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In Vitro and *In Vivo* Characterization of the Non-peptide NK_3 Receptor Antagonist SB-223412 (Talnetant): Potential Therapeutic Utility in the Treatment of Schizophrenia

Lee A Dawson*[,1], Katherine J Cato[1], Claire Scott[1], Jeannette M Watson[1], Martyn D Wood[1], Richard Foxton[1], Raúl de la Flor[1], Gareth A Jones[1], James NC Kew[1], Jane E Cluderay[1], Eric Southam[1], Graham S Murkitt[1], Jane Gartlon[1], Darrel J Pemberton[1], Declan NC Jones[1], Ceri H Davies[1] and Jim Hagan[1]

[1]*Psychiatry Centre of Excellence for Drug Discovery, GlaxoSmithKline, Essex, UK*

Neurokinin-3 (NK_3) receptors are concentrated in forebrain and basal ganglia structures within the mammalian CNS. This distribution, together with the modulatory influence of NK_3 receptors on monoaminergic neurotransmission, has led to the hypothesis that NK_3 receptor antagonists may have therapeutic efficacy in the treatment of psychiatric disorders. Here we describe the *in vitro* and *in vivo* characterization of the highly selective NK_3 receptor antagonist talnetant (SB-223412). Talnetant has high affinity for recombinant human NK_3 receptors (pK_i 8.7) and demonstrates selectivity over other neurokinin receptors (pK_i $NK_2 = 6.6$ and $NK_1 < 4$). In native tissue-binding studies, talnetant displayed high affinity for the guinea pig NK_3 receptor (pK_i 8.5). Functionally, talnetant competitively antagonized neurokinin B (NKB)-induced responses at the human recombinant receptor in both calcium and phosphoinositol second messenger assay systems (pA_2 of 8.1 and 7.7, respectively). In guinea pig brain slices, talnetant antagonized NKB-induced increases in neuronal firing in the medial habenula ($pK_B = 7.9$) and senktide-induced increases in neuronal firing in the substantia nigra pars compacta ($pK_B = 7.7$) with no diminution of maximal agonist efficacy, suggesting competitive antagonism at native NK_3 receptors. Talnetant (3–30 mg/kg i.p.) significantly attenuated senktide-induced 'wet dog shake' behaviors in the guinea pig in a dose-dependent manner. Microdialysis studies demonstrated that acute administration of talnetant (30 mg/kg i.p.) produced significant increases in extracellular dopamine and norepinephrine in the medial prefrontal cortex and attenuated haloperidol-induced increases in nucleus accumbens dopamine levels in the freely moving guinea pigs. Taken together, these data demonstrate that talnetant is a selective, competitive, brain-penetrant NK_3 receptor antagonist with the ability to modulate mesolimbic and mesocortical dopaminergic neurotransmission and hence support its potential therapeutic utility in the treatment of schizophrenia.

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INTRODUCTION

Tachykinins are a family of structurally related peptide neurotransmitters that share a common amino acid sequence at their C-terminal region. There are five mammalian tachykinins, substance P (SP), neurokinin (NK) A, neurokinin B (NKB), neuropeptide (NP) K and NPγ, which exert their diverse physiological actions via interaction with three family 1 (rhodopsin-like) Gq protein–coupled receptors: NK_1, NK_2, and NK_3. The interaction of the tachykinins with its receptor results in a Gq-mediated activity which ultimately leads to elevation of intracellular Ca^{2+} via a phospholipase C—IP_3/DAG signaling pathway (for review see Maggi, 1995).

*Correspondence: Dr LA Dawson, Psychiatry Centre of Excellence for Drug Discovery, GlaxoSmithKline, New Frontiers Science Park (North), Third Avenue, Harlow, Essex, CM19 5AW, UK, Tel: +44 0 1279 622000, Fax: +44 0 1279 875389, E-mail: Lee.A.Dawson@gsk.com
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The rank order of affinity of tachykinins for the NK_3 receptor is NKB > > NKA > SP. Studies of function and localization have demonstrated the presence of NK_3 receptors in mammalian central, peripheral, and enteric nervous systems, where their activation influences the release of a variety of neurotransmitters (for review see Maggi, 1995). In the mammalian brain, NK_3 receptors are expressed within medial prefrontal cortex (mPFC), thalamus, and amygdala, while their presence in midbrain dopaminergic nuclei (the ventral tegmental area (VTA) and substantia nigra (SN)), locus coeruleus, and septal and basal nuclei suggest a role in modulating central monoaminergic systems (Langlois *et al*, 2001). However, there are considerable species differences in NK_3 receptor expression within the brain (Langlois *et al*, 2001; Rigby *et al*, 2005) which can complicate preclinical investigations of this receptor.

Neurochemical studies have shown that NK_3 receptor activation can augment dopamine (DA) and acetylcholine (ACh) efflux in the nucleus accumbens (NAcc) and caudate putamen (Marco *et al*, 1998), norepinephrine (NE) efflux in

the mPFC (Jung *et al*, 1996; Bert *et al*, 2002) and ACh in the hippocampus (Marco *et al*, 1998) of the guinea pig, and γ-aminobutyric acid (GABA) in the NAcc and caudate putamen of the rat (Preston *et al*, 2000). Similarly, electrophysiological data have demonstrated that NK_3 receptor activation can augment NE (Jung *et al*, 1996) and DA cell firing (Nalivaiko *et al*, 1997) in the guinea pig brain. Furthermore, NK_3 receptor antagonists have been shown to attenuate dopaminergic cell firing in the guinea pig VTA and SN, induced by DA D_2 receptor and neurotensin receptor blockade (Gueudet *et al*, 1999).

Taken together, these data suggest a possible role for NK_3 receptors in a number of psychiatric diseases and moreover, may indicate that this receptor could be targeted in the treatment of these disorders (for review see Spooren *et al*, 2005). This hypothesis has recently gained credence with the report that the NK_3 receptor antagonist SR-142801 (osanetant) (Meltzer *et al*, 2004) showed efficacy in schizophrenic patients. Specifically, in a 6-week multicenter, double-blind, placebo-controlled, clinical study in hospitalized schizophrenic and schizoaffective disorder patients osanetant improved four primary efficacy measures, ie the total score from the Positive and Negative Scale for Schizophrenia, the derived score for the Brief Psychiatric Rating Scale (BPRS), the BPRS positive symptom subscale (delusions, hallucinations, conceptual disorganization, and bizarre behavior), and the Clinical Global Impression Scale. Furthermore, the improvement in these patients was not significantly different from that achieved with the antipsychotic haloperidol and the magnitude of the haloperidol-induced improvement was consistent with that observed previously (Meltzer *et al*, 2004).

We now report the *in vitro* characterization of the nonpeptide tachykinin receptor antagonist ((S)-(−)-N (α-ethylbenzyl)-3-hydroxy-2-phenylquinoline-4-carboxamide), talnetant (SB-223412; Sarau *et al*, 1997) at human recombinant and guinea pig native NK_3 receptors and present evidence of *in vivo* activity at brain NK_3 receptors. Furthermore, we have investigated the mechanism of action of this compound on central neurochemical function that indicates that this molecule may have therapeutic utility in the treatment of psychiatric disorders.

MATERIALS AND METHODS

Animals

Male Dunkin–Hartley guinea pigs (David Hall or B&K Universal, UK) were housed four per cage in a temperature-($18 \pm 2^{\circ}C$) and humidity ($55 \pm 5\%$)-controlled environment on a 12 h light/dark cycle with lights on at 0700 hours. Food and water were available *ad libitum*. All experiments were carried out in accordance with the United Kingdom Animals (Scientific Procedures) Act, 1986 and with GlaxoSmithKline ethical standards.

