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[^{3}H]GR205171 displays similar NK1 receptor binding profile in gerbil and human brain

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1 In this study, [^{3}H]GR205171 (3(*S*)-(2-methoxy-5-(5-trifluoromethyltetrazol-1-yl)-phenylmethyl-amino)-2(*S*)-phenylpiperidine), a potent and selective NK1 receptor antagonist, was characterised in autoradiographic studies in gerbil brain and in binding experiments on homogenates from gerbil and human brain cortex and striatum.

2 In autoradiographic studies in gerbil brain, highest levels of [^{3}H]GR205171 binding sites were observed in caudate putamen, nucleus accumbens, medial and cortical nuclei of the amygdala and intermediate levels were detected in the hypothalamus, basolateral amygdala, septum, and cortex.

3 Saturation experiments in homogenates of brain striatum from gerbil showed that [^{3}H]GR205171 binds to a single receptor population with a pK_d value of 10.8 ± 0.2 and a B_{max} value of 607 ± 40 fmol mg^{-1}. A lower number of NK1 receptor sites was found in cortex, where a B_{max} of 94 ± 6 fmol mg^{-1} protein was obtained. Saturation experiments performed on homogenates from brain striatum of two human subjects and brain cortex of three human subjects showed that [^{3}H]GR205171 binds with pK_d values not different from gerbil and B_{max} values ranging from 318 ± 51 to 432 ± 27 fmol mg^{-1} protein in striatum and from 59 ± 1 to 74 ± 21 fmol mg^{-1} protein in cortex. The natural ligand [^{3}H]Substance P (SP) bound with sub-nanomolar affinity to 15 and 6% sites compared to [^{3}H]GR205171 in gerbil and human striatum, respectively.

4 In competition binding experiments, GR205171 and the NK1 receptor antagonists aprepitant (MK-869), L-733,060 and NKP-608 bound with similar pK_i values in gerbil and human striatum, irrespective of the use of [^{3}H]GR205171 or [^{3}H]SP as radioligand. The following rank order was found in terms of pK_i values: GR205171 > aprepitant $\geqslant$ L-733,060 > NKP-608. In homologous displacement experiments in gerbil and human striatum, SP showed nanomolar affinity, whereas in [^{3}H]GR205171 competition experiments SP bound with pIC_{50} values in the micromolar range and Hill slopes significantly lower than one.

5 It is concluded that the similarities of [^{3}H]GR205171 binding characteristics and pharmacology between gerbil and human in cortex and striatum support the use of gerbil in preclinical models to study the effects of NK1 receptor antagonists in the central nervous system.
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Keywords: GR205171; substance P; NK1 receptor; binding; gerbil brain; human brain

Abbreviations: B_{max}, receptor density; BSA, bovine serum albumin; DMSO, dimethyl sulphoxide; GPCR, G-protein-coupled receptors; GR205171, 3(*S*)-(2-methoxy-5-(5-trifluoromethyltetrazol-1-yl)-phenylmethylamino)-2(*S*)-phenylpiperidine; HEPES, 4-2-(hydroxyethyl)piperazine-1-ethanesulfonic acid; IC_{50}, half-maximal inhibitory concentration; L-733,060, (2*S*,3*S*)-3-((3,5-bis(trifluoromethyl)phenyl)methyloxy)-2-phenyl piperidine; MK-869, aprepitant, 5-[[2(*R*)-[1(*R*)-[3,5-bis(trifluoromethyl)phenyl]ethoxy]-3(*S*)-(4-fluorophenyl)-4-morpholinyl]methyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-one; NKP-608, (quinoline-4-carboxilic acid [*trans*-(2*R*, 4*S*)-1-(3,5-bistrifluoromethyl-benzoyl)-2-(4-chloro-benzyl)-piperidin-4-yl]-amide); PET, positron emission tomography; SP, Substance P

