyl-N[4-(4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl)butyl]benzamide) and CP 99994 ((+)-(2S,3S)-*cis*-(2-methoxybenzylamino)-2-phenylpiperidine dihydrochloride) were synthesized in the Department of Medicinal Chemistry, SmithKline Beecham, Milan, Italy.

2.2. Receptor cloning and expression

2.2.1. Mouse NK_3 receptor

Mouse brain and 15-day embryo Marathon Ready cDNA were purchased from Clontech Laboratories (Palo Alto, CA). RACE PCR was performed according to the manufacturer's protocol using the Advantage GC cDNA polymerase mix (Clontech Laboratories). A cDNA fragment was amplified from both cDNAs using a murine NK_3 receptor internal primer (5′GTATTTCATTCTCACTGCGATCTACC3′) and the anchored Marathon adaptor primer. The amplified product was subcloned into the pCR2.1 vector (Invitrogen, Carlsbad, CA). Several clones were sequenced and found to overlap and be in agreement with the partial murine NK_3 receptor cDNA (Genbank X87823). Using mouse brain Marathon cDNA and the Expand polymerase (Roche Molecular Biochemicals, Indianapolis, IN) the full-length coding region of murine NK_3 receptor was amplified by PCR using primers designed at the extreme ends of the predicted coding sequence (5′ATGGCCTCGGTTCCCACCGGCG3′ and 5′TTAGGAATATTCATCCACAGAGG3′). Clones from independent PCR reaction were sequenced and found to be identical. The insert was subcloned into the pCR3.1 vector (Invitrogen, Carlsbad, CA) for transient transfection in human embryonic kidney (HEK) 293 cells and subsequent binding and functional studies. The sequence data has been deposited with Genbank under accession number AF233341.

2.2.2. Human NK_3 receptor

Human cDNA for NK_3 receptor was isolated from human placenta poly A + RNA using reverse transcriptase-PCR technology and site-directed mutagenesis. The sequence of the human NK_3 receptor was confirmed by sequence analysis. The receptor was transiently expressed in HEK 293 cells as outlined previously (Sarau et al., 1997).

2.3. Radioligand binding assays

Receptor binding assays were performed with crude membranes from HEK 293 cells transiently expressing the human NK_3 receptor (HEK 293-human NK_3 receptor) or murine NK_3 receptor (HEK 293-murine NK3 receptor) as detailed previously (Sarau et al., 1997). Competition binding studies were performed using [^{125}I]-[MePhe^7]neurokinin B (0.15 nM) binding to HEK 293-murine NK_3 receptor and HEK 293-human NK_3 receptor cell membranes incubated in 150 μl of 50 mM Tris, pH 7.4, containing 4 mM $MnCl_2$, 1 μM phosphoramidon and 0.1% ovalbumin, with or without antagonist, for 90 min at 25°C as previously described (Sarau et al., 1997). Concentration–response curves for each compound were run using duplicate samples in at least three independent experiments. Non-specific binding was assessed as the binding in the presence of 0.5 μM cold [MePhe^7]neurokinin B. The IC_{50}, defined as the concentration required to inhibit 50% of the specific binding, was obtained from concentration–response curves. Values presented are the apparent inhibition constant (K_i), which was calculated from the IC_{50} as described by Cheng and Prusoff (1973).

2.4. Calcium mobilization assay

Ca^{2+} mobilization studies were carried out using Fluo 3-loaded HEK 293-murine NK_3 receptor and HEK 293-human NK_3 receptor cells and a microtiter plate-based assay using FLIPR (Fluorescent Image Plate Reader) (Molecular Devices, Sunnyvale, CA). Briefly, cells (about 80% confluent) were harvested and plated in 96-well black wall/clear bottom plates (Biocoat plates, Becton Dickinson Labs, Franklin Lakes, NJ) at approximately 40,000 cells/well and grown in the incubator at 37°C for 18–24 h. On the day of assay, the media was aspirated and replaced with 100 μl Eagle's minimum essential medium with Earl's salts, L-glutamine, 0.1% bovine serum albumin,