In Vitro Binding to Human Recombinant and Guinea Pig Native NK_3 Receptors

Human cloned NK_3 receptors (Sarau *et al*, 1997) stably expressed in HEK293 cells were harvested at confluence, resuspended in 20 volumes of phosphate-buffered saline (PBS), and then pelleted by centrifugation (1000*g*/10 min). For preparation of membranes, cell pellets were homogenized (Polytron, 15 s, setting 5) and centrifuged (50 000*g*, 15 min, 4°C) in 30 volumes of Tris-HCl (50 mM, pH 7.4, 4°C). The membranes were washed with ice-cold buffer, homogenization and centrifuged (50 000*g*, 12 min, 4°C) three times, and the resultant membranes were finally resuspended at a membrane concentration equivalent to 2×10^7 cells/ml ($\sim$1 mg protein/ml) and stored at −80°C prior to use.

Guinea pig cerebral cortices were dissected and homogenized (Polytron, 15 s, setting 5) in 30 volumes (based on wet weight of tissue) of 50 mM Tris (pH 7.4 at 37°C) and membranes prepared as above.

Recombinant (10–20 μg protein per tube) or cerebral cortex membranes (100–200 μg protein per tube) were incubated in Tris-HCl buffer (50 mM, pH 7.4 at 37°C) containing $MgCl_2$ (10 mM), and bovine serum albumin (BSA, 0.1%) with [^{3}H]SB-222200 (1 nM for competition analysis and eight concentrations within the range 0.1–100 nM for saturation analysis) in the presence or absence of the test compound for 60 min at 37°C. For both saturation and competition analyzes, nonspecific binding was determined using 1 μM SB-787976 (a selective, nonpeptide NK_3 receptor antagonist; Blaney *et al*, 2001). The reaction was terminated by rapid filtration through Whatman GF/B grade filters (presoaked with 0.3% polyethyleneimine) followed by 5×1 ml ice-cold buffer washes and bound radioactivity was determined by liquid scintillation counting.

Intracellular Ca^{2+} Mobilization in HEK293 Cells Stably Expressing the Human NK_3 Receptor (hNK_3/HEK293)

Changes in intracellular Ca^{2+} concentrations were monitored using a fluorescence imaging plate reader (FLIPR, Molecular Devices Corp., Sunnyvale, CA, USA) by a method similar to that described by Jerman *et al* (2001). HEK293 cells were preincubated (37°C for 60 min) with Calcium Plus reagent (Molecular Devices Corp.) in Tyrodes buffer (NaCl, 145 mM; KCl, 2.5 mM; HEPES, 10 mM; glucose, 10 mM; $MgCl_2$, 1.2 mM; $CaCl_2$, 1.5 mM) containing probenecid (2.5 mM) before incubation (30 min, 37°C) with either buffer or antagonist (50 μl). The plates were then placed in the FLIPR, NKB (10 pM–1 μM final concentration) added, and changes in fluorescence monitored.

Inositol Phosphate (IP) Accumulation in U-2OS Cells Transiently Expressing the Human NK_3 Receptor

Human osteosarcoma (U-2OS) cells previously transduced with the human NK_3 receptor (transduction utilized a recombinant baculovirus under the control of a mammalian promoter generated using vectors and methods described in Ames *et al*, 2004) were incubated for 16 h with inositol-free DMEM in the presence of [^{3}H]myo-inositol 1 μCi/well (Amersham, UK). The growth medium was then aspirated and the cells washed with 2×200 μl inositol-free DMEM/3% BSA. The cells were then preincubated (30 min, 37°C) in the absence or presence of talnetant (1, 3, or 10 nM) and NKB was then added (0.1 nM–10 mM; 30 min, 37°C) in the presence of LiCl (5 mM). In experiments investigating the

reversibility of antagonism, a washing stage (3 ×) was included following antagonist incubation and prior to NKB addition. The assay was terminated and cells solubilized by the addition of 0.1 M formic acid (200 μl) and 20 μl aliquots were transferred to Picoplates (Perkin Elmer Life Sciences) containing 80 μl of RNA-Ysi SPA beads (Amersham) previously diluted 1 : 8 (vol:vol) with double deionized water. The Picoplates were shaken for 60 min and then centrifuged (10 min, 1000*g*) and counted using a scintillation counter.

In Vitro Electrophysiology

Guinea pigs were killed by decapitation and their brains rapidly removed and immersed in an ice-cold sucrose-containing artificial cerebrospinal fluid (aCSF), composed of sucrose 189 mM, glucose 10 mM, $NaHCO_3$ 26 mM, KCl 2.5 mM, $MgCl_2$ 5 mM, $CaCl_2$ 0.1 mM, NaH_2PO_4 1.2 mM, bubbled continuously with an O_2/CO_2 mixture (95/5%) to maintain the pH 7.4, 400 μm. Coronal slices containing the dorsal substantia nigra pars compacta (SNpc) or the medial habenula (mHb) were cut using a Vibroslice (Camden Instruments Inc.). Brain slices were subsequently immersed in aCSF containing: NaCl 120 mM, KCl 2.5 mM, $CaCl_2$ 1.2 mM, $MgCl_2$ 1.5 mM$NaHCO_3$ 25 mM, $NaHPO_4$ 2.5 mM, glucose 10 mM, again bubbled with 95/5% O_2/CO_2, for at least 30 min prior to initiation of recording. A single slice was then transferred to a recording chamber (submerged configuration) that was continuously perfused with oxygenated aCSF at a rate of 2–4 ml/min and maintained at 33°C.

Extracellular recordings were obtained using microelectrodes (3–5 MΩ; filled with aCSF) placed in the SNpc or mHb under visual control. Presumptive dopaminergic neurons in the SNpc were identified by a low-frequency (~2 Hz) regular firing pattern and reversible depression of spontaneous activity in response to application of the DA receptor agonist quinpirole (3 μM; Nalivaiko *et al*, 1997). Neurons from the mHb were identified as those which exhibited spontaneous activity in the range of 2–8 Hz and which displayed an increase in activity in response to 1 min applications of 300 nM NKB (Boden and Woodruff, 1994). Only neurons that met the criteria for their respective nuclei were selected for further analysis. Electrical signals were fed into a high-input impedance preamplifier (AxoClamp 2B, Axon Instruments Inc.), amplifier (Brownlee), and digitizer (Axon Instruments Inc.). Baseline activity was recorded for 5–10 min before drugs were applied via Tyvek tubing connected to the recording chamber, allowing complete exchange of fluids within 2 min of the arrival of a new solution. Senktide (3 nM–1 μM) was bath applied for 5–8 min, until a steady-state effect was reached, with a 15–20 min washout between concentrations. NKB (10 nM–10 μM) was applied for 1 min to minimize desensitization, with 10–20 min washout between concentrations. Antagonists were pre-applied for 30 min.

Autoradiographical Determination of Cortical *Ex Vivo* Receptor Occupancy

Guinea pigs were administered vehicle (1% methylcellulose, 2 ml/kg) or talnetant (1, 3, 10, 30, or 100 mg/kg i.p., $n = 4$ per group). After 1 h of dose administration, animals were killed and brains were rapidly removed and frozen in isopentane at approximately −40°C. Hindbrain and trunk blood samples were collected and analyzed for drug concentration (see below). Subsequently, 14 μm thick coronal sections, from between 15.4 and 15.8 mm anterior to the frontal zero plane as defined by Rapisarda and Bacchelli (1977), were cut using a Leica CM3050 cryostat and thaw-mounted onto Superfrost Plus slides (BDH) then stored at −80°C until use.