Introduction

The tachykinin receptors are G protein-coupled receptors characterized by seven trans-membrane domains. They are classified in neurokinin type 1 (NK1), 2 (NK2), and 3 (NK3) receptors at which the endogenous neuropeptides substance P (SP), Neurokinin A and Neurokinin B bind with highest affinity, respectively. NK1 receptors are widely distributed in both the central and peripheral nervous system, where the undecapeptide SP functions as a neurotransmitter and neuromodulator in a variety of physiological processes such as neuronal excitation, vasodilatation and smooth muscle contraction (Quartara & Maggi, 1998). Over recent years, interest in central NK1 receptors has increased based on reports on the therapeutic potential for NK1 receptor antagonists in psychiatric disorders. The first clinical evidence of the ability of NK1 receptor antagonists to alleviate depression and anxiety came from a double blind study with aprepitant (MK-869), which showed efficacy similar to the selective serotonin reuptake inhibitor paroxetine but without the side-effects of this class of molecules (Kramer *et al*., 1998). Following that study, other two independent clinical trials with two different NK1 receptor antagonists (L-759274 and

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CP-122,721) showed antidepressant effects in depressed patients. However, the recent negative findings with aprepitant in a depression phase III study made controversial the efficacy of NK1 receptor antagonist in depression (Herpfer & Lieb, 2005) and rendered crucial the identification of new potent and brain penetrant NK1 receptor antagonists to further explore the antidepressant and anxiolytic activity of NK1 receptor antagonists. The preclinical assessment of the efficacy of NK1 receptor antagonists has been partially hindered by the low pharmacological homology between human and rat or mouse NK1 receptors. NK1 receptor antagonists which display high affinity at human receptors, such as CP-96,345 and GR205171 show lower affinity in mouse and rat (Beresford *et al*., 1991; Zocchi *et al*., 2003). Vice versa, antagonists with high affinity at rat or mouse receptors, such as RP67580, show a lower affinity in human (Fong *et al*., 1992).

Guinea pig and gerbil are the preclinical species most commonly used to test efficacy of NK1 receptor antagonists since they share higher homology to human in terms of NK1 receptor pharmacology as compared to other rodents (Beresford *et al*., 1991; Saria, 1999). Over recent years, a number of different models have been developed in gerbil to assess brain penetrant and anxiolytic and antidepressant-like properties of NK1 receptor antagonists such as the agonist-induced foot tapping (Bristow & Young, 1994; Rupniak & Williams, 1994), the social interaction test (Cheeta *et al*., 2001), the elevated plus maze test (Varty *et al*., 2002), the fear conditioning test (Rupniak *et al*., 2003a) and the tail suspension test (Varty *et al*., 2003).

Autoradiographic and *in situ* mRNA hybridisation studies performed in mammalian brain showed that NK1 receptors are most abundant in striatum, nucleus accumbens, hippocampus, raphe nuclei and medulla oblongata, whereas expression in cortex is moderate (Saria, 1999; Caberlotto *et al*., 2003; Rigby *et al*., 2005). However, no data are available to compare the NK1 receptor binding pharmacology and expression between human and gerbil brain despite the large use of the latter in preclinical models.

In this study, autoradiographic experiments with the NK1 receptor antagonist [^{3}H]GR205171 (Gardner *et al*., 1996) were performed in brain slices of gerbil to determine NK1 receptor distribution. Moreover, [^{3}H]GR205171 saturation binding experiments were performed on homogenates from cortex and striatum of gerbil and human in order to assess the relative amount of NK1 receptor sites. [^{3}H]SP saturation experiments were also performed on striatum from both gerbil and human in order to determine the amount of agonist compared to antagonist binding sites. Finally, [^{3}H]SP and [^{3}H]GR205171 displacement binding experiments by known NK1 receptor antagonists such as aprepitant, NKP-608 and L-733,060 (Seabrook *et al*., 1996; Kramer *et al*., 1998; Vassout *et al*., 2000) were performed in gerbil and human striatum.

Methods

Materials

GR205171, aprepitant and NKP-608 were synthesised in GSK Chemical Department, Verona, Italy. [^{3}H]GR205171 (3.07 TBq mmol^{-1}), di-trifluoroacetic acid salt was synthesised in GSK Chemical Department and labelled with [^{3}H] by Amersham International Plc., Little Chalfont, Buckinghamshire, U.K. [^{3}H]SP (1–2 TBq mmol^{-1}) was purchased from Amersham Biosciences, Freiburg, Germany. Leupeptin, Bacitracin, Pefabloc, Pepstatin, Chymostatin, Phosphoramidon, Bovine serum albumin (BSA), L-733,060 and SP were purchased from Sigma, Milan, Italy. All drugs to be tested in filtration binding assays were diluted primarily in dimethyl sulfoxide (DMSO) and further diluted in the assay buffer to give a final DMSO concentration of 1%.