4 μM Fluo 3 acetoxymethyl ester (Fluo 3 AM, Molecular Probes, Eugene, OR) and 2.5 mM probenecid. Plates were incubated for 60 min at 37°C, then media aspirated and replaced with the same media without Fluo-3 AM, and incubated for 10 min at 37°C. The cells were washed three times and then incubated for 10 min at 37°C in 100 μl assay buffer (120 mM NaCl, 4.6 mM KCl, 1.03 mM KH_2PO_4, 25 mM $NaHCO_3$, 1.0 mM $CaCl_2$, 1.1 mM $MgCl_2$, 11 mM glucose, 20 mM HEPES (pH 7.4) with 2.5 mM probenecid. Plates were placed into FLIPR for analysis as described previously (Sarau et al., 1999). The maximal change in fluorescence after agonist addition was quantitated. The percent of maximal neurokinin B-induced Ca^{2+} mobilization was determined for each concentration of antagonist and the $IC_{50,}$ defined as the concentration of test compound that inhibits 50% of the maximal response induced by 1 nM neurokinin B, assessed. For agonist potency, the EC_{50} is defined as the concentration that produces 50% of the maximal neurokinin B-induced response.

3. Results

3.1. Molecular biological characterization

A partial sequence for murine NK_3 receptor was obtained from Genbank (X87823, L27827). Alignment of this sequence with the rat (Shigemoto et al., 1989; Genbank J05189) and human (Huang et al., 1992; Genbank M89473) NK_3 receptor cDNA suggested that approximately 100 amino acids were missing from the 3′ end of

