Biochemical Pharmacology 77 (2009) 1522–1530

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Characterization of species-related differences in the pharmacology of tachykinin NK receptors 1, 2 and 3

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ARTICLE INFO

Article history:
Received 19 November 2008
Accepted 30 January 2009

Keywords:
Aprepitant
Saredutant
Talnetant
NK receptors
Species difference
NK-receptor function
Chemistry
NK-receptor sequences

ABSTRACT

Tachykinin NK receptors (NKRs) differ to a large degree among species with respect to their affinities for small molecule antagonists. The aims of the present study were to clone NKRs from gerbil (NK_2R and NK_3R) and dog (NK_1R, NK_2R and NK_3R) in which the sequence was previously unknown and to investigate the potency of several NKR antagonists at all known human, dog, gerbil and rat NKRs.

The NKR protein coding sequences were cloned and expressed in CHO cells. The inhibitory concentrations of selective and non-selective NKR antagonists were determined by inhibition of agonist-induced mobilization of intracellular Ca^{2+}. Receptor homology models were constructed based on the rhodopsin crystal structure to investigate and identify the antagonist binding sites and interaction points in the transmembrane (TM) regions of the NKRs.

Data collected using the cloned dog NK_1R confirmed that the dog NK_1R displays similar pharmacology as the human and the gerbil NK_1R, but differs greatly from the mouse and the rat NK_1R. Despite species-related amino acid (AA) differences located close to the antagonist binding pocket of the NK_2R, they did not affect the potency of the antagonists ZD6021 and saredutant. Two AA differences located close to the antagonist binding site of NK_3R likely influence the NK_3R antagonist potency, explaining the 3–10-fold decrease in potency observed for the rat NK_3R. For the first time, detailed pharmacological experiments *in vitro* with cloned NKRs demonstrate that not only human, but also dog and gerbil NKR displays similar antagonist pharmacology while rat diverges significantly with respect to NK_1R and NK_3R.

1. Introduction

Several small molecule antagonists selective for tachykinin NK_1R, NK_2R and NK_3R are in clinical development and the selective NK_1R antagonist aprepitant is approved for treatment of emesis in response to cytostatic treatment in cancer patients [1]. During the development of selective NK_1R antagonists, Beresford et al. discovered large discrepancies in the affinity for NK_1R from different species [2]. This led to the NK_1R family being grouped into two sub-families based on the orthologous receptor's affinity to small molecule antagonists. The first sub-family consists of the human, guinea pig, rabbit, dog, gerbil and ferret NK_1R, and the second sub-family of the rat and mouse homologues [2–5]. Thus, several disease-related animal models in species other than rat or mouse have been developed for evaluation of NK_1R antagonists [6–10]. Amino acid (AA) residues in the NK_1R responsible for species-dependent differences in antagonist pharmacology have been studied in detail, especially human Glu97, Val116 and Ser290 [5,11–14]. However, although the dog appears to be an appropriate species in detecting anti-emetic effects with selective NK_1R antagonists intended for clinical use [15], the pharmacology of the dog NK_1R at the molecular level, and its homology to the human NK_1R, remain unknown to our knowledge.

Consistent species-related differences in the pharmacology of NK_2R have not been reported either, although recent publications demonstrated that the selective NK_2R antagonist MEN15596 and analogues MEN14268 and MEN13918 had a marked species selectivity for inhibiting NK_2R-mediated effects in human, guinea pig and pig urinary bladder, while being 1000-fold less potent at the rat and mouse NK_2R expressed in urinary bladder [16,17]. By contrast, species-dependent differences were not observed with MEN11420 (nepadutant) [18] or saredutant [19]. Detailed

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Abbreviations: NKR, tachykinin NK receptor; SP, substance P; NKA, neurokinin A; NKB, neurokinin B; AA, amino acids; FLIPR^{TM}, Fluorometric Imaging Plate Reader; TM, transmembrane.

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doi:10.1016/j.bcp.2009.01.020

site-directed mutagenesis studies suggested that the Ile202 residue, located in the upper part of TM5 in the human NK_2R was, at least in part, responsible for these differences [16]. Still, the homology between the human NK_2R and that of species commonly used in tachykinin receptor pharmacology studies, such as dog and gerbil, has not been reported.

Furthermore, species-related differences in functional response profiles between human and mouse/rat have been reported for selective tachykinin NK_3R antagonists. Compounds from different structural classes have 5–10-fold lower potency and 10–50-fold lower affinity for rodent NK_3R compared to human counterparts [20,21]. Site-directed mutagenesis studies indicate that two AA in the second transmembrane domain of the human NK_3R (Met134 and Ala146) were responsible for these species differences [20,22]. However, as is the case for NK_2R, the identity of NK_3R from dog and gerbil remain unknown.

Thus, there are clearly gaps in our knowledge with respect to which species most likely will predict clinical efficacy and selectivity for NK_1R, NK_2R or NK_3R antagonists. Furthermore, the lack of antagonist affinity data will complicate species selection for toxicological studies intended to detect adverse effects upon blocking of receptor signalling. In the current study, we have cloned and sequenced the dog NK_1R, NK_2R and NK_3R and the gerbil NK_2R. This should increase the understanding of the molecular mechanisms underlying the species-related differences in NKR pharmacology, which would facilitate more relevant model system selection.

2. Material and methods

2.1. Chemicals

Substance P (SP), neurokinin A (NKA) and Pro7neurokinin B (NKB) were purchased from Bachem (Peninsula Laboratories Inc., San Carlos, CA). ZD6021 was synthesized at AstraZeneca, Wilmington, USA [23]. Aprepitant [24], RP67580 [25], CP99,994 [26], saredutant [19], talnetant [27,28] and osanetant [29,30] were synthesized at AstraZeneca Mölndal, Sweden.