Slides were thawed and rapidly dried under a stream of cold air then subjected to a 10 min incubation at room temperature with 10 nM [^{3}H]senktide (Perkin Elmer; 51.9 Ci/mmol) in 50 mM Tris-HCl (pH 7.5) containing 3 mM $MnCl_2$, 0.02% (w/v) BSA (Roche), 40 μg/ml bacitracin (Sigma), 2 μg/ml chymostatin (Sigma), and 4 μg/ml leupeptin (Roche). Adjacent sections were incubated in the same solution with the addition of 10 μM SR-142801 to define nonspecific binding. Slides were then subjected to a series of four short (1 min) washes in ice-cold 50 mM Tris-HCl (pH 7.5) followed by two rinses in ice-cold deionized water and were then rapidly dried under a stream of cold air.

Sections were analyzed by direct β-imaging (Beta Imager, BioSpace, Paris) for 12 h and bound radioactivity was measured (counts per minute per square millimetre) in the mPFC using Beta Vision Plus software (BioSpace).

Blood and brain samples and standards were extracted by protein precipitation by addition of 'precipitation buffer' (95 : 5 MeCN: EtOH containing 0.1% formic acid and an internal standard) followed by centrifugation at 3000 r.p.m. for 15 min. The resulting supernatant (100 μL) was removed and analyzed for talnetant using LC-MS-MS.

Chromatographic separations were performed using gradient HPLC on Acquity UPLC BEH C18 columns (50 × 2.1 mm) and Waters Acquity UPLC (Waters, Mildford, USA). Separation was achieve via a gradient (mobile phase A = 5% MeCN/95% water/0.1% formic acid, mobile phase B = 95% MeCN/5% Water/0.1% formic acid) transition from 85 : 15 to 5 : 95% over 1.1 min at a flow rate of 0.5 ml/min. Talnetant was detected using Waters Micromass Quattro Premier VAA301 mass spectrometer (Waters) and quantified using Masslynx v 4.0 software for the PC.

Guinea Pig 'Wet Dog Shake' Behavior

Guinea pigs were anesthetized with a 3% isoflurane and 0.5 l/min oxygen mixture. A unilateral intracerebroventricular (i.c.v.) cannula (5 mm length, 22 G; coordinates AP = +9.8; L = ±2.3; DV = −4.0 from intra-aural line and skull surface) was directed at the lateral ventricle and fixed in position using three tether screws and cyanoacrylate adhesive. Animals were allowed 7 days' post-operative care prior to experimentation.

Prior to testing, guinea pigs were handled daily and habituated to clear plastic observation cages on at least five occasions. On test days, guinea pigs were administered senktide (0.3 μg, i.c.v.) (Bachem, UK) and 180 s later wet dog shake (WDS) behavior was scored for 30 min by trained observers who were blind to treatment group. Senktide was dissolved in 20 mM acetic acid (0.1%)/PBS solution and administered using an injection needle extending 1 mm beyond the tip of the i.c.v. cannula. Senktide or vehicle

solution was injected in a volume of 5 µl. Talnetant (3, 10, or 30 mg/kg, $n = 9$–10) or vehicle (1% methyl cellulose in water) was administered by i.p. injection 60 min prior to senktide administration.

Microdialysis

Guinea pigs were anesthetized by gaseous administration of isofluorane. Microdialysis guide cannulae (CMA 11, CMA, UK) were implanted for sampling from either the NAcc or mPFC and dorsal hippocampus (dHipp). Implantation coordinates were measured from bregma (AP and ML) and the surface of the dura (DV) and are listed in Table 1. Guide cannulae were secured with dental cement and tether anchor screw (Instech, Presearch, Hitchin, UK) attached. Animals were allowed 7 days' postoperative care prior to experimentation.

Microdialysis was performed according to the methods described by Hughes and Dawson (2004). In brief, microdialysis probes (2 mm cuprophane membrane, CMA11 14/02, CMA) were implanted and perfused with aCSF containing NaCl 145 mM, KCl 2.7 mM, $MgCl_2$ 1.0 mM, $CaCl_2$ 1.2 mM, Na_2HPO_4 2.0 mM at 1 µl/min. After 2 h of equilibration, four basal microdialysis samples were collected. Animals were simultaneously implanted with mPFC and dHipp probes then received either vehicle or talnetant (30 mg/kg, i.p.) or the positive comparator clozapine (10 mg/kg, s.c.). Guinea pigs implanted with NAcc probes were initially dosed (1 ml/kg, i.p.) with a pretreatment of vehicle (1% methylcellulose) or talnetant (10 or 30 mg/kg) followed 30 min later with either haloperidol (0.5 mg/kg, i.p.) or vehicle. A 30 min sampling regime was used throughout and all microdialysate samples were collected and analyzed for DA, NE and 5-HT content. At the end of the experiment, probes were removed and animals returned to their home cages. Animals were reused in a randomized crossover design with 7 days between each use and on a maximum of four occasions (Hughes and Dawson, 2004). After the final microdialysis experiment, animals were killed and their brains were removed and stored in formalin before verification of probe placement. Brains were sectioned (50 µm) using a cooled cryostat and sections stained with cresyl violet to visualize sites of probe implantation. Data from animals with incorrect probe placement were discarded from the analysis.

Chromatographic separations were performed using a Capcell PAK, strong cation exchange column (5 µm UG80, 1.5 × 150 mm; Shiseido, Tokyo, Japan). The mobile phase, consisted of NaCl 5 mM, Na_2HPO_4 13 mM, NaH_2PO_4 87 mM, EDTA.Na_2 0.1 mM, in 20% methanol buffered to pH 6 was delivered via a Jasco PU-980 HPLC pump (Jasco, Tokyo, Japan) at a flow rate of 0.2 ml/min at a temperature of 40°C. DA, NE, and 5-HT were detected via an electrochemical amperometric detector (Decade, Antec-Leyden, Leyden, The Netherlands) fitted with a 3 mm glassy carbon electrode set a +500 mV *vs* Ag/AgCl reference. The analog data output was smoothed at 40 Hz (LINK, Antec-Leyden) before collection. Samples (10 µl) were injected via a cooled (4°C) Gilson model 234 autosampler (Gilson, Villiers-le-Bel, France) fitted with a six-port rotary valve (Model 7125, Rheodyne, Berkeley, CA, USA) with a 20 µl injection loop. All data were acquired using Millenium[32] software (Waters).

Table 1 Co-ordinates for Microdialysis Guide Cannula Implantation (mm) into the Guinea Pig (Rapisarda and Bacchelli, 1977)

NAcc	mPFC	dHipp
AP+4.0	AP+6.1	AP−4.1
ML−1.5	ML−0.5	ML+2.5
DV−6.0	DV−5.0	DV−5.0

Abbreviations: AP, anteroposterior; dHipp, dorsal hippocampus; DV, dorsoventral; ML, mediolateral; mPFC, medial prefrontal cortex; NAcc, nucleus accumbens.

Data Analysis

The concentration of drug which inhibited the specific binding of [^{3}H]SB-222200 by 50% (IC_{50}) was derived using a four-parameter logistic equation (GraphPad Prism, GraphPad Software Inc.). pK_i values (−log of the inhibition constant) were then calculated from the IC_{50} values as described by Cheng and Prusoff (1973). Data are expressed as the mean ± SEM of at least three separate experiments each performed using duplicate determinations.

In FLIPR experiments, peak changes in fluorescence occurred within the first 5 s and were reported following baseline subtraction. Concentration–response curves were analyzed using a four-parameter logistic equation (GraphPad Prism, GraphPad Software Inc.) to obtain pEC_{50} values (−log EC_{50}). A pA_2 value for talnetant was obtained by Schild analysis (Schild, 1947). Data are expressed as mean ± SEM of three separate experiments.