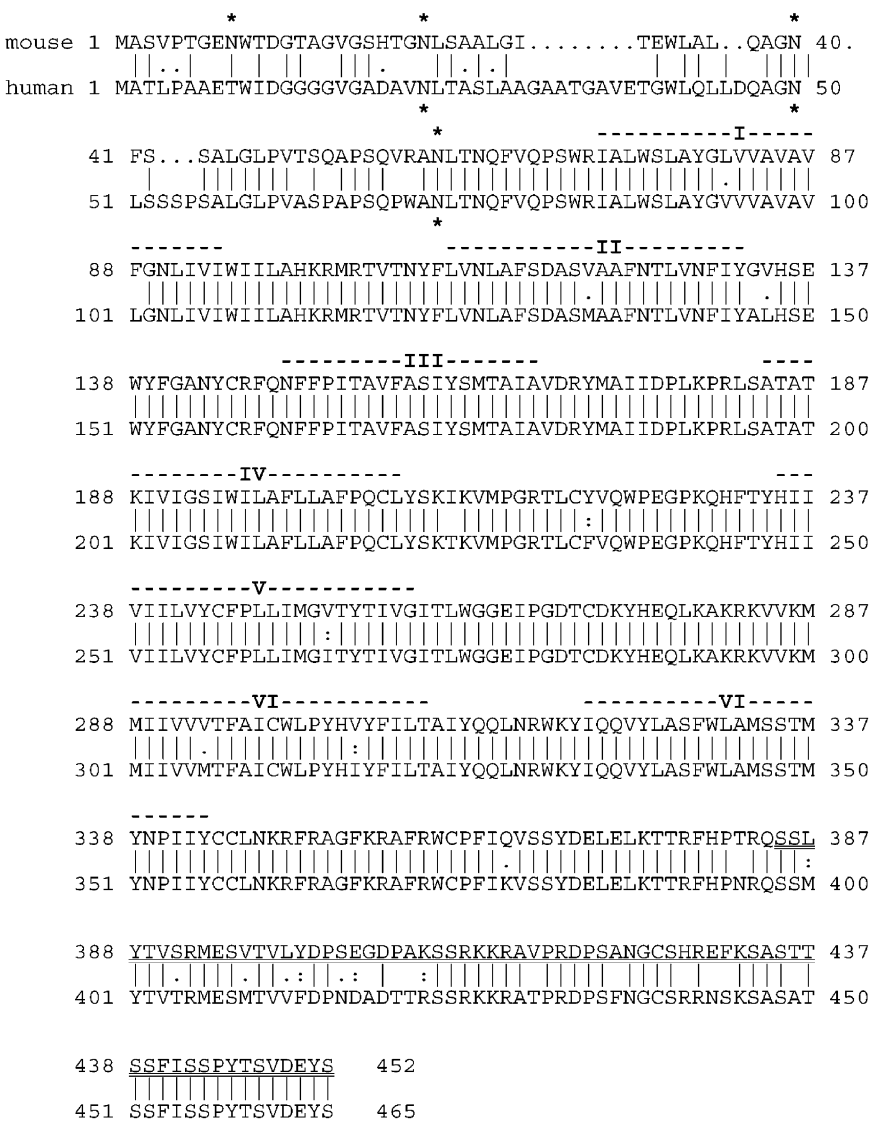

Fig. 1. Sequence alignment of the full-length murine NK_3 receptor compared to human NK_3 receptor (M89473). The C-terminal portion of the sequence added by RACE-PCR is underlined and the potential extracellular N-glycosylation sites are marked by an asterisk (*). The predicted transmembrane spanning regions are denoted by an overscore.

the murine NK_3 receptor clone. 3′ RACE PCR was used to amplify a 550 base pair fragment from mouse brain or 15-day mouse embryo cDNA. The sequence of this fragment from either source is identical, and highly homologous to the corresponding region of the rat NK_3 receptor (93% identity over the last 68 amino acids), including a stop codon which corresponds to that found in the rat, human and rabbit (Genbank AF133908) cDNA sequences. Using the new sequence information, a full-length cDNA was PCR amplified from mouse brain by RT-PCR. This cDNA adds an additional 68 amino acids to the 3′ end of the partial sequence and is predicted to encode a 452 amino acid polypeptide. The full-length murine cDNA (Fig. 1) is highly homologous to rat (96%) with less identity to human (86%) and rabbit (88%). The greatest sequence divergence is seen in the N-terminus where the mouse (and rat) polypeptide is 13 amino acids shorter than human, leaving this region prior to the first transmembrane domain only 55% identical (23/42 residues). The mouse sequence has four potential N-glycosylation sites in the N-terminal extracellular domain whereas the human has three (Fig. 1).

3.2. Pharmacological characterization

3.2.1. Binding experiments

Initially studies were performed to assess the binding characteristics of [^{125}I]-[MePhe^7]neurokinin B to membranes prepared from HEK 293-murine NK_3 receptor cells. Using this heterologous assay the binding of the radioligand was saturable, specific and of high affinity affinity; the K_d and B_{max} were determined to be 0.47 ± 0.05 nM and 2.98 ± 0.70 pmol/mg protein ($n = 3$), respectively. The heterologous binding of [^{125}I]-[MePhe^7]neurokinin B to HEK 293-human NK_3 receptor cell membranes was reported previously to be saturable, specific and of high affinity ($K_d = 0.9$ nM and $B_{max} = 0.6$ pmol/mg) (Sarau et al., 2000).

A comparison was made of the ability of tachykinin receptor selective agonists and antagonists (see Fig. 2 for structural diversity) to inhibit binding of [^{125}I]-[MePhe^7]neurokinin B to HEK 293-murine NK_3 receptor; the results are summarized in Fig. 3 and Table 1. The natural ligand with the highest affinity for the human NK_3 receptor, neurokinin B, and the human NK_3 receptor-selective agonists, senktide and [MePhe^7]neurokinin B, produced concentration-dependent inhibition of [^{125}I]-[MePhe^7]neurokinin B binding to HEK 293 murine NK_3 receptor cell membranes, with respective K_i's of 2.3 ± 0.3 nM, 11.9 ± 2.1 nM and 0.6 ± 0.1 nM ($n = 3$) (Fig. 3A; Table 1). In contrast, substance P (NK_1 receptor-preferring natural ligand) and neurokinin A (NK_2 receptor-preferring natural ligand) demonstrated much lower affinity for [^{125}I]-[MePhe^7]neurokinin B binding to HEK 293-murine NK_3 receptor cell membranes with IC_{50}s of 4921 nM and 785 nM, respectively, ($n = 3$) (Fig. 3A; Table 1). Thus, the relative rank order potency was [MePhe^7]neurokinin B > neurokinin B > senktide ≫ neurokinin A > substance P. The NK_3 receptor selective antagonists from two distinct chemical classes, SB 223412, SB 222200 and SR 142801, produced concentration-dependent inhibition of [^{125}I]-[MePhe^7]neurokinin B binding to HEK 293-murine NK_3 receptor cell membranes with respective K_i's of 43.9 ± 9.8,