2.2. Molecular cloning of the gerbil and dog NK receptors

The sequences for the human and rat NKR subtypes and the gerbil NK_1R have been published previously (see Table 1) for accession numbers [5]. The receptor sequences for the gerbil NK_2R and the dog NK_1R, NK_2R and NK_3R were largely unknown and are presented in this paper and submitted to the EMBL GenBank database under the accession numbers listed in Table 2. The gerbil NK_3R sequence has been cloned and was presented in a recent study [31].

Dog hypothalamus was used as a source for cloning NK_1R and NK_3R. Dog ileum and gerbil colon were used as sources for cloning of the respective NK_2R. Total RNA was prepared from the different tissues with RNA-STAT-60 (Tel-Test Inc., Friendswood, TX, USA). One μg of total RNA from each tissue sample was used for the first strand cDNA synthesis using SMART RACE cDNA Amplification kit (BD Biosciences, Mountain View, CA, USA). ClustalW alignment of NK_1R, NK_2R and NK_3R sequences from human, rat, mouse and guinea pig was used to select primers with high homology between different species. Primers used in the 3′RACE and in the 5′RACE are listed in Table 2. The RACE fragments were characterized and cloned fragments containing gerbil and dog specific NK_1R, NK_2R and NK_3R sequences spanning the open reading frame were identified.

Complementary DNA (2.5 μl) from the harvested tissues indicated above was used in the optimized full-length PCR with forward and reverse primers (20 μM of each), 1× PCR buffer, 5 mM of each dNTP and 1 U Pfu Ultra (Stratagene, La Jolla, CA, USA). A Kozak sequence (GCCACC) was introduced before the ATG in each construct. Conditions were optimized for each primer pair used. The resulting PCR products for the gerbil and dog NK_1R and NK_2R were cloned into pIREShyg2 expression vector (Clontech, Palo Alto, CA, USA). The full-length cDNA of dog and gerbil NK_3 receptor was cloned into pCDNA/FRT expression vector (Invitrogen, Carlsbad, CA, USA). In order to construct a full-length clone of dog NK_3R, the 5′-end of the dog NK_3R was cloned using genomic sequence data (TI number 356163905) from a trace file as a template for PCR reactions.

Multiple sequence alignments were constructed using ClustalX version 2.0 [32], and the TM domains were predicted using the TMHMM server version 2.0 (http://www.cbs.dtu.dk/services/TMHMM/). A position in the alignments is considered TM if the majority of the individual sequences are predicted to be TM at that position.

Table 1
Accession numbers for the cloned NKR from human, rat, gerbil and dog and the accession numbers for NKR from mouse and guinea pig used in the alignments of NK receptors.

Receptor	Species	Accession number
NK_1	Human	NM_001058
NK_2	Human	AY322545
NK_3	Human	M89473
NK_1	Rat	J05097
NK_2	Rat	M31838
NK_3	Rat	NM_017053
NK_1	Gerbil	AJ884917
NK_2	Gerbil	AJ884918
NK_3	Gerbil	AM157740
NK_1	Dog	AJ884915
NK_2	Dog	AJ884916
NK_3	Dog	AM423140
NK_1	Mouse	NM_009313
NK_2	Mouse	NM_009314
NK_3	Mouse	NM_021382
NK_1	Guinea pig	P30547
NK_2	Guinea pig	Q64077
NK_3	Guinea pig	P30098

Table 2
Primers used in the 3′RACE and in the 5′RACE of NK_1R, NK_2R and NK_3R.

Receptor	Species	Primer sequence	Reaction	Original sequence
NK_1	Dog	CCCTCGTAGTCGCCGGCGCTGATAAAG	5′RACE	Dog S75109
NK_1	Dog	CCCTTTATCAGCGCCGGCGACTACGAG	3′RACE	Dog S75109
NK_2	Dog	CACTGTAGGCGACGATCATCACCAAGAG	5′RACE	Dog S75024
NK_2	Dog	TCTCTTGGTGATGATCGTCGCCTACAGTG	3′RACE	Dog S75024
NK_3	Dog	GGGACCTTCTGGCCATTGCACATAACA	5′RACE	Dog S75029
NK_3	Dog	CATGCCAGGCCGTACCCTTTGTTATGTGC	3′RACE	Dog S75029
NK_2	Gerbil	GGAAAGCAAGCCGGAATCCAGAGCG	5′RACE	Rat and mouse alignment
NK_2	Gerbil	GGCTGCCCTACCACCTCTACTTCATCCT	3′RACE	Gerbil

2.3. Cell culture and transfection

Chinese Hamster Ovary (CHO) cells (ATCC, Middlesex, UK) or CHO-FlpIN cells (Invitrogen, Carlsbad, CA, USA) were transfected with the different constructs. All accession numbers for the sequences used to transfect the CHO cells are listed in Table 2. $NK_{1-3}R$-containing clones were selected by growth in appropriate selection media and tested for functionality in a Ca^{2+} mobilization assay.

CHO cells stably expressing human NK_1R was supplied by AstraZeneca R&D, Wilmington, USA and human NK_2R, rat NK_1R, rat NK_2R and rat NK_3R were transfected in house (see Table 1 for gene accession numbers). Stable transfectants were maintained by supplementing culture media (NutMix F12 (HAM) with glutamax I and 10% FBS) (Invitrogen, Carlsbad, CA, USA) with suitable selection depending on the expressing vector. Cultures were kept at 37 °C in a 5% CO_2-incubator and routinely passaged when 70–80% confluent for up to 20–25 passages.