Extracellular recordings of action potentials were collected and analyzed using pClamp 9.0 software (Axon Instruments Inc.) or Spike 2 (Cambridge Electronic Design, UK). The effect of senktide was evaluated by measuring the mean action potential discharge frequency during the final 2 min of exposure to each concentration of drug, when a steady-state effect had been reached. In the case of NKB, no steady state was reached, so the 60 s period of maximal activity was used to assess drug effects. Neuronal firing frequency in the presence of senktide was expressed as a percentage of baseline (pre-drug). NKB effects were normalized to the baseline (pre-drug) response to 300 nM NKB. Concentration–response curves were fitted and IC_{50} values calculated using Origin 5.0 (Microcal) or Prism 4 (Graphpad). pK_B values were calculated using the Gaddum equation (Gaddum, 1937).

Data for total number of WDS are expressed as mean ± SEM. Data were analyzed by one-way ANOVA followed by Dunnet's *t*-test.

For occupancy determinations, specific binding was determined as the mean of bilateral measurements from two sections per animal (non-specific binding) subtracted from the mean of bilateral measurements from four sections per animal (total binding). Percent occupancy values were calculated using the following formula: ((vehicle group mean-specific binding−individual animal-specific binding)/vehicle group mean-specific binding) × 100. Curve fitting was carried out using one-site binding nonlinear regression in Prism v4.02.

In microdialysis experiments, the mean concentration of neurotransmitter in the first four baseline samples was calculated and this value denoted as 100%. Values for all samples were expressed as a percentage of this mean preinjection control value. These transformed data were analyzed by two-way ANOVA with repeated measures followed by *post hoc* Fisher's test where appropriate. Postdrug administration of total DA efflux was calculated as area under the curve using a trapezoidal calculation. These transformed data were analyzed by one-way ANOVA with repeated measures followed by Fisher's least squares differences test.

RESULTS

Saturation Analysis of [^{3}H]SB-222200 Binding to Human-Cloned NK$_3$ Receptors

SB-222200 has previously been reported to be a potent NK$_3$ receptor antagonist (Sarau *et al*, 2000) and to displace [^{125}I][MePhe7] NKB binding to the human-cloned receptor with a K_i of 4.4 nM. SB-222200 was tritiated (48 Ci/mmol, Tocris, UK) and used to radiolabel the human-cloned and native NK$_3$ receptor from guinea pig. The NK$_3$ receptor affinity for talnetant was determined from [^{3}H]SB-222200 competition binding. Talnetant displayed high affinity for both the human (pK_i 8.7 ± 0.10) and guinea pig (pK_i 8.5 ± 0.06) NK$_3$ receptors.

Selectivity Screening

As previously reported (Sarau *et al*, 1997) talnetant is >100-fold selective for the NK$_3$ receptor *vs* the other members of the tachykinin receptor subfamily. Furthermore, talnetant was cross-screened at concentrations of 1 or 10 μM and was without affinity/effect at 68 enzymes, ion channels, and receptors includingPDE_{1-4}, PKC, PLA_2, ionotropic glutamate receptors, L-type Ca^{2+} channels, adenosine (A_1, A_2) receptors, serotonin (5-HT_{1A}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_4) receptors, DA (D_1, D_2, D_3, D_4) receptors, histamine (H_1, H_2) receptors, muscarinic (M_1, M_2) receptors, $GABA_A$ or $GABA_B$ receptors, and opiod receptor. Although not specifically examined for talnetant, all subsequent quinoline-like NK$_3$ receptor antagonists (the chemical class to which talnetant belongs; Sarau *et al*, 1997; Blaney *et al*, 2001) have shown no affinity for DA, serotonin or NE transporters, or NE receptors (α1, α2) (data not shown).

Intracellular Ca^{2+} Mobilization Using FLIPR

Talnetant showed no evidence of agonist activity at concentrations up to 300 nM (data not shown). However, talnetant (30 nM–1 μM) produced a parallel rightward shift of the NKB concentration–response curve that was surmountable at all concentrations tested (Figure 1). Schild analysis of the data gave a pA_2 of 8.20 ± 0.2 and a slope of 0.94 ± 0.09 which was not significantly different from unity. Talnetant, therefore, showed a profile consistent with potent competitive NK$_3$ receptor antagonist activity in this assay system.

Inositol Phosphate Accumulation in U-2OS Cells Transiently Expressing the Human NK$_3$ Receptor

Talnetant (0.1–1 μM) attenuated the NKB-induced accumulation of IP in hNK$_3$/U-2OS cells producing a rightward shift of the NKB concentration–response curve, again with no effect on the agonist-induced maximal response. Schild analysis of the data gave a pA_2 of 7.7 ± 0.3. Repeated (×3) cell washes (following talnetant incubation and prior to agonist application) completely reversed the antagonist effect of talnetant suggesting that the antagonism by talnetant was reversible (Figure 2).

Effects of Talnetant on NKB-Induced Neuronal Firing in the Medial Habenula

Under control conditions, the mean (± SEM) firing rate of NKB-sensitive neurons in the mHb was 4.4 ± 0.4 Hz (n = 11). NKB (10 nM–3 μM) produced a concentration-dependent increase in neuronal firing with an EC_{50} of 91 nM

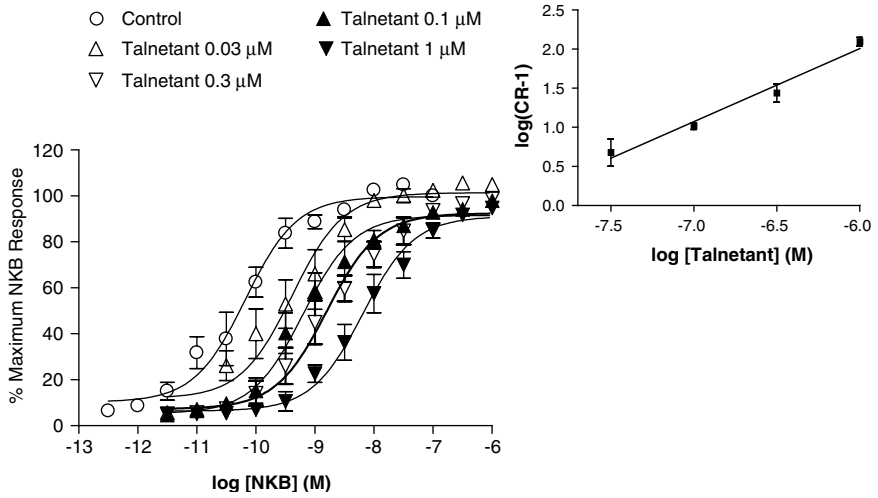

Figure 1 The effect of talnetant (30 nM–1 μM) on the neurokinin B (NKB)-stimulated increase in $(Ca^{2+})_i$ in HEK293 cells stably expressing the human NK$_3$ receptor. Data points represent the peak increase in fluorescence and are the mean ± SEM from at least three separate experiments and are shown as the percentage of the fitted maximal response to NKB. Inlay shows Schild plot of these data.

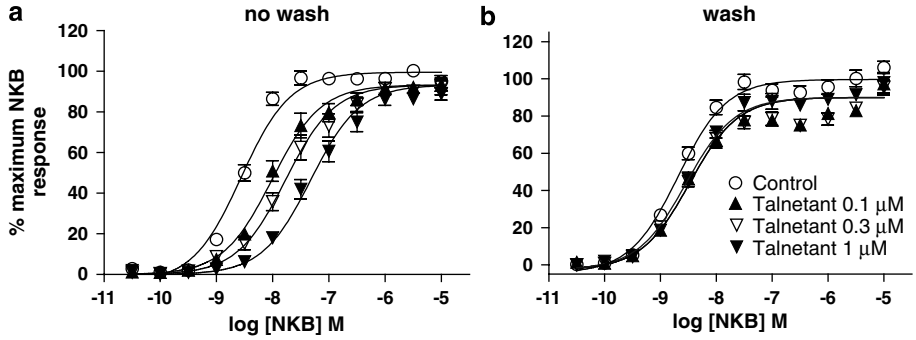

Figure 2 The effect of talnetant (0.1–10 μM) on the neurokinin B (NKB)-induced IP accumulation in U-2OS cells transiently expressing the human NK_3 receptor. (a) Effect of talnetant on NKB responses and (b) effects of talnetant following a wash procedure prior to NKB addition. Data points are the mean ± SEM from three separate experiments each performed using duplicate determinations.