[^{3}H]GR205171 autoradiography in gerbil brain Animal manipulations were performed according to Italian law (art. 7, Legislative Decree No. 116, 27 January 1992), which acknowledged the European Directive 86/609/EEC, and to GlaxoSmithKline policy on the care and use of laboratory animals and related codes of practice. Mongolian gerbils (60–70 g, Charles River, Sulzfeld, Germany) were killed by decapitation and brains were quickly removed and frozen in isopentane at −30°C. Coronal brain sections were thawed, air dried and preincubated for 15 min at room temperature in Tris-HCl (50 mM, pH 7.4) containing 0.02% BSA. Sections were then incubated for 90 min at room temperature in the same buffer containing $MnCl_2$ (3 mM), Bacitracin (0.04 mg ml^{-1}), Leupeptin (0.004 mg ml^{-1}), Chymostatin (0.002 mg ml^{-1}), and 0.2 nM [^{3}H]GR205171. Nonspecific binding was determined in the presence of 100 nM unlabeled GR205171 in the incubation buffer. At the end of the incubation, sections were washed four times (60 s each) in ice-cold Tris-HCl (50 mM, pH 7.4) containing 0.02% BSA, briefly dipped in ice-cold distilled water and then dried under a stream of cool air, and exposed for one week to imaging plates (Fuji Photo Film, Tokyo, Japan).

Quantification of autoradiographic results Films from the autoradiography experiments were scanned using a BAS-5000 Bio-Imaging Analyzer (Fuji Photo Film, Tokyo, Japan) and quantified using an image analysis software system (AIS 4.0, Imaging Research Inc., St Catharines, Ontario, Canada). Regional densities of binding were determined with reference with tritium standards (RPA 510, Amersham, Freiburg, Germany) on the same film and converted to fmol mg^{-1} tissue.

Preparation of brain homogenates from human and gerbil Brain cortex and striatum from mongolian gerbils (pools of five animals for each preparation) were dissected immediately after animal decapitations. The following brain samples from a total of four human donors were obtained under approved ethical guidelines and homogenates were prepared separately: cortex and striatum from subject A, male, aged 58 years, died of a cardio-respiratory failure (post-mortem interval 23 h); striatum from subject B, male aged 17 years, died of brain tumor (post-mortem interval 8 h); cortex from subject C, female aged 60 years, died of acute tracheobronchitis (post-mortem interval 7 h 30 min); cortex from subject D, female aged 93 years, died of natural causes (post-mortem interval not known). Brain homogenates were prepared as follows: fresh (gerbil) or frozen (human) tissues were weighed, crumbled and homogenised in 10 volumes of membrane-preparation buffer (HEPES 50 mM pH 7.4, Leupeptin 0.1 mM, Bacitracin 40 μg ml^{-1}, EDTA 1 mM, Pefabloc 0.2 mM, Pepstatin 2 μM). The homogenate was then centri-

fuged at 48,000 × *g* for 20 min, and the pellet was washed once more by resuspension in 10 volumes of membrane preparation buffer and centrifugation again at 48,000 × *g* for 20 min. The final pellet was resuspended in 7–10 volumes of membrane preparation buffer and frozen at −80°C until use. Protein concentration was determined by the Bio-Rad Protein assay (Milan, Italy) using BSA as the standard.

Binding experiments with [^{3}H]GR205171 and [^{3}H]SP In saturation experiments, increasing concentrations of [^{3}H]GR205171 (0.01–1 nM) or [^{3}H]SP (0.02–5 nM) were incubated with homogenates from striatum (20–50 μg well^{-1} for [^{3}H]GR205171, 200–500 μg well^{-1} for [^{3}H]-SP) or cortex (100 μg well^{-1}) for 60 min at room temperature in a final volume of 400 μl of 50 mM HEPES, pH 7.4, 3 mM $MnCl_2$, 0.02% BSA, 2 μg ml^{-1} Leupeptin, 20 μg ml^{-1} Bacitracin, and 0.5 μM Phosphoramidon. Nonspecific binding was determined in the presence of 1 μM GR205171. Reactions were stopped by filtration over GF/C filters presoaked in 0.5% polyetylenimmine followed by three washings with cold 0.9% NaCl.