2.4. Calcium mobilization assay

Ca^{2+} mobilization was studied in CHO cells or in CHO-FlpIn cells stably expressing human, rat, gerbil or dog NK_{1-3} receptors using the cytoplasmic Ca_i^{2+} indicator Fluo-4 (TEFLABS 0152, Austin, TX, USA). Cells were seeded into black-walled clear-base 96-well plates (Costar, #3904) at a density of 35,000 cells per well in culture media and grown for 24 h in a 37 °C CO_2-incubator. The cells were incubated with 4 μM of Fluo-4 (TEFLABS 0152) in loading media (Nut Mix F12 (HAM), glutamax I, 22 mM HEPES, 2.5 mM probenicid (P-8761, Sigma, St. Louis, MO, USA)) and 0.04% pluronic F-127 (P-2443 Sigma, St. Louis, MO, USA) for 30 min in a 37 °C CO_2-incubator. The Fluo-4-loaded cells were then washed three times in assay buffer (Hanks Balanced Salt Solution, 20 mM HEPES, 2.5 mM probenicid and 0.1% BSA). The plates were placed into the Fluorometric Imaging Plate Reader (FLIPR™) to monitor cell fluorescence (λ_{ex} = 488 nm; λ_{em} = 540 nm) before and after the addition of antagonists and/or agonists. Antagonists and agonists were dissolved in assay buffer (final DMSO concentration kept below 1%, D2650, Sigma, St. Louis, MO, USA) in 96-well plates and added to the loaded cells by the automated pipettor in the FLIPR™. Loaded cells were pre-incubated with antagonists for 2 min before addition of agonist (0.08 nM SP for NK_1R, 0.15 nM NKA for NK_2R and 1.0 nM Pro7NKB for NK_3R). Ca_i^{2+} responses were measured as peak fluorescence intensity minus basal fluorescence after agonist addition.

2.5. Rhodopsin homology model

The sequences of the human $NK_{1-3}R$ were aligned to the bovine rhodopsin and a subset of rhodopsin sequences, covering different

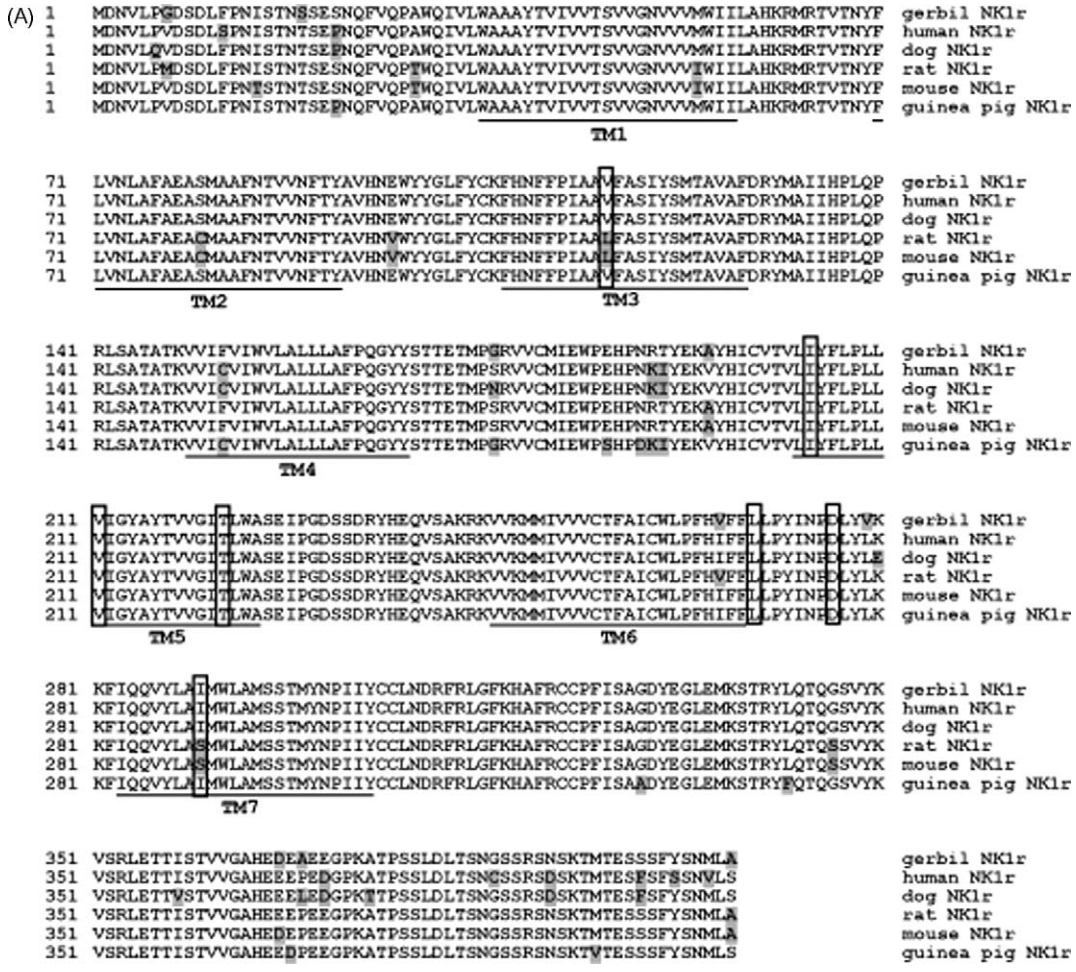


Fig. 1. (A) Sequence alignments of NK_1Rs from gerbil, human, dog, rat, mouse and guinea pig. TM regions are underlined, sequence differences indicated by gray shading and amino acids predicted to be important for antagonist binding are boxed. (B) Dose-dependent inhibitory effect of the selective NK_1R antagonist aprepitant on SP-evoked mobilization of intracellular Ca^{2+} in cells expressing the NK_1R from various species. Representative curves from each animal from three experiments done, are shown, all performed in the same experiment (see Table 3). (C) Chemical sequences of amino acids interacting in the NK_1R binding site in the presence of aprepitant. Bold numbers on AA indicate change as compared to the human AA. AA marked in bold show a TM region species difference.