($n = 6$; Figure 3a). In a separate set of recordings, application of 100 nM talnetant resulted in a nonsignificant reduction in spontaneous firing from 3.4 ± 0.3 to 2.6 ± 0.5 Hz ($n = 5$, $p = 0.08$, paired *t*-test). In the presence of 100 nM talnetant, there was a rightward shift in the concentration–response curve with no significant effect on the maximum agonist efficacy ($n = 5$, $p = 0.93$, F-test). The EC_{50} of NKB under these conditions was 788 nM, resulting in an apparent pK_B for talnetant of 7.9 (Figure 3a).

Effects of Talnetant on Senktide-Induced Dopamine Neuronal Firing in the Substantia Nigra

Under control conditions, the mean (± SEM) firing rate of presumptive dopaminergic SNpc neurons was 2.2 ± 0. 8 Hz ($n = 27$). Senktide (1 nM–1 μM) produced a concentration-dependent increase in neuronal firing yielding an EC_{50} of 26 nM ($n = 15$) (Figure 3b). In separate recordings, the concentration–response curve of senktide was determined in the presence of talnetant (100 nM) following a 45 min preincubation with the antagonist ($n = 3$). Application of talnetant (100 nM) to the bath perfusion medium was without effect on baseline neuronal firing. In the presence of talnetant, a parallel shift in the senktide concentration–response curve was observed with no change in maximum response and yielding an EC_{50} for senktide of 140 nM and an apparent pK_B for talnetant of 7.4 (Figure 3b).

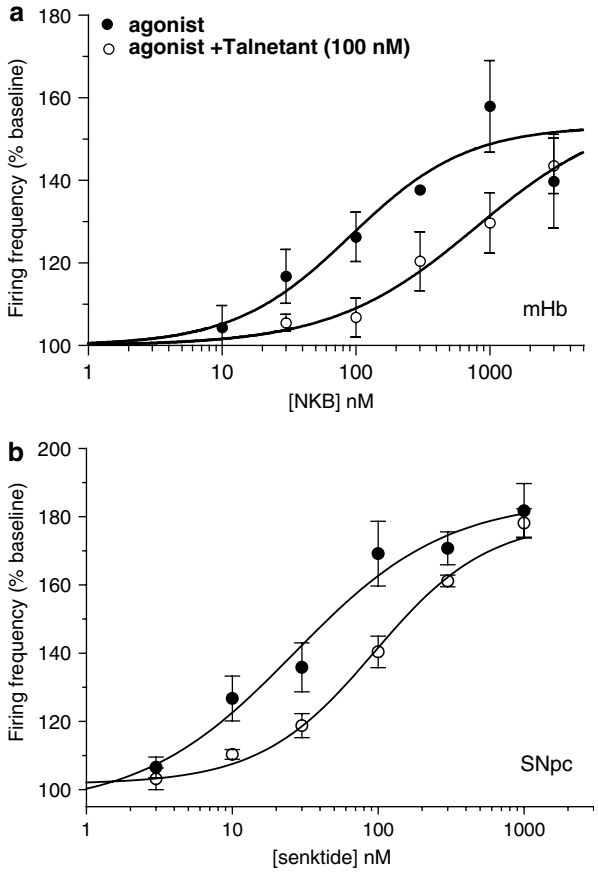

Figure 3 The effect of talnetant on the mean concentration-dependent increase in medial habenula (mHb) neuronal firing elicited by neurokinin B (NKB) (a) and substantia nigra pars compacta (SNpc) neuronal firing elicited by senktide (b) in the guinea pig slice. Under control conditions and in the presence of talnetant (100 nM) that yielded respective EC_{50} values of 91 nM ($n = 6$) and 788 nM ($n = 5$) in the mHb. Under control conditions and in the presence of talnetant (100 nM) that yielded EC_{50} values of 26 ($n = 15$) and 140 nM ($n = 3$) in the SNpc. Mean neuronal firing frequencies are expressed as a mean ± SEM percentage of baseline (predrug).

Occupancy of Guinea Pig Cortical NK_3 Receptors

As demonstrated by the dose-dependent decrease in specific binding of the NK_3-specific radioligand [^{3}H]senktide (Figure 4a), i.p. administration of talnetant produced dose-dependent occupancy of NK_3 receptors in guinea pig mPFC (Figure 4b) with a calculated ID_{50} of 3.3 mg/kg. Concurrent measurement of concentrations of talnetant within the whole brain showed that this correlated with an IC_{50} of 9.6 ng/g and that concentrations above 100 ng/g produce maximal occupancy (Figure 4c).

Effects of Talnetant on Senktide-Induced Guinea Pig WDS Behaviors

Talnetant (30 mg/kg i.p.) alone produced a small but significant reduction in baseline behaviors (from 2.4 ± 0.5 to 0.6 ± 0.3; $p < 0.05$; Figure 5). The NK_3 receptor-selective agonist senktide significantly increased WDS behavior from 2.4 ± 0.5 to 31.1 ± 5.1 ($p < 0.05$; Figure 5). Talnetant (3–30 mg/kg i.p.) produced a significant ($F_{5,51}$ 46.6, $p < 0.05$) attenuation of this senktide-induced WDS behavior at 30 mg/kg only (from 31.1 ± 5.1 to 16.7 ± 4.1; $p < 0.05$; Figure 5).

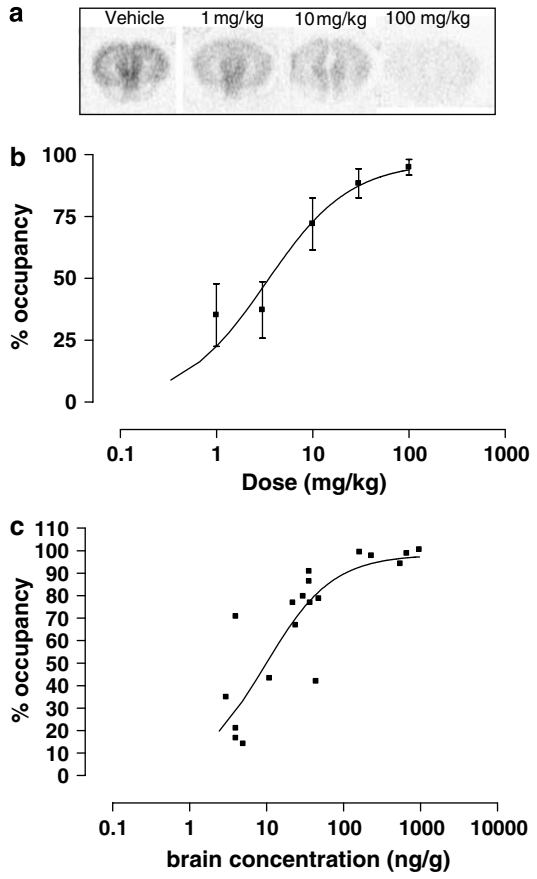

Figure 4 Relationship between *ex vivo* cortical NK_3 receptor occupancy and dose of talnetant administered and subsequent concentration measured in brain. (a) [^{3}H]senktide *ex vivo* binding to brain sections from guinea pigs treated with vehicle, 1, 10, or 100 mg/kg talnetant. (b) NK_3 receptor occupancy (mean ± SEM, $n = 6$ per group) by talnetant in guinea pig mPFC determined by *ex vivo* [^{3}H]senktide autoradiography. (c) The relationship between NK_3 receptor occupancy and concentration of talnetant in brain samples from the same experimental animals. Each point represents data from an individual animal.