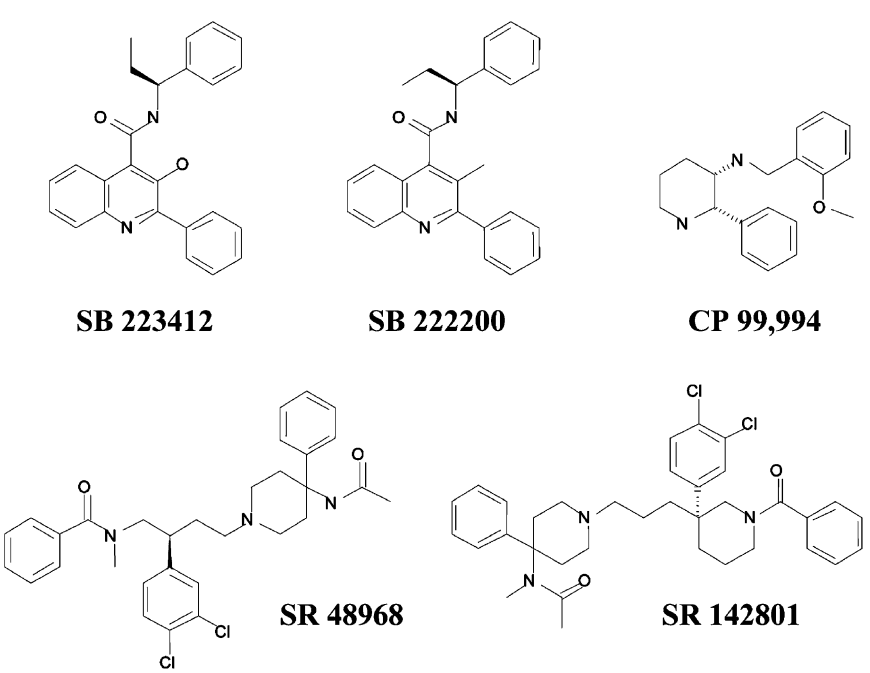

Fig. 2. Structures showing the chemical diversity of the selective tachykinin antagonists.

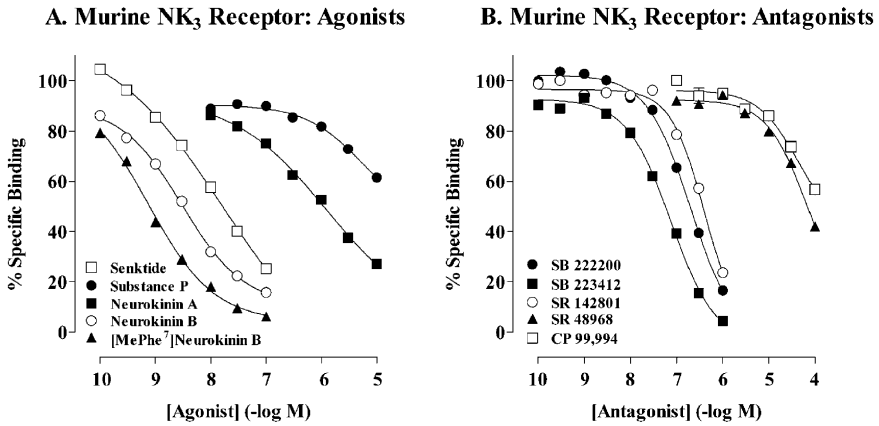

Fig. 3. Competition binding of [^{125}I]-[MePhe^7]neurokinin B to HEK 293-murine NK_3 receptor by tachykinin (A) agonists or (B) antagonists. HEK 293-murine NK_3 receptor cell membranes were incubated with 0.15 nM [^{125}I]-[MePhe^7]neurokinin B in the absence and presence of various concentrations of agonists (A): neurokinin A (■); neurokinin B (○); [MePhe^7]neurokinin B (▲); senktide (□); substance P (●); or antagonists (B): SB 222200 (●); SB 223412 (■); SR 142801 (○); SR 48968 (▲) or CP 99,994 (□) as described in Section 2. Values presented are the mean of three experiments. Standard errors are omitted for clarity, but are given for K_i's in Table 1.