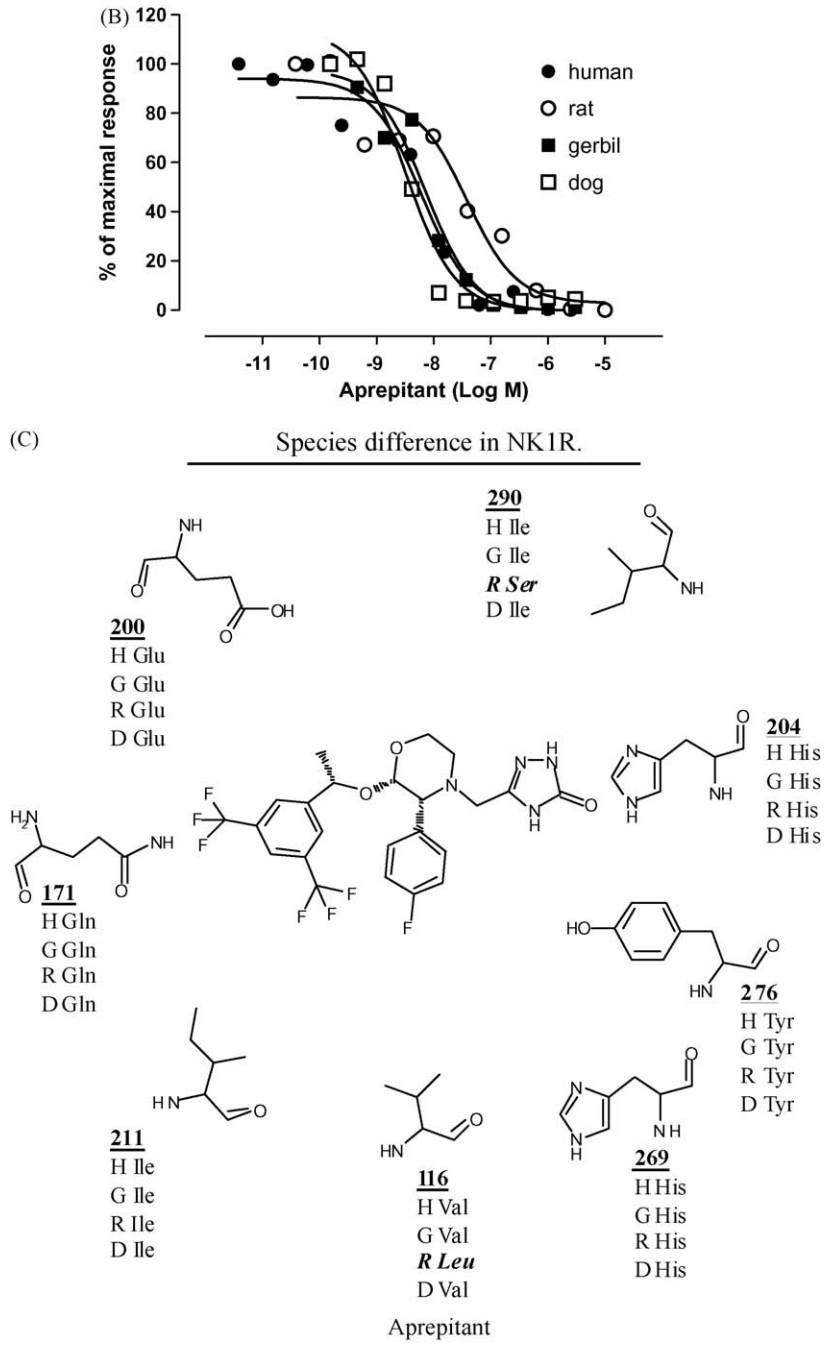


Fig. 1. (*Continued*).

degrees of sequence identity. The pairwise alignments of $NK_{1-3}R$ and rhodopsin, were extracted and adjusted manually to optimize compatibility with structure and frequently occurring sequence motifs amongst GPCRs. The rhodopsin–$NK_{1-3}R$ alignment was used as input for the automatic modeling and used for the evaluation of the WhatCheck module [33]. All binding site results and discussions are based on predictions drawn from NKR modified rhodopsin homology model. A limitation of the Whatif modeling tool is that insertions and gaps are not considered. In this case, however, the active site should not be affected by this limitation.

2.6. Data analysis

The calcium mobilization data generated *in vitro* were fitted to a four-parameter equation using Excel Fit. IC_{50}'s for antagonists were determined from concentration–response curves for each compound. Potency (K_B)-values for antagonists were calculated using the Cheng–Prusoff equation [34] and expressed as pK_B-values ($pK_B = -\log K_B$). All data are expressed as mean ± S.E.M.

3. Results

3.1. Sequence characterization and antagonist pharmacology of the tachykinin NK_1 receptor

Multiple sequence alignments of the gerbil, human, dog, rat, mouse and guinea pig NK_1R are shown in Fig. 1A. Unlike rat and gerbil, the dog NK_1R displays 100% homology with human in the TM regions. Thus, the Val116 and Ile290 residues, previously identified as important for antagonist binding, are identical between

the dog and the human NK_1 receptor sequences. Also extracellular residues such as Glu97 have also been suggested as being responsible for species selectivity of antagonists. Indeed, gerbil and dog NK_1R also contained a Glu residue in this position while rat and mouse counterparts contained Val97.

The selective NK_1R antagonists aprepitant (Fig. 1B and Table 3), CP99,994 (Table 3), and the pan-NK antagonist ZD6021 (Table 3) were slightly more potent (5–10-fold) inhibitors of the SP-induced responses in cells expressing the dog compared to the human NK_1R. RP67580 displayed similar potency at NK_1R from all species evaluated (Table 3).