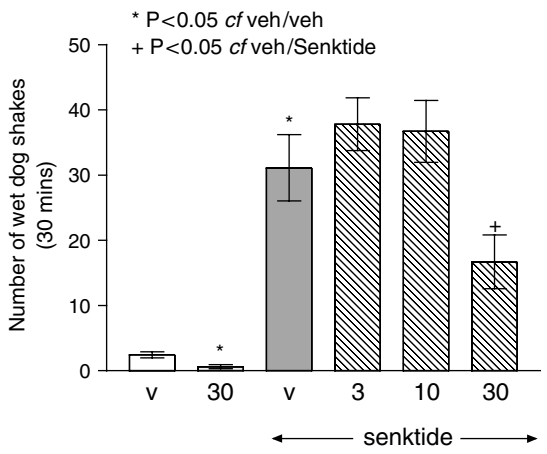

Figure 5 Effects of talnetant on senktide-induced 'wet dog shaking' behaviors in the GP. Data expressed as mean ± SEM ($n = 9$–10) *$p < 0.05$ *vs* senktide alone.

Effects of Talnetant on Extracellular Levels of DA, NE, and 5-HT in the mPFC and dHipp

Mean (± SEM) basal levels (pM) of DA, NE, and 5-HT in the guinea pig mPFC were 289.2 ± 27.0 ($n = 27$), 313.6 ± 15.0 ($n = 31$), and 196.0 ± 11.7 ($n = 29$), respectively. Talnetant (30 mg/kg, i.p.) produced a significant increase in extracellular levels of DA, reaching a maximum level of 149.4 ± 22% of preinjection control levels at $t = 180$ min ($F_{1,12} = 10.5$; $p < 0.01$). Similarly, NE increased to a maximum of 165.4 ± 14% of preinjection control levels at $t = 30$ min ($F_{1,12} = 8.6$; $p < 0.05$) in the mPFC (Figure 6). Similarly, the positive comparator clozapine (10 mg/kg, s.c.) produced significant ($F_{1,15} = 13.4$; $p < 0.005$ and $F_{1,15} = 19.3$; $p < 0.001$) increases in both neurotransmitters (maximum increases: DA, 238 ± 65%, $t = 120$ min; NE, 227.1 ± 42%, $t = 60$ min) (Figure 6). No changes were observed in extracellular levels of 5-HT with either drug treatment (data not shown).

Mean basal levels (pM) of NE and 5-HT in the guinea pig dHipp were 319.2 ± 19.3 ($n = 29$) and 255.5 ± 18.8 ($n = 30$), respectively. Basal levels of DA were below the limits of consistent detection in this brain region and as such data have not been included. Talnetant (30 mg/kg, i.p.) produced a significant ($F_{1,12} = 23.3$, $p < 0.001$) increase in extracellular levels of NE that reached a maximum of 204.3 ± 32% of preinjection control levels while having no effect on extracellular 5-HT (Figure 6). Similarly, clozapine (10 mg/kg, s.c.) produced a significant ($F_{1,14} = 46.7$, $p < 0.0001$) increase in extracellular NE (maximum increases: 224.9 ± 28%, $t = 90$ min) but produced no change in 5-HT (data not shown).

Effects of Talnetant on Haloperidol-Induced Increases in NAcc Extracellular Levels of Dopamine: *In Vivo* Microdialysis

Basal levels of DA in the guinea pig NAcc were 1071 ± 78 pM ($n = 32$). Repeated measures ANOVA revealed a significant overall effect of treatment for the interaction study between talnetant and haloperidol ($F_{4,91} = 7.7$, $p < 0.0001$; Figure 7). *Post hoc* analysis revealed no significant effect of talnetant (30 mg/kg, i.p.) alone. Haloperidol (0.5 mg/kg, s.c.) produced a significant ($p < 0.0001$) increase in extracellular levels of DA reaching a maximum value of 202.2 ± 18.5% ($t = 150$ min). Haloperidol-induced increases were significantly ($p = 0.0002$) attenuated by 30 mg/kg of talnetant. A similar trend was also seen at 10 mg/kg of talnetant but this failed to reach statistical significance ($p = 0.16$).

DISCUSSION

Here we describe the *in vitro* and *in vivo* characterization of the highly selective NK_3 receptor antagonist talnetant (SB-223412; Sarau *et al*, 1997) and provide evidence in support of this hypothesis that NK_3 receptors may be useful as a therapeutic target for the treatment of psychotic disorders.

Talnetant exhibits high affinity for the human NK_3 receptor as determined using antagonist radioligand ([^{3}H]SB-222200) binding. This value (pK_i 8.7) was not substantially different from that previously reported using the endogenous agonist radioligand NKB (pK_i 9.0; Sarau *et al*, 1997). Cross-species native tissue binding, using the

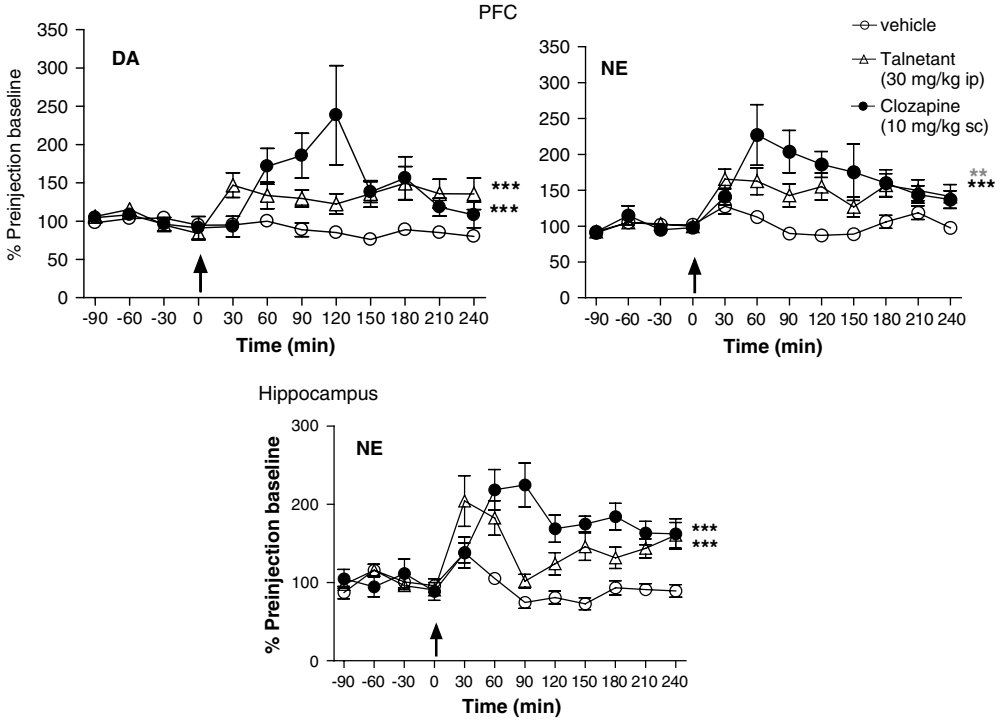

Figure 6 Comparative effects of talnetant and clozapine on dopamine (DA) and norepinephrine (NE) efflux in prefrontal cortex (PFC) and hippocampus of the guinea pig. Data expressed as mean ± SEM ($n = 8$–12 per group). $^{**}p < 0.01$, $^{***}p < 0.001$ *vs* vehicle. Note that basal levels of DA in the hippocampus were not consistently above the limits of detection of the analytical systems used, thus data on this transmitter have not been presented.