In competition binding experiments, 0.2 nM [^{3}H]GR205171 or 0.6 nM [^{3}H]SP were incubated with homogenates of gerbil or human (subject A) striatum as aforementioned, in the presence of increasing concentration of displacing compounds (1 pM–1 μM GR205171, L-733,060, aprepitant and NKP-608, 10 pM–10 μM SP).

Data analysis

Radioligand binding data were analysed by nonlinear regression analysis using GraphPad Prism 4.0 (GraphPad Software, CA, U.S.A.). Determination of K_D and B_{max} of [^{3}H]GR205171 and [^{3}H]SP was assessed by elaborating saturation experiments using one site binding (hyperbola) equation. Curve fitting from competition-binding experiments was determined by using one site competition equation after checking with *F* test ($P<0.05$) that Hill slope in the four parameter logistic equation was not statistically different from 1.0. In this condition, IC_{50} values were converted to K_i using the Cheng–Prusoff equation (Cheng & Prusoff, 1973). Results are expressed as mean pK_i ± s.e.m.

Results

[^{3}H]GR205171 autoradiography on gerbil brain

The distribution of [^{3}H]GR205171 binding in gerbil brain was heterogeneous (Figure 1). The highest levels of binding sites were observed in caudate putamen, nucleus accumbens, medial and cortical nuclei of the amygdala. Intermediate levels were detected in the hypothalamus, basolateral amygdala, septum, and cortex, while low levels were observed in hippocampus and thalamus. The number of receptor sites (fmol mg^{-1} tissue) labelled by 0.2 nM [^{3}H]GR205171 were 69.6 ± 2.8 in striatum, 8.5 ± 0.5 in cortex, 171.6 ± 6.9 in medial amygdala, 30.1 ± 2.0 in basolateral amygdala, 8.2 ± 0.7 in thalamus, and 34.3 ± 1.5 in hypothalamus ($n=3$).

Saturation binding experiments

In saturation-binding experiments performed in gerbil and human striatum and cortex, [^{3}H]GR205171 bound to a single site with similar and high affinity (Figure 2a and b). B_{max} values obtained for gerbil (607 ± 40 fmol mg^{-1} protein in striatum and 94 ± 6 fmol mg^{-1} protein in cortex) were in agreement with the number of receptor sites labelled by [^{3}H]GR205171 in autoradiography experiments, assuming 1 mg of protein corresponds approximately to 10 mg of tissue. Human striatum and cortex showed lower B_{max} values compared to gerbil (from 318 ± 51 to 432 ± 27 fmol mg^{-1} protein in striatum and from 59 ± 1 to 74 ± 21 fmol mg^{-1} protein in cortex). However, the ratio between the number of receptors in the two tissues was similar in the two species, the striatum showing approximately six fold higher receptor expression than cortex. No significant difference in B_{max} and pK_d for [^{3}H]GR205171 was observed either between subjects A and B in striatum or among subjects A, C, and D in cortex (Table 1).

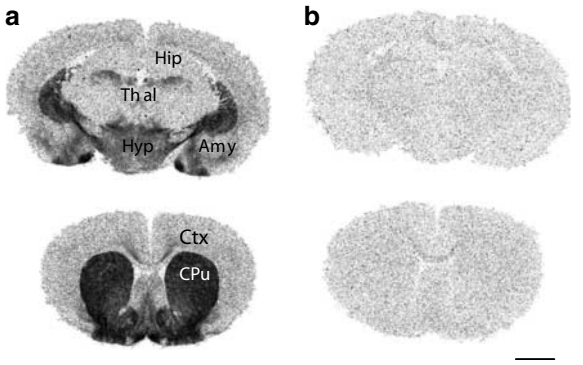

Figure 1 (a) Representative autoradiographic localisation of [^{3}H]GR205171 binding sites at two gerbil brain levels. Brain regions containing substantial levels of binding include caudate putamen (Cpu), nucleus acccumbens, amygdala (Amy) and hypothalamus (Hyp). Medium/low levels of binding were detected in cortex (Ctx), hippocampus (Hip), thalamus (Thal) and septum. (b) Nonspecific binding in the presence of 100 nM unlabeled GR205171. Scale bar: 0.25 cm.