Fig. 1C illustrates a rhodopsin–NK_1R homology model which emphasizes the role of AA 116 and 290 in dictating species differences in NK_1R antagonist pharmacology. The polar 290Ser in rat is smaller and less hydrophobic compared to the non-polar 290Ile present in human and dog NK_1R. In contrast, the Leu116Val (rat/human) is a rather conservative AA exchange. There are no other species-related differences in any of the other residues found to be close to the aprepitant binding site using this model (Fig. 1C).

Table 3
Potency of selective NK receptor antagonists and the pan-NKR antagonist ZD6021 on substance P (NK_1R), NKA (NK_2R) and Pro7NKB (NK_3R) evoked increases in intracellular Ca^{2+} mobilization. Data are expressed as pK_B values ± S.E.M., n = 3–5.

Compound	Human	Dog	Gerbil	Rat
NK_1R				
ZD6021	8.6 ± 0.4	9.5 ± 0.2	9.0 ± 0.2	<6
RP67580	7.1 ± 0.4	7.1 ± 0.6	6.5 ± 0.2	7.3 ± 0.4
CP99,994	8.7 ± 0.2	9.8 ± 0.3	8.9 ± 0.3	5.9 ± 0.2
Aprepitant	8.7 ± 0.2	9.2 ± 0.1	8.8 ± 0.2	7.3 ± 0.1
NK_2R				
ZD6021	8.3 ± 0.4	8.4 ± 0.2	8.4 ± 0.3	8.1 ± 0.1
Saredutant	9.1	9.4 ± 0.1	9.3 ± 0.2	9.4 ± 0.1
NK_3R				
ZD6021	7.9 ± 0.3	7.8 ± 0.1	7.9 ± 0.1	6.7 ± 0.2
Talnetant	8.6 ± 0.3	8.4 ± 0.2	8.4 ± 0.1	7.4 ± 0.2
Osanetant	8.4 ± 0.5	8.2 ± 0.2	8.0 ± 0.2	7.4 ± 0.3

3.2. Sequence characterization and antagonist pharmacology at the tachykinin NK_2 receptor

The alignments of the gerbil, human, dog, rat, mouse and guinea pig NK_2Rs, are shown in Fig. 2A. The residues located in the proposed saredutant binding site differ between species. Position 202 located within TM5 is an Ile in the human and dog NK_2R while the gerbil and rat NK_2R have a Phe in this position. Position 205, also located in TM5, is Ile in human, rat and dog NK_2R and Val in gerbil NK_2R, while position 267 (located in TM6) has Leu in the human, rat and gerbil NK_2R, which is replaced by a Phe in the dog NK_2R.