174 ± 55 and 327 ± 138 nM ($n = 3$). The NK_1 receptor selective antagonist, CP 99,994, and the NK_2 receptor selective antagonist, SR 48968, were weak competitors, with K_i's of $86{,}667 \pm 7{,}431$ and $49{,}833 \pm 2{,}088$ nM, respectively ($n = 3$) (Fig. 3B; Table 1).

The rank order for the agonist displacement of [^{125}I]-[MePhe^7]neurokinin B binding to HEK 293-human NK_3 receptor cell membranes was similar to that obtained with the HEK 293-murine NK_3 receptor membranes: [MePhe^7]neurokinin B > neurokinin B > senktide ≫ neurokinin A = substance P (Table 1; Sarau et al., 2000). Furthermore, the antagonists also showed a similar rank-order potency for inhibition of [^{125}I]-[MePhe^7]neurokinin B binding to HEK 293-human NK_3 receptor compared to HEK 293-murine NK_3 receptor cell membranes: SB 223412 = SR 142801 > SB 222200 > > SR 48968 > CP 99,994. However, the NK_3 receptor selective antagonists had about 10- to 100-fold higher affinities for the human NK_3 receptor compared to the murine NK_3 receptor (Table 1; Sarau et al., 2000).

3.2.2. Ca^{2+} mobilization studies

Cellular functional activity of agonists and antagonists was determined by assessment of their effects on Ca^{2+} mobilization in HEK 293-murine NK_3 receptor cells (Fig. 4). In HEK 293-murine NK_3 receptor cells, the tachykinin receptor agonists were potent stimulators of Ca^{2+} mobilization with rank order potencies of neurokinin B =

Table 1
Effects of tachykinin receptor ligands on [^{125}I]-[MePhe^7]neurokinin B binding and Ca^{2+} mobilization in HEK 293-murine NK_3 receptor and HEK 293-human NK_3 receptor cells. The experimental protocols for these studies are given in Section 2.

Agonists	Binding (Inhibition of [^{125}I]-[MePhe^7]neurokinin B)		Ca^{2+} Mobilization	
	Murine NK_3 receptor; K_i (nM)	Human NK_3 receptor; K_i (nM)	Murine NK_3 receptor; EC_{50} (nM)	Human NK_3 receptor; EC_{50} (nM)
Neurokinin B	2.3 ± 0.3	8.9 ± 0.2	0.09 ± 0.02	0.3 ± 0.2
[MePhe^7]neurokinin B	0.6 ± 0.1	1.8 ± 1.0	0.26 ± 0.13	0.3 ± 0.1
Senktide	11.9 ± 2.1	55 ± 19	0.15 ± 0.09	0.1 ± 0.03
Neurokinin A	785 ± 195	3300 ± 240	0.82 ± 0.24	22 ± 4
Substance P	4921 ± 2251	7660 ± 3090	5.4 ± 2.4	97 ± 16
Antagonists			Inhibition of Neurokinin B (1 nM)-induced Responses; IC_{50} (nM)	
SB 223412	43.9 ± 9.8	1.1 ± 0.1	1030 ± 590	128 ± 29
SR 142801	327 ± 138	3.8 ± 0.3	2825 ± 1590	244 ± 36
SR 222200	174 ± 55	18.1 ± 5.4	1470 ± 813	308 ± 85
SR 48968	$49{,}833 \pm 2088$	1250 ± 160	> 33,000 (2)	15,000 (2)
CP 99,994	$86{,}667 \pm 7431$	$23{,}000 \pm 4100$	> 33,000 (2)	> 33,000 (2)

Results are presented as EC_{50} or K_i's (for agonists) or IC_{50} or K_i's (for antagonists) and are the mean ± standard error of the mean or the mean; $n = 3$ unless indicated in parentheses. Some of the values for the experiments using HEK 293-human NK_3 receptor cells were taken from Sarau et al., 2000.