In striatum, [^{3}H]SP bound with similar and high affinity to a single site both in human (subject A) and gerbil (Figure 2c, Table 1), with pK_d values of 9.4 ± 0.1 and 9.5 ± 0.2, respectively. In both species, [^{3}H]SP bound to a smaller amount of receptor sites compared with the antagonist ($B_{max}=93\pm20$ in gerbil striatum, $B_{max}=18\pm2$ in human striatum). The percentage of sites recognised with high affinity by [^{3}H]SP were 6% in human and 15% in gerbil in comparison with the sites recognised by [^{3}H]GR205171.

Competition-binding experiments

Affinity values of compounds tested in displacement experiments vs [^{3}H]GR205171 and [^{3}H]SP are shown in Table 2. The NK1 receptor antagonists GR205171, aprepitant, L-733,060 and NKP-608 showed similar pK_i values in gerbil and human (subject A) striatum, irrespective of the radioligand used. The following rank order was found in terms of pK_i values: GR205171 > aprepitant ≥ L-733,060 > NKP-608 (Figures 3 and 4). In homologous displacement experiments, SP showed pK_i values similar to pK_d obtained in [^{3}H]SP saturation experiments (Figure 3), whereas a considerable drop in the potency was observed when tested against [^{3}H]GR205171. SP competition curves did not fully displace [^{3}H]GR205171 even