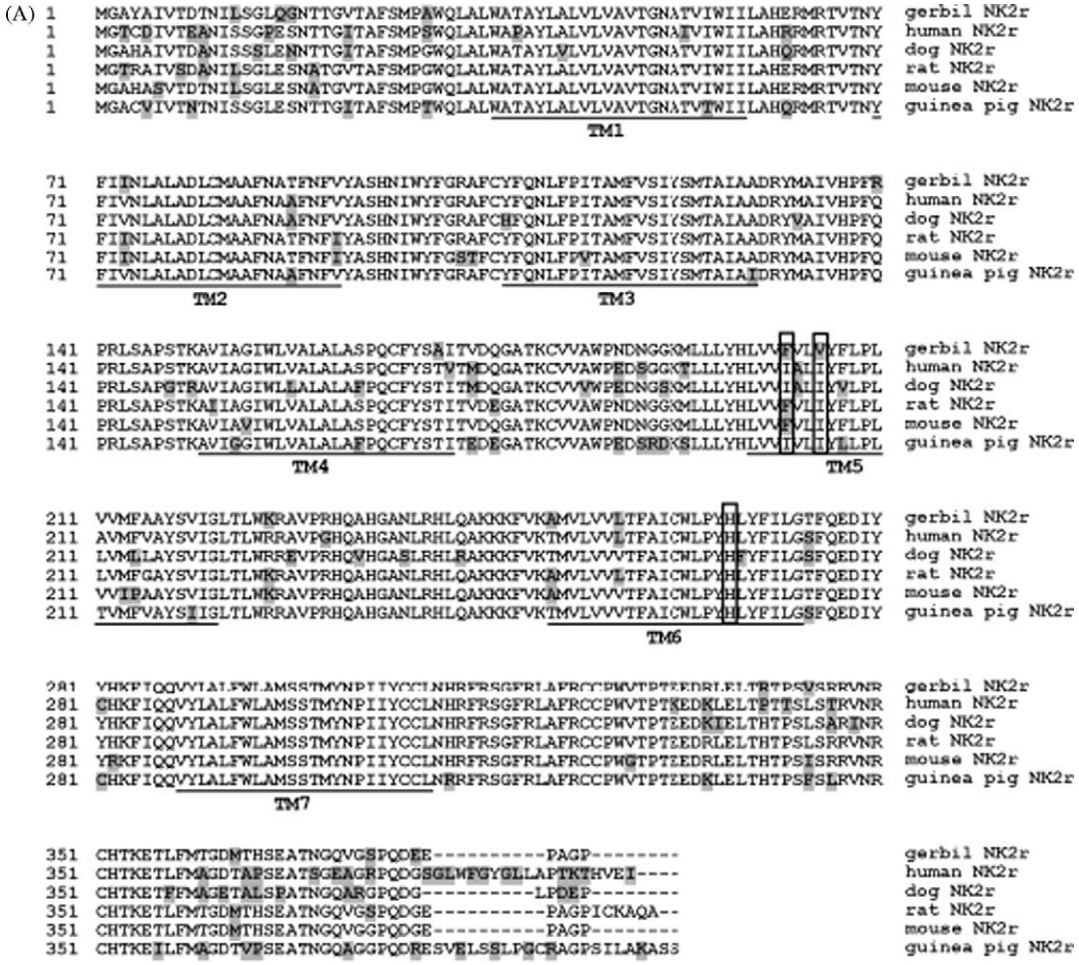


Fig. 2. (A) Sequence alignments of NK_2Rs from gerbil, human, dog, rat, mouse and guinea pig. TM regions are underlined, sequence differences indicated by gray shading and amino acids predicted to be important for antagonist binding are boxed. (B) Representative curve illustrating the dose-dependent inhibitory effect of the selective NK_2 receptor antagonist saredutant on NKA-evoked mobilization of intracellular Ca^{2+} in cells expressing the NK_2 receptor from various species. Representative curves from each animal from three experiments done, are shown, all performed in the same experiment (see Table 3). (C) Chemical sequences of amino acids interacting in the NK_2R binding site in the presence of saredutant. Bold numbers on AA indicate change as compared to the human AA. AA marked in bold show a TM region species difference.

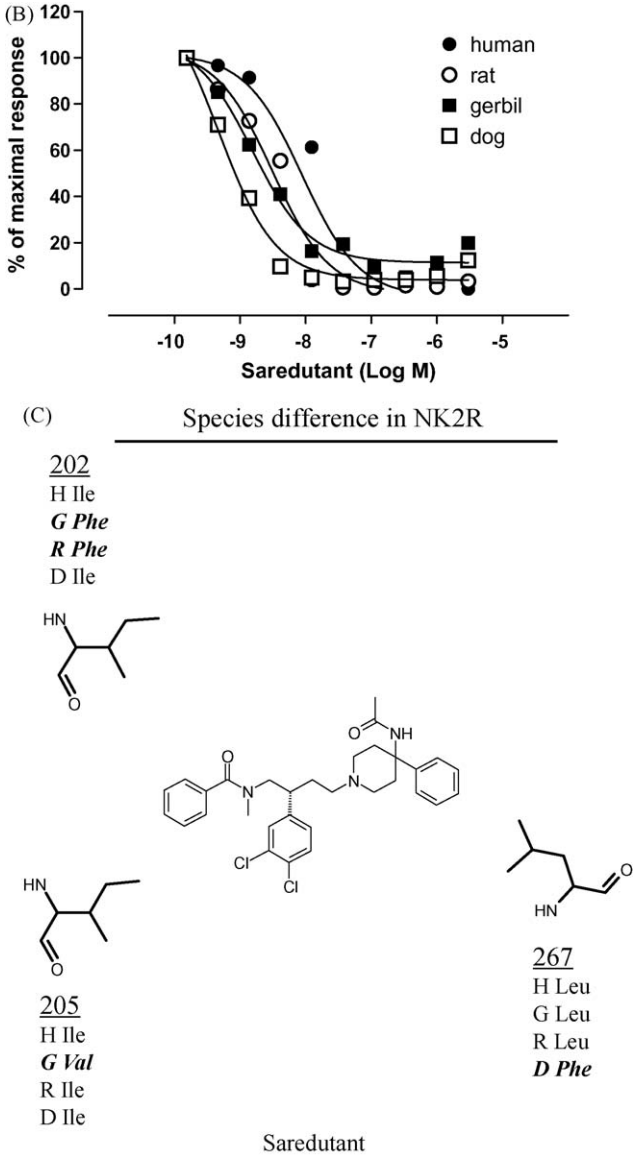


Fig. 2. (*Continued*).

Despite the interspecies differences in AA in TM regions, the selective NK_2R antagonist saredutant (Fig. 2B and Table 3), and the pan-NKR antagonist ZD6021 (Table 3), had a similar potency at the NK_2R from the various species.

Fig. 2C illustrates the chemical structures of AA that differ between species and that are presumed to be involved in NK_2R binding of saredutant. All the differences between AA in the NK_2R binding site contain neutral, non-polar AA. Even though there are differences in the hydrophobicity among residues, they do not seem to affect the potency of the tested NK_2R antagonists (Table 3).

3.3. Sequence characterization and antagonist pharmacology at the tachykinin NK_3 receptor

Alignments of the gerbil, human, dog, rat, mouse and guinea pig NK_3R sequences are shown in Fig. 3A. Key residues important for the binding of talnetant (Met134, Ala146 and Ile317) are identical between gerbil, dog and human, while the corresponding residues in the rat and mouse sequences are Val134, Gly146 and Val317 respectively.

The selective NK_3R antagonists talnetant (Fig. 3B and Table 3) and osanetant (Table 3) had similar potency at human, dog and gerbil NK_3R while being approximately 10-fold less potent for the rat NK_3R. Furthermore, the pan-NKR antagonist ZD6021 also displayed 10-fold higher potency at human, dog and gerbil NK_3R compared to rat NK_3R (Table 3).

Fig. 3C illustrates the rhodopsin–NK_3R homology model which emphasizes the role of key residues for talnetant binding. The human NK_3R positions 265 and 306 differ from all other species studied. The dog NK_3R has a Met at position 202 while the other species have an Ile. Nevertheless, based on the pharmacological profile of antagonists at NK_3R for the various species it appears as if the rat/mouse-specific AA at residues 134, 146 and 317 may play a role in the lower potency of NK_3R antagonists at rat NK_3R.