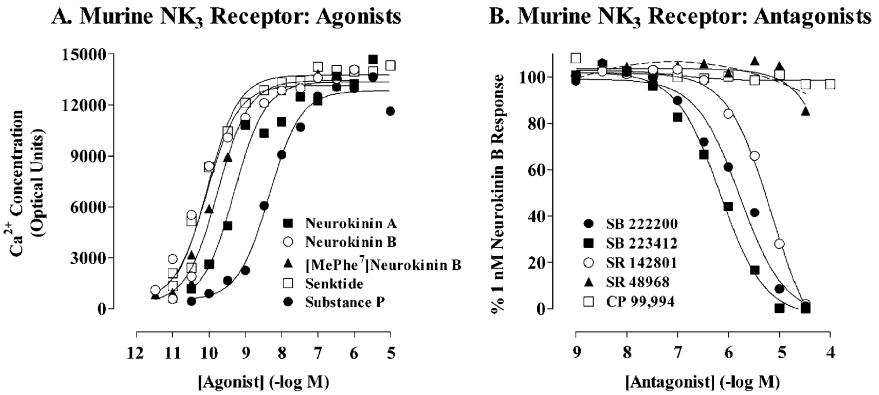

Fig. 4. (A) Tachykinin agonist-induced calcium mobilization in HEK 293-murine NK_3 receptor cells or (B) inhibition by the tachykinin receptor antagonists, SB 223412, SB 222200, SR 142801, SR 48968 and CP 99,994, of neurokinin B-induced calcium mobilization in HEK 293-murine NK_3 receptor. (A) Fluo 3-loaded or HEK 293-murine NK_3 receptor cells were stimulated with increasing concentrations of agonists neurokinin A (■); neurokinin B (○); [MePhe^7]neurokinin B (▲); senktide (□); substance P (●), with results expressed as Ca^{2+} concentrations (optical units). (B) Fluo-3 loaded HEK 293-human NK_3 receptor cells were stimulated with neurokinin B after the addition of increasing concentrations of antagonists SB 222200 (●); SB 223412 (■); SR 142801 (○); SR 48968 (▲) or CP 99,994 (□), with results expressed as a percentage of the maximum Ca^{2+} concentrations elicited by 1 nM in the absence of any antagonist. Values presented are the mean for two to three experiments. Standard errors are omitted for clarity but are given for the EC_{50}s in Table 1.

senktide = [MePhe^7]neurokinin B > neurokinin A > substance P. All agonists had the same efficacy, eliciting a similar maximum response (Fig. 4A). Substance P and neurokinin A had higher potencies than expected in the murine NK_3 receptor cells, producing Ca^{2+} mobilization with EC_{50}s of 1–5 nM. In fact, all the agonists, except [MePhe^7]neurokinin B, have much higher potencies (25- to 95-fold) in the Ca^{2+} mobilization compared to the binding experiments. This may reflect, at least in part, the utilization of membranes (binding experiments) versus whole cells (Ca^{2+} mobilization studies). A similar rank order of potencies for the agonists has been demonstrated for Ca^{2+} mobilization in HEK 293-human NK_3 receptor. However, neurokinin A and substance P were less potent agonists in HEK 293-human NK_3 receptor cells with EC_{50}'s of 22 ± 4 and 97 ± 16 nM, respectively (Table 1; Sarau et al., 2000).

In studies examining the effects of antagonists against neurokinin B-induced Ca^{2+} mobilization an approximate EC_{80} agonist concentration was utilized (1 nM neurokinin B). SB 223412, SB 222200 and SR 142801 demonstrated concentration-dependent inhibition of neurokinin B-induced Ca^{2+} mobilization in HEK 293-murine NK_3 receptor cells, with IC_{50}s of 1030 ± 590 nM ($n = 3$), 1470 ± 813 nM ($n = 3$) and 2825 ± 1590 nM ($n = 3$), respectively. SR 48968 and CP 99,994 were essentially inactive with IC_{50}'s of > 33 μM ($n = 2$) (Fig. 4B). A similar rank-order profile for the antagonists was evident against neurokinin B-induced Ca^{2+} mobilization in the HEK 293-human NK_3 receptor cells (Table 1; Sarau et al., 2000). However, SB 223412, SB 222200 and SR 142801 were 5- to 10-fold more potent, compared to their potencies in HEK 293-murine NK_3 receptor cells with IC_{50}'s of 128, 308 and 244 nM, respectively. Significant but weak inhibition at the human NK_3 receptor was demonstrated with SR 48968 ($IC_{50} = 15{,}000$, $n = 2$), whereas CP 99,994, in concentrations up to 33 μM, was without effect ($n = 2$) (Table 1).