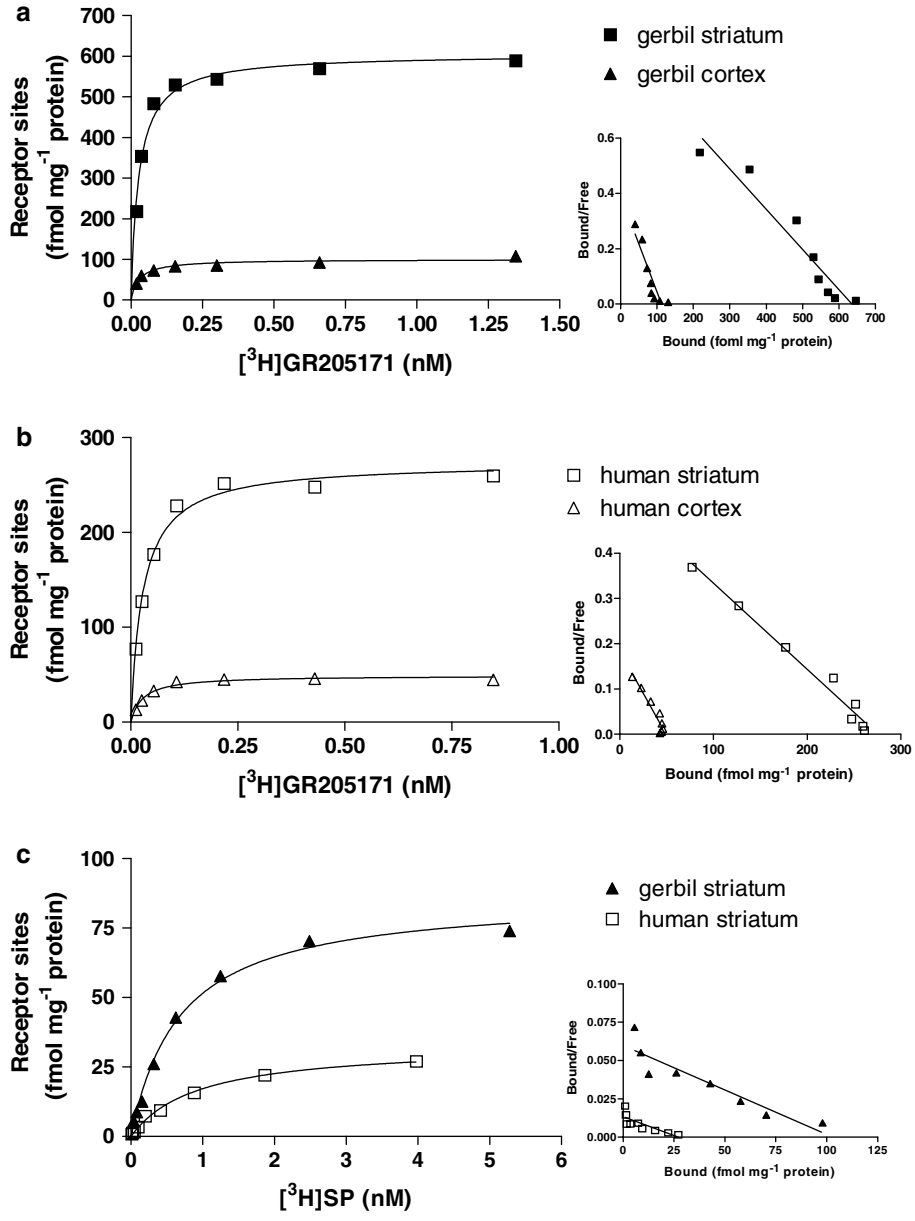

Figure 2 Representative saturation binding experiments with the NK1 receptor antagonist [^{3}H]GR205171 in gerbil (a) and human (b) striatum and cortex and with the endogenous agonist [^{3}H]SP (c) in gerbil and human striatum. Relative Scatchard plot is shown on the right of each saturation curve. Brain samples of subject A were used in these experiments. See Methods for experimental procedures.

at concentration of agonist up to 10 μM. Moreover, Hill slopes of the SP competition curves were significantly lower than one, according to a multi-site binding (Table 2, Figure 4).

Discussion

This study was undertaken with the goals to compare the levels of NK1 receptors in brain cortex and striatum of gerbil and human by using the radiolabelled selective NK1 receptor antagonist, [^{3}H]GR205171 and the endogenous ligand, [^{3}H]SP. Moreover, displacement binding experiments of the two radioligands by standard NK1 receptor antagonists and SP were carried out in both species. Indeed, a high pharmacological homology between gerbil and human NK1 receptors was already proposed by Beresford *et al*. (1991), although it derived from the test of a single NK1 receptor antagonist. Over recent years gerbil has also become the elective species by many groups to assess the central activity of novel NK1 receptor antagonists (Cheeta *et al*., 2001; Duffy *et al*., 2002), even though a more extensive comparison of receptor binding properties and pharmacology of the NK1 receptors in the brain between gerbil and human was still lacking.

GR205171 is a potent and selective nonpeptide NK1 receptor antagonist which is widely used as reference NK1 receptor antagonist both in *in vitro* and *in vivo* studies (Gardner *et al*., 1996; Rupniak *et al*., 2001). Furthermore, [^{11}C]GR205171 and its [^{18}F]-analogue have been extensively used in positron emission tomography (PET) experiments in rhesus monkey and human, respectively, to determine receptor

occupancy of NK1 receptor antagonists at potential therapeutic doses (Bergstrom *et al*., 2000; Hargreaves, 2002; Zamuner *et al*., 2002).

The distribution of [^{3}H]GR205171 binding sites in gerbil brain found in the present study was particularly high in caudate putamen, nucleus accumbens, medial and cortical nuclei of the amygdala. It was intermediate in the hypothalamus, basolateral amygdala, septum and cortex, and low in hippocampus and thalamus. These data closely matched the distribution of NK1 receptors labeled by [^{125}I]-SP, as reported by Duffy *et al*. (2002) and more recently by Rigby *et al*. (2005) in gerbil brain. Furthermore, the high level of [^{3}H]GR205171specific binding in striatum endorses the findings of PET experiments with [^{11}C]-GR205171 and the [^{18}F]-analogue in rhesus monkey and human, where this region shows the most intense radioligand uptake and thus is considered the preferred region for the assessment of NK1 receptor blockade by brain-penetrant NK1 receptor antagonists.

[^{3}H]GR205171 and [^{3}H]SP filtration binding assays on homogenates from brain cortex and striatum of gerbil and human were carried out to gain information about NK1 receptors density in the two different brain areas and to compare the pharmacology of known NK1 receptor ligands in the two different species. Saturation experiments with [^{3}H]GR205171 showed that striatum and cortex of both gerbil and human have the same affinity values for the radioligand used. Moreover, striatum displayed more binding sites with respect to cortex by a factor of approximately six-folds in both gerbil and human. Human striatum and cortex showed lower B_{max} values compared to gerbil. However, when human brain samples are used there are potential concerns that need to be taken into account such as the potential degradation of receptor proteins due to the post mortem intervals. A partial

Table 1 [^{3}H]GR205171 and [^{3}H]SP saturation experiments on brain striatum and cortex from gerbil and different human subjects