4. Discussion

It has been known for quite some time that there are major differences in the affinity of many selective NK_1R antagonists between human and rat/mouse NK_1R. Therefore, since these commonly used experimental animals are not always suitable for evaluation of clinically relevant compounds, other species have been used instead, including gerbils and dogs. Indeed, gerbils have been used for detecting potential behavioural [6] anti-depressive [9], visceral anti-hyper algesic [10] and gut motility [7] effects of NK_1R antagonists. We recently showed that the gerbil NK_1R shares key residues (Glu97, Val116 and Ile290) with the human NK_1R homologue and that it has similar affinity for several known NK_1R antagonists [5] (Fig. 1C). Dogs are often used to study the efficacy of anti-emetic compounds like NK_1R antagonists [15]. The current study extends our previous findings in that the dog NK_1R also contains the same important residues for antagonist function as human and gerbil NK_1R. This provides confirmation at the molecular level that the dog belongs to the human-like NK_1R sub-family which has been previously suggested based on experiments *in vivo* [35]. Interestingly, the compounds ZD6021, CP99,994 and aprepitant were actually slightly more potent (5–10-fold) at dog NK_1R compared to gerbil and man. The reason for this is unclear since the receptors display 100% homology in the TM region. RP67580, often referred to as a rat-selective NK_1R antagonist, was equipotent at dog, rat and man. Hence, the rat-selectivity is not supported in the current study and is in-line with previous data [5]. Aprepitant is clearly less potent at rat NK_1R than at NK_1R from other species. However, based on our data, aprepitant could be useful as a tool compound in rat models since it displays similar potency as RP67580, an NK_1R antagonist commonly used in rat models, but appears to have better DMPK properties such as CNS penetration and metabolic half-life [36].

Cloning and sequencing of gerbil and dog NK_2R provides data for a more thorough comparison between species. In contrast to NK_1R, rather large discrepancies were found in the TM region in the NK_2R. Residues 202, 205 and 267 had a variety of amino acids in this position among species (Fig. 2C). Interestingly, all three of these residues have been implicated in the binding of NK_2R antagonists [37]. Despite the potential importance of these residues for binding and the rather large degree of variation between species, we were unable to identify significant differences of the potency of saredutant and ZD6021 at NK_2R among the species studied. However, it cannot be excluded that the potency of structurally different compounds could have different potency at NK_2R from gerbil and dog [16].

Consistent with previous findings by Ref. [38], both talnetant and osanetant displayed about a 5–10-fold lower potency at the rat NK_3R compared to the human NK_3R. ZD6021 also displayed a relatively weak potency for rat NK_3R (30-fold weaker than at human NK_3R). The current study extends these findings demonstrating that the antagonists tested have similar potency at dog and gerbil NK_3R as to human NK_3R. Recent data show that talnetant

and osanetant interact within overlapping but not identical binding pockets in the human tachykinin NK_3R transmembrane domains [39]. The human-like pharmacology of gerbil and dog NK_3R is consistent with the presence of a methionine located in position 134, a residue that has previously been shown to be important for talnetant and osanetant binding to the human NK_3R (Fig. 3C). The Ile317Val AA change may induce less sterical hindrance, and may affect the functional potency of the tested compounds (Fig. 3C). The above suggestions support that gerbils and dog represent appropriate species for evaluating the efficacy of tachykinin NK_3R antagonists intended for clinical use. Indeed, osanetant has demonstrated anxiolytic-like and antidepressant-like effects in gerbils [40].

Single nucleotide polymorphisms in genes encoding receptors can affect many aspects of receptor function and antagonist binding. For instance, four variants of the human NK_2R are common within the human population [41]. The current study utilized the Thr23_Arg375 variant, however saredutant and ZD6021 display similar potency at all four human variants. If AA-exchanging polymorphisms in NKRs occur within or between strains of the experimental animals compared here is unknown to our knowledge.

Given the above species difference in NKR pharmacology the study of receptor-mediated toxicology of any NKR modulator, both agonist and antagonists, is not trivial. Ideally, for such studies to be meaningful, selected species for toxicology testing should express the receptor in a similar way and have similar functions as in humans. Concomitantly, selected species should be well characterized with historical data, in order to differentiate any treatment related lesions from spontaneous (background) pathology. For these reasons, studies of potential toxicology of NK_2R antagonists seem to