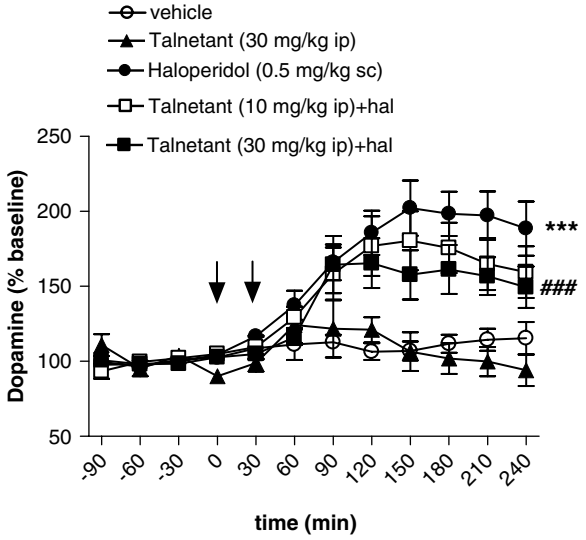

Figure 7 Effects of talnetant alone and on haloperidol-mediated DA efflux in the guinea pig NAcc. Data expressed as mean ± SEM ($n = 8$–12 per group). Significant increase: $^{***}p < 0.001$ *vs* vehicle, $^{###}p < 0.001$ *vs* haloperidol.

same antagonist radioligand, revealed that the affinity of talnetant for the guinea pig cortical NK_3 receptor (pK_i 8.5) was very similar to that at the human receptor. Differences in the pharmacological properties of peptidomimetic small molecule ligands for tachykinin receptors across species is well documented (for review see Maggi, 1995) and other NK_3 receptor antagonist ligands, such as PD-154740, PD-157672 (Maggi, 1995), and SR-142801 (Chung *et al*, 1995), have all been demonstrated to have a much reduced affinity for rodent *vs* human receptors. The implications of interspecies variation for preclinical models will be discussed later.

Both Ca^{2+} imaging and IP accumulation assays were used to probe the functional consequences of NK_3 receptor ligands on the Gq signaling cascade, although it could be argued that because the IP assay permits both agonist (NKB) and antagonist to attain an equilibrium status that this assay environment is more akin to the physiological situation. In both assay systems, talnetant was revealed to be a high affinity NK_3 receptor antagonist that produced a concentration-dependent parallel rightward shift in the NKB concentration–response curve with no appreciable decrease in the excitatory maximum response (E_{max}), suggestive of a competitive mechanism of action. Furthermore, antagonism was lost following a washout procedure, demonstrating a reversible activity. Thus, talnetant is a competitive, reversible antagonist at the recombinant human NK_3 receptor *in vitro*.

The functional activity of talnetant was further evaluated in native tissue preparations. Since the affinity of talnetant for the guinea pig NK_3 receptor appears to be similar to the human homolog, we employed electrophysiological recordings in guinea pig brain slices. Neurons within the guinea pig mHb have previously been described as having a high sensitivity to NK_3 receptor agonists and an apparent absence of NK_1 and NK_2 receptor-mediated responses which makes this nucleus ideal for studies of central NK_3-mediated events (Boden and Woodruff, 1994). Talnetant produced a parallel rightward shift in the NKB-induced neuronal excitations with no appreciable decrease in E_{max}, again indicative of competitive antagonist activity, in this case in a native tissue preparation.

Wet dog shake is a form of stereotypical behavior that may be induced in guinea pigs through the i.c.v. administration of the potent peptide NK_3 receptor agonist senktide (Renzetti *et al*, 1991). This behavior has been previously shown to be blocked by the NK_3 receptor antagonist SR-142801 (Yip and Chahl, 1997) and we now show that systemic administration of talnetant produced significant attenuation, thereby demonstrating the central blockade of NK_3 receptors at a 30 mg/kg dose. Based on our *ex vivo* occupancy studies, systemic administration of talnetant to guinea pigs also resulted in a dose- and brain concentration-dependent increase in occupancy of mPFC NK_3 receptors (as measured by displacement of the agonist radioligand senktide; Langlois *et al*, 2001) with a 30 mg/kg dose generating mean total brain concentrations of talnetant of 111 ng/g and effectively achieving maximal NK_3 receptor occupancy.

The DA hypothesis of schizophrenia has guided some aspects of schizophrenia research for the past four decades. Although the hypothesis has gained in complexity, and to a degree is now yielding to a multifactorial hypothesis involving monoamines, glutamate and GABA, the underlying hyperfunctionality of the mesolimbic DA system and a hypofrontality are still believed to be core facets of schizophrenia pathology (for review see Carlsson *et al*, 2001). Thus, direct or indirect modulation of forebrain dopaminergic systems provides a valid focus for research into the development of novel therapeutics for the disease. The expression of NK_3 receptors in ventral mesencephalic DA cells and their apparent localization on dopaminergic cell bodies (Dam *et al*, 1990; Stoessl, 1994; Chen *et al*, 1998) was the first indication that these tachykinin receptors may be potential modulators of dopaminergic neurotransmission. Biochemical and electrophysiological studies with tachykinin receptor agonists provided supportive evidence (Humpel *et al*, 1991; Keegan *et al*, 1992; Bannon *et al*, 1986; Alonso *et al*, 1996) and the more recent development of selective, small molecule NK_3 receptor antagonists has confirmed this hypothesis. In this regard, the NK_3 receptor agonists senktide and the endogenous ligand NKB were demonstrated to stimulate dopaminergic cell firing in guinea pig slice preparations (Nalivaiko *et al*, 1997). These responses were blocked by the NK_3 receptor antagonist SR-142801 but not NK_1 or NK_2 receptor antagonists. Using *in vivo* microdialysis techniques in guinea pigs, local administration of senktide into dopaminergic cell bodies of the mesencephalic and SNpc resulted in an enhanced DA output in terminal projection regions such as mPFC, NAcc, and striatum. Again, these responses were blocked by SR-142801 confirming the NK_3 receptor-mediated dopaminergic responses (Marco *et al*, 1998).