		[^{3}H]GR205171 binding		*[^{3}H]SP binding*	
		pK_d	B_{max}	pK_d	B_{max}
Brain striatum	Gerbil	10.8±0.2	607±40	9.5±0.2	93±20
	Subject A	10.6±0.1	318±51	9.4±0.1	18±2
	Subject B	10.7±0.1	432±27		
Brain cortex	Gerbil	10.5±0.2	94±6		
	Subject A	10.5±0.2	74±21		
	Subject C	10.6±0.1	59±1		
	Subject D	10.7±0.1	60±2		

B_{max} values are expressed as fmol mg^{-1} protein. Data presented are the mean±s.e.m. of at least three independent experiments performed in duplicate. See Methods for experimental procedures. No significant difference in [^{3}H]GR205171 pK_d and B_{max} values in striatum and cortex was observed among subjects ($P>0.05$, *t*-test).

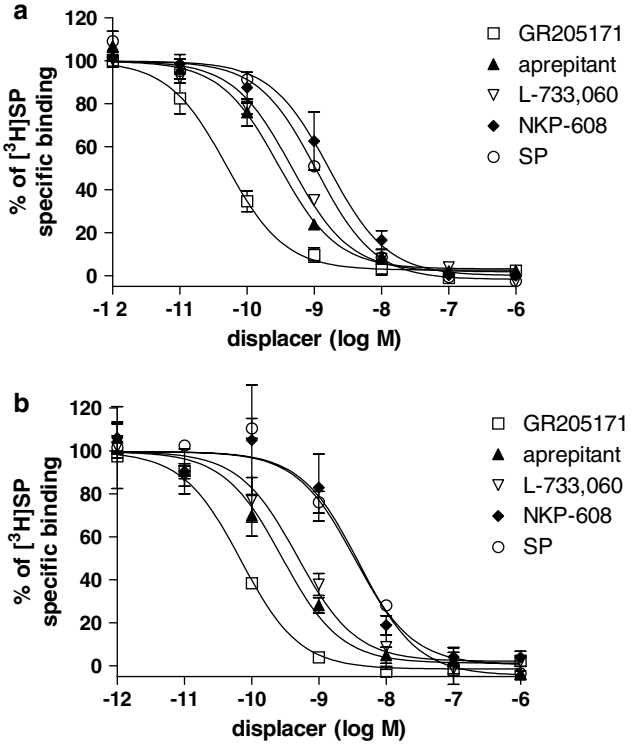

Figure 3 Inhibition of [^{3}H]SP binding by GR205171, aprepitant (MK-869), L-733,060, NKP-608 and SP in homogenates prepared from gerbil (a) and human (b) striatum. Results are expressed as % of specific radioligand binding against the log concentration of each compound and values are mean±s.e.m. These data were taken from three independent experiments. Brain striatum of subject A was used in these experiments.

Table 2 [^{3}H]GR205171 and [^{3}H]SP competition experiments on brain striatum of human (subject A) and gerbil

	pK_i vs [^{3}H]GR205171		*pK_i vs [^{3}H]SP*	
	Human	*Gerbil*	*Human*	*Gerbil*
SP	6.34±0.22[a] (Hill slope=0.38±0.09) (*n*=4)	6.61±0.16[a] (Hill slope=0.57±0.10) (*n*=3)	9.06±0.11 (*n*=3)	9.49±0.03 (*n*=3)
GR205171	10.50±0.09 (*n*=3)	10.79±0.02 (*n*=5)	10.65±0.10 (*n*=4)	10.48±0.10 (*n*=3)
L-733,060	9.81±0.14 (*n*=4)	9.94±0.15 (*n*=4)	9.71±0.15 (*n*=4)	9.77±0.04 (*n*=4)
Aprepitant	10.11±0.04 (*n*=4)	10.34±0.07 (*n*=4)	10.11±0.20 (*n*=4)	10.06±0.12 (*n*=4)
NKP-608	9.31±0.13 (*n*=3)	9.34±0.28 (*n*=3)	9.09±0.14 (*n*=3)	9.30±0.23 (*n*=3)

n represents the number of independent experiments. See Methods for experimental procedures.
[a]pIC_{50} values.