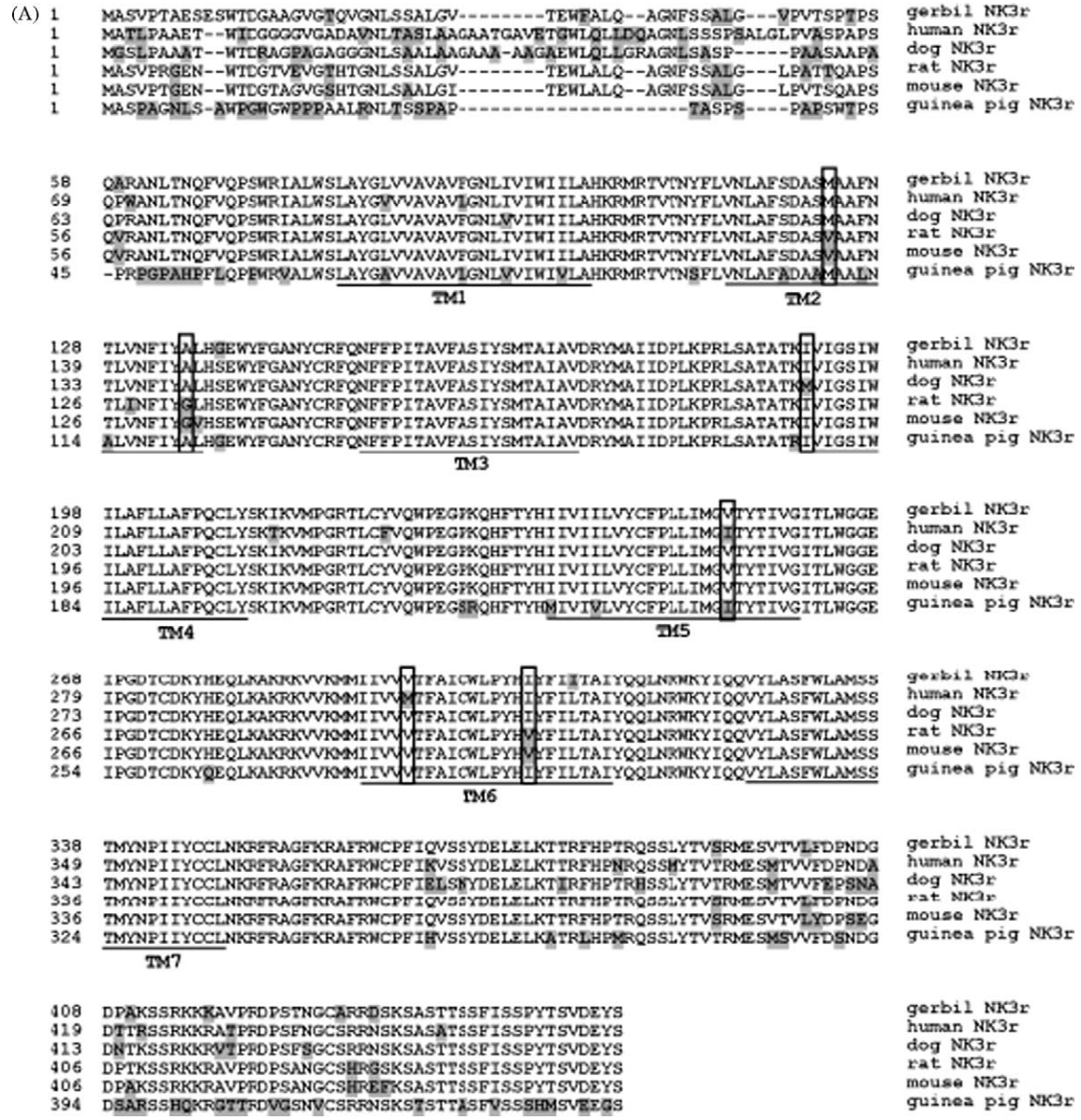
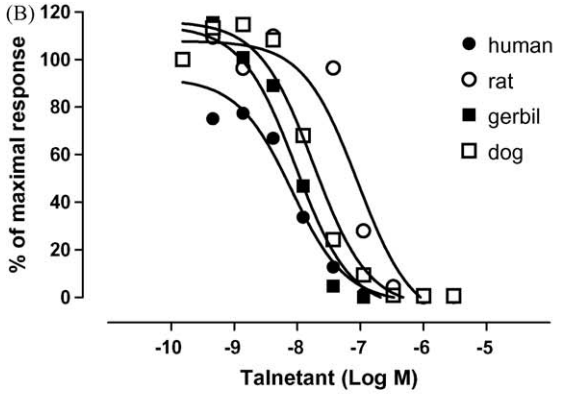


Fig. 3. (A) Sequence alignments of NK_3Rs from gerbil, human, dog, rat, mouse and guinea pig. TM regions are underlined, sequence differences indicated by gray shading and amino acids predicted to be important for antagonist binding are boxed. (B) Representative curve illustrating the dose-dependent inhibitory effect of the selective NK_3R antagonist talnetant on NKB-evoked mobilization of intracellular Ca^{2+} in cells expressing the NK_3R from various species. Representative curves from each animal from three experiments done, are shown, all performed in the same experiment (see Table 3). (C) Chemical sequences of amino acids interacting in the NK_3R binding site in the presence of talnetant. Bold numbers on AA indicate change as compared to the human AA. AA marked in bold show a TM region species difference.



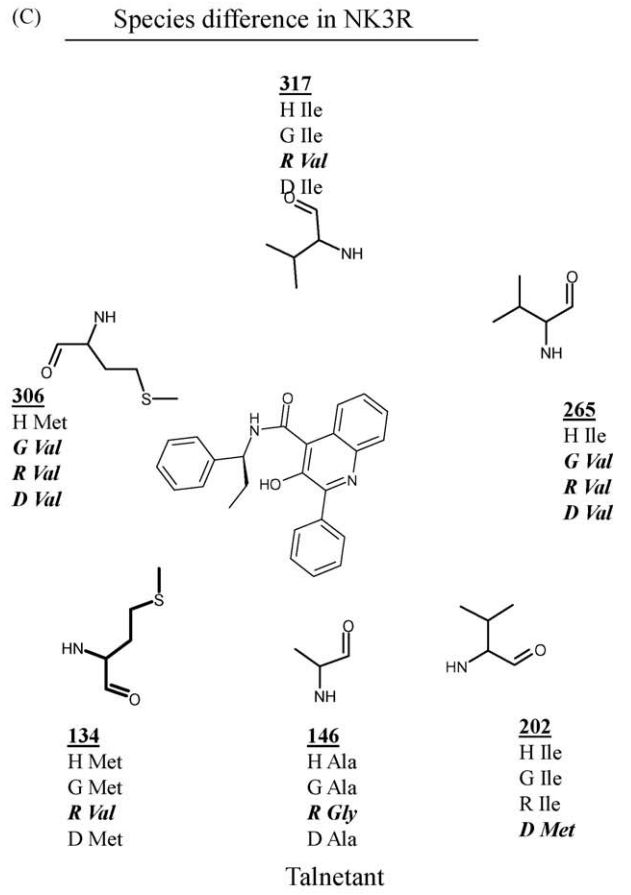


Fig. 3. (*Continued*).

be appropriately conducted in well-known species such as the rat and dog, while dogs appear to suffice for detecting potential unwanted effects of NK_1R and NK_3R antagonism.

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