4. Discussion

In this report the cloning, expression and characterization of the murine NK_3 receptor is described. Molecular biological analysis reveals that the murine NK_3 receptor cDNA encodes a 452 amino acid polypeptide, which compares with 465, 452 and 467 amino acids for human NK_3 receptor, rat NK_3 receptor and rabbit NK_3 receptor, respectively (Shigemoto et al., 1989; Huang et al., 1992; Medhurst et al., 1999). The murine NK_3 receptor cDNA has 96% identity to the rat variant, 86% identity to the human NK_3 receptor cDNA and 88% identity to the rabbit variant, with the greatest divergence in the N-terminus, in particular the region adjacent to the first transmembrane domain. The murine NK_3 receptor possesses a greater number (four) of N-glycosylation sites than the human NK_3 receptor (three).

Differences have been reported in ligand binding and functional response profiles for NK_3 receptor antagonists between human and rat NK_3 receptors. For example, binding studies revealed that NK_3 receptor antagonists from structurally distinct classes (Fig. 2), including SR 142801 (Chung et al., 1995; Emonds-Alt et al., 1995) and SB 223412 (Sarau et al., 1997), had > 25-fold lower affinities for the rat NK_3 receptor versus the human NK_3 receptor. Site-directed mutagenesis studies indicated that two variant amino acids in the second transmembrane domain of the human receptor (Met134 and Ala146) were responsible for these species differences (Wu et al., 1994; Chung et al., 1995). The corresponding amino acids in the murine sequence are identical to the rat and therefore, the

NK_3 receptor antagonists examined in this study, SR 142801 (Emonds-Alt et al., 1995; Chung et al., 1995), SB 223412 (Sarau et al., 1997) and SB 222200 (Sarau et al., 2000), could be anticipated to demonstrate a similar lower affinity for the murine NK_3 receptor. Indeed, binding experiments showed that the compounds exhibited about 10- to 100-fold lower affinities for the murine NK_3 receptor compared to the human NK_3 receptor, and 5- to 10-fold reduced potencies for the murine NK_3 receptor in calcium mobilization studies. The results of this study, and previous reports (Chung et al., 1995; Emonds-Alt et al., 1995; Sarau et al., 1997), indicate that the selective NK_3 receptor antagonists identified to date have lower affinities for murine NK_3 receptor and rat NK_3 receptor compared the human NK_3 receptor. This pharmacological profile of closer similarity between the murine and rat NK_3 receptors compared to the human variant is mirrored by the closer homology between the former receptors at the cDNA level.

Species differences have been reported in the affinities of NK_1 receptor antagonists for mouse and rat NK_1 receptor compared to variants of this receptor in human and other species (Snider et al., 1991; Barr and Watson, 1993; Beaujouan et al., 1993; Watling et al., 1994; Maggi, 1995). In some cases the molecular basis for these differences have been elucidated (Fong et al., 1992; Sachais et al., 1993; Jensen et al., 1994). Differences in the potencies of antagonists for NK_2 receptors from various species is also apparent (Watling et al., 1994; Maggi, 1995). Collectively, the data provide accumulating evidence that the species sensitivity of tachykinin receptors to antagonists can be broadly divided into two groups: mouse, rat and other rodents (lower sensitivity), and human and other species, including guinea pig and rabbit (higher sensitivity).

The current results highlight further the need for evaluation of species differences in antagonist potencies to be taken into consideration in the interpretation of data examining the effects of tachykinin receptors antagonists, including NK_3 receptor antagonists, in animal models of disease, in particular in the mouse and rat. Nevertheless, the potencies of SR 142801, SB 223412 and SB 222200 would appear to be of the appropriate magnitude in mouse and rat (Chung et al., 1995; Sarau et al., 1997) to anticipate efficacy in vivo in these species. The identification of the molecular characteristics of the murine NK_3 receptor will assist in the profiling of NK_3 receptor antagonists, and will likely be of value in the search for potential NK_3 receptor subtypes.

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