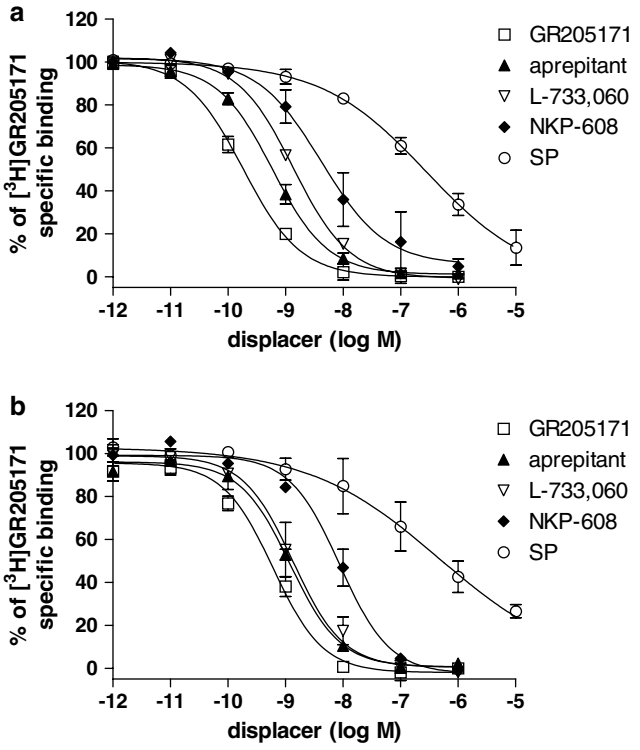

Figure 4 Inhibition of [^{3}H]GR205171 binding by GR205171, aprepitant (MK-869), L-733,060, NKP-608 and SP in homogenates prepared from gerbil (a) and human (b) striatum. Results are expressed as % of specific radioligand binding against the log concentration of each compound and values are mean ± s.e.m. These data were taken from three independent experiments. Brain striatum of subject A was used in these experiments.

degradation of NK1 receptors may have occurred during the human post-mortem interval, causing an overall underestimation of NK1 receptor density. Nevertheless, B_{max} values obtained for two subjects in striatum and three subjects in cortex revealed a similarity of NK1 receptor expression across subjects investigated.

Interestingly, a similar ratio of [^{3}H]GR205171 binding sites in gerbil striatum with respect to cortex was also observed when the autoradiography technique was used in gerbil. In autoradiography experiments, the density of sites in striatum and cortex is in agreement with numbers of specific binding sites calculated for the corresponding brain regions in gerbil brain homogenates. Saturation binding experiments were then performed with the NK1 receptor preferred agonist SP as radioligand in striatum, one of the brain regions with the highest level of receptor expression. [^{3}H]SP was found to bind with high affinity at six- to 18-fold lower number of a binding sites with respect to [^{3}H]GR205171 in gerbil and human, respectively, suggesting that only a small fraction of receptors in native tissues are in the SP-preferred conformation. These data support the findings of Sagan *et al*. (1999) that the NK1 receptor may exist in agonist preferring conformations and accordingly to this, SP displaced [^{3}H]GR205171 with extremely low affinity, which was not the case for GR205171, which displaced [^{3}H]SP and [^{3}H]GR205171 with similar potency. This latter finding further support the valuable properties of the radioligand [^{3}H]GR205171, since it does not discriminate across NK1 receptor conformations and produces a higher signal to noise ratio with respected to [^{3}H]SP in receptor binding experiments.

In competition binding experiments, the selective NK1 receptor antagonists GR205171, aprepitant, L-733,060 and NKP-608 showed similar affinities in human and gerbil brain striatum, irrespective of the use of [^{3}H]GR205171 or [^{3}H]SP as radioligand, confirming the high pharmacological homology between the gerbil and the human NK1 receptors. The pK_i values obtained for GR205171, aprepitant and L-733,060 are in agreement with those found by Duffy *et al*. (2002) in gerbil striatum by using [^{125}I]-SP. On the other hand, the affinity we observed for NKP-608 is intermediate between the pIC_{50} value of 7.9 obtained by Vassout *et al*. (2000) in gerbil midbrain against [^{3}H]SP and the pK_i value of 10.1 found by Rupniak *et al*. (2003b) at recombinant human NK1 receptors against [^{125}I]-Tyr^8-SP. Methodological differences may account for the discrepancy among the published affinities of NKP-608 and the data produced in this work.

In conclusion, the close correspondence in the NK1 receptor binding properties in gerbil and human brain supports the use of this animal species to investigate the physiology of NK1 receptors and characterise novel NK1 receptor ligands.

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