Using *in vitro* electrophysiological recordings of dopaminergic cell firing in the guinea pig SNpc, we demonstrated that talnetant produced a parallel rightward shift in the senktide-induced excitations with no appreciable decrease in E_{max}. This response is, again, indicative of the competitive receptor antagonist activity of talnetant in this native tissue assay. The pK_B was 7.4, which was in line with the data in the mHb (pK_B 7.9). These values are, however, somewhat lower than those generated *in vitro* at the human receptor. As the binding affinity for the guinea pig receptor is similar to that at the human NK_3 receptor, this discrepancy is likely to be the result of incomplete equilibrium of talnetant within the slice. Nevertheless, these observations do confirm that talnetant can block NK_3 receptors that are presumably located on dopaminergic cell bodies of the SNpc. To determine if these receptors play a role in modulating dopaminergic neurotransmission *in vivo*, we investigated the impact of talnetant on DA efflux in a region receiving afferent projections from the midbrain dopaminergic nuclei. The NAcc (separation of core and shell substructures of the NAcc is not feasible due to the poor anatomical differentiation in the guinea pig (Rapisarda and Bacchelli, 1977)) receives mixed innervation from both the SNpc and VTAs, and is implicated in the hypothesized hyperfunction of the mesolimbic projection systems in schizophrenia. Talnetant alone had no effect on basal DA efflux. This observation is consistent with previous *in vivo* studies, utilizing both microdialysis (Marco *et al*, 1998) and electrophysiology (Gueudet *et al*, 1999), which demonstrated that SR-142801 was without effect when administered alone. Taken together, these data suggest that there is no detectable tonic activation of NK_3 receptors on dopaminergic cell bodies in the guinea pig under these conditions. SNpc/VTA cell firing can be increased by acute administration of haloperidol, an effect that could be attenuated with SR-142801 (Gueudet *et al*, 1999). We have shown that haloperidol increased extracellular DA efflux in the NAcc, presumably via blockade of the tonically active DA D_2 autoreceptor, and that pretreatment with talnetant produced a dose-related attenuation of the haloperidol-induced increase in extracellular DA. These data confirm and extend the findings of Gueudet *et al* (1999) that NK_3 receptor antagonism can reduce hyperactivity in dopaminergic cell firing and consequently attenuate a hyperactive mesolimbic DA output. The reason that the NK_3-mediated depression of DA efflux in the NAcc is revealed in the presence of haloperidol may simply be that blockade of the primary dopaminergic D_2 autoreceptor increases the activity of the dopaminergic nuclei (Gueudet *et al*, 1999) revealing the NKB-mediated drive on the system. Interestingly, both the haloperidol-induced increases in DA efflux in the present study and the neuronal firing data (Gueudet *et al*, 1999) appear to be only partially reversed by NK_3 receptor antagonism. The simplest explanation for this is that a proportion of the haloperidol-induced response is mediated through removal of tonic negative-feedback circuitry, mediated by endogenous DA acting at the D_2 autoreceptor. As NK_3 receptor antagonists do not modulate basal firing or DA levels *per se,* they are, thus, unlikely to fully reverse the D_2 receptor-mediated effects of haloperidol.

NK_3 receptors have also been shown to have modulatory actions on other neurotransmitter systems including NE (Jung *et al*, 1996; Bert *et al*, 2002) and 5-HT (Liu *et al*, 2002). Thus, talnetant was examined for its ability to modulate efflux of NE, 5-HT, and DA in the mPFC and dHipp. Previous data have suggested that direct activation of NK_3 receptors, via application of senktide either directly to cell bodies (Marco *et al*, 1998; Bert *et al*, 2002; Liu *et al*, 2002) or i.c.v. (Jung *et al*, 1996), resulted in an increase in extracellular levels of NE, DA, and 5-HT in cortical structures. It was therefore somewhat surprising that talnetant induced increases in mPFC DA and NE, and

enhanced dHipp NE levels. Notably, these changes were similar to those induced by the atypical antipsychotic clozapine (which has no reported affinity for NK_3 receptors) in the same study. However, unlike clozapine, talnetant possesses no affinity for the monoaminergic sites of action (ie D_{1-4}, 5-HT_2, 5-HT_1 etc) generally associated with this class of antipsychotics.

These findings indicate that there is a tonic activation of NK_3 receptors that impact upon dopaminergic and noradrenergic projections to cortical and hippocampal structures. Based on published studies (Marco *et al*, 1998; Bert *et al*, 2002) and our own observations, the populations of NK_3 receptors that mediate this effect are not likely to be on the cell bodies of the locus coeruleus or midbrain dopaminergic nuclei, since administration of NK_3 receptor agonists directly to these areas also enhanced output of monoamines in the cerebral cortex and hippocampus. NK_3 receptors have been demonstrated to regulate GABAergic neurotransmission (Preston *et al*, 2000) and colocalization studies indicate that NK_3 receptors are expressed on GABAergic interneurons of basal ganglia nuclei including striatum, globus pallidus, ventral pallidum, and substantia inominata (Preston *et al*, 2000; Furuta *et al*, 2004). Furthermore, subpopulations of basal ganglia GABA neurons appear to send projections to the cerebral cortex (Furuta *et al*, 2004). Interestingly, a proportion of these GABAergic neurons are parvalbumin positive and this subpopulation of interneurons has been implicated in the pathophysiology of schizophrenia (for review see Lewis *et al*, 2005). Therefore, one possible explanation for talnetant-induced stimulation of monoamine efflux in the cerebral cortex and hippocampus is that NK_3 receptors, localized on GABAergic interneurons, are tonically active under normal physiological conditions and exert a tonic inhibitory action on basal ganglion and cortical neurotransmission. Thus, antagonism of these populations of NK_3 receptors disinhibits basal ganglia GABAergic neurotransmission thereby enhancing cortical dopaminergic and noradrenergic neurotransmission. Further work is required to establish to confirm this hypothesis.

Interspecies variation in NK_3 receptor location and function adds to the complexity of unraveling NK_3 receptor biology. NK_3 receptors are distributed in the medial and deep-layer structures of the cortex, hypothalamus, and midbrain dopaminergic structures such as the SN and VTA of the rat. In the guinea pig distribution in mid- and deep-layer cortical structures is largely similar to the rat but differs in the midbrain dopaminergic neurons where NK_3 receptors have not been detected (Yip and Chahl, 2001). However, NK_3-mediated functional changes in these nuclei, measured using electrophysiological recordings and subsequent neurochemical output, have been demonstrated (Navilaiko *et al*, 1997; Marco *et al*, 1998). PCR (Buell *et al*, 1992) and, more recently, immunocytochemistry techniques (Mileusnic *et al*, 1999; Koutcherov *et al*, 2000) have established that NK_3 receptors are also expressed, albeit in low abundance, in the primate CNS and human distribution studies have shown that the deep-layer cortical distribution appears to be maintained (Tooney *et al*, 2000). However, no NK_3 receptors have been detected, to date, within the mesencephalic dopaminergic neurons (Dietl and Palacios, 1991; Whitty *et al*, 1994, 1997). Thus, in addition to the complications of using preclinical species to evaluate the functional role of the NK_3 receptors, there is also some controversy with regard to the specific localization and density of NK_3 receptors within the human brain (Rigby *et al*, 2005). Although, in this regard our in house studies, using autoradiographic and immunohistochemical techniques, have detected widespread but low-level expression of the NK_3 receptors within human brain (unpublished data). Despite these remaining uncertainties, the hypothesis that NK_3 receptor antagonists have antipsychotic activity (Spooren *et al*, 2005) has gained support following preliminary reports suggesting that the NK_3 receptor antagonists SR-142801 (osanetant; Meltzer *et al*, 2004) may be clinically efficacious. The ability of NK_3 receptor antagonists to attenuate dopaminergic cell firing (Gueudet *et al*, 1999) and the present demonstration of reduced DA output from the mesolimbic system in the guinea pig may provide a mechanism which accounts for the amelioration of positive symptoms (PANNS and BPRS) reported in the SR-142801 trial. Additionally, the enhancement of guinea pig prefrontal and hippocampal monoaminergic neurotransmission may also suggest the potential to modulate the hypofrontality associated with schizophrenia (Carlsson *et al*, 2001). Thus, the neurochemical profile of talnetant is suggestive of potential therapeutic activity in the treatment of multiple symptom domains of schizophrenia and we await the outcome of further clinical trials with interest.

In conclusion, talnetant is a competitive antagonist at both the human and guinea pig NK_3 receptors. *In vivo*, the systemic administration of talnetant produced occupancy and antagonism of central NK_3 receptors. Dopaminergic hyperactivity in the mesolimbic projection systems was stimulated by NK_3 receptor activation in the guinea pig SNpc and attenuated by talnetant. Furthermore, talnetant blocked tonically active NK_3 receptors resulting in enhanced noradrenergic and dopaminergic neurotransmission in forebrain structures producing a neurochemical profile similar to the atypical antipsychotic clozapine. The present data offer support for the hypothesis that NK_3 receptor antagonists may be efficacious in the treatment of schizophrenia.

AUTHORSHIP DISCLOSURE

All authors are employees of GlaxoSmithKline Plc.

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