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The pharmacology of GR203040, a novel, potent and selective non-peptide tachykinin NK_1 receptor antagonist

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1 The *in vitro* and *in vivo* pharmacology of GR203040 ((2S, 3S)-2-methoxy-5-tetrazol-1-yl-benzyl-(2-phenyl-piperidin-3-yl)-amine), a novel, highly potent and selective non-peptide tachykinin NK_1 receptor antagonist, was investigated in the present study.

2 GR203040 potently inhibited [^{3}H]-substance P binding to human NK_1 receptors expressed in Chinese hamster ovary (CHO) and U373 MG astrocytoma cells, and NK_1 receptors in ferret and gerbil cortex (pK_i values of 10.3, 10.5, 10.1 and 10.1 respectively). GR203040 had lower affinity at rat NK_1 receptors ($pK_i = 8.6$) and little affinity for human NK_2 receptors ($pK_i < 5.0$) in CHO cells and NK_3 receptors in guinea-pig cortex ($pK_i < 6.0$). With the exception of the histamine H_1 receptor ($pIC_{50} = 7.5$), GR203040 had little affinity ($pIC_{50} < 6.0$) at all non-NK_1 receptors and ion channels examined. Furthermore, GR203040 produced only weak inhibition of Na^+ currents in SH-SY5Y neuroblastoma and superior cervical ganglion cells (pIC_{50} values < 4.0). GR203040 produced only weak antagonism of Ca^{2+}-evoked contractions of rat isolated portal vein ($pK_B = 4.1$). The enantiomer of GR203040, GR205608 ((2**R**, 3**R**)-2-methoxy-5-tetrazol-1-yl-benzyl-(2-phenyl-piperidin-3-yl)-amine), had 10,000 fold lower affinity at the human NK_1 receptor expressed in CHO cells ($pK_i = 6.3$).

3 In gerbil *ex vivo* binding experiments, GR203040 produced a dose-dependent inhibition of the binding of [^{3}H]-substance P to cerebral cortical membranes ($ED_{50} = 15$ $\mu g\ kg^{-1}$ s.c. and 0.42 mg kg^{-1} p.o.). At 10 $\mu g\ kg^{-1}$ s.c., the inhibition of [^{3}H]-substance P binding was maintained for > 6 h. In the rat, GR203040 was less potent ($ED_{50} = 15.4$ mg kg^{-1} s.c.) probably reflecting, at least in part, its lower affinity at the rat NK_1 receptor.

4 In guinea-pig isolated ileum and dog isolated middle cerebral and basilar arteries, GR203040 produced a rightward displacement of the concentration-effect curves to substance P methyl ester (SPOMe) with suppression of the maximum agonist response (apparent pK_B values of 11.9, 11.2 and 11.1 respectively).

5 In anaesthetized rabbits, GR203040 antagonized reductions in carotid arterial vascular resistance evoked by SPOMe, injected via the lingual artery (DR_{10} (i.e. the dose producing a dose-ratio of 10) = 1.1 $\mu g\ kg^{-1}$, i.v.). At a dose 20 fold greater than its DR_{10} value (i.e. 22 $\mu g\ kg^{-1}$, i.v.), significant antagonism was evident more than 2 h after GR203040 administration.

6 In anaesthetized rats, GR203040 (3 and 10 mg kg^{-1}, i.v.) produced a dose-dependent inhibition of plasma protein extravasation in dura mater, conjunctiva, eyelid and lip in response to electrical stimulation of the trigeminal ganglion.

7 It is concluded that GR203040 is one of the most potent and selective NK_1 receptor antagonists yet described, and as such, has considerable potential as a pharmacological tool to characterize the physiological and pathological roles of substance P and NK_1 receptors. GR203040 may also have potential as a novel therapeutic agent for the treatment of conditions such as migraine, emesis and pain.

Keywords: GR203040; NK_1 receptor antagonist; migraine; substance P; receptor selectivity

Introduction

The undecapeptide, substance P, was first isolated by von Euler and Gaddum (1931) from extracts of brain and intestine. A member of the tachykinin family, substance P is the endogenous ligand for the tachykinin NK_1 receptor, while neurokinins A and B are endogenous ligands for NK_2 and NK_3 receptors, respectively (Regoli & Nantel, 1991; Maggi *et al.*, 1993). Substance P elicits a variety of biological responses via NK_1 receptor activation, such as smooth muscle contraction or relaxation, neuronal depolarization and exocrine gland secretion (Pernow, 1983; Payan *et al.*, 1984). Indeed, there is now considerable evidence that implicates an involvement of substance P in a number of physiological and pathological processes. Thus, substance P may be an important mediator in the pathogenesis of migraine and related headaches (Moskowitz *et al.*, 1989). Substance P is distributed throughout the cranial vasculature in the trigeminal sensory afferent nerve fibres and its release can be demonstrated following activation of the trigeminovascular system (Edvinsson *et al.*, 1983; Goadsby *et al.*, 1988). Following its release, and NK_1 receptor activation, substance P is thought to contribute to the cranial vasodilatation and oedema formation that are implicated in migraine headache (Edvinsson *et al.*, 1981; Markowitz *et al.*, 1987). Substance P may also have a neurotransmitter or neuromodulator role in nociceptive transmission generally (Otsuka & Yanagisawa, 1987), and is implicated in inflammation (Pernow, 1983) and emesis (Bountra *et al.*, 1993).

Our understanding of the biological actions of substance P and the involvement of NK_1 receptors has progressed significantly following the identification of several non-peptide NK_1 receptor antagonists with high affinity and selectivity. The

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pharmacology of compounds such as CP-96,345, CP-99,994, SR140333 and RP67580, has been described extensively (Snider *et al.*, 1991; Garret *et al.*, 1991; McLean *et al.*, 1993; Emonds-Alt *et al.*, 1993). The present paper describes the pharmacological properties of a novel non-peptide NK_1 receptor antagonist, GR203040 ((2S, 3S)-2-methoxy-5-tetrazol-1-yl-benzyl-(2-phenyl-piperidin-3-yl)-amine; Figure 1). GR203040 is one of the most potent and selective NK_1 receptor antagonists yet described, and offers considerable potential as a pharmacological tool to characterize the physiological and pathological roles of substance P and NK_1 receptors. In the present study, the pharmacology of GR203040 has been examined in a variety of *in vitro* and *in vivo* preparations. In some experiments, the activities of the NK_1 receptor antagonists, CP-96,345 and CP-99,994, were examined for comparison.

Methods

In vitro *experiments*

Radioligand binding experiments The affinity of GR203040 was evaluated at human NK_1 receptors, endogenously expressed in U373 MG astrocytoma cells (Heuillet *et al.*, 1993), and stably transfected into Chinese hamster ovary (CHO) cells (Turcatti *et al.*, 1993), and in ferret, gerbil and rat cortex (Beresford *et al.*, 1991) using [^{3}H]-substance P as the radioligand. Affinity at human NK_2 receptors was evaluated with [^{3}H]-GR100679 in stably transfected CHO cells (Beresford *et al.*, 1995) and at NK_3 receptors with [^{3}H]-senktide in guinea-pig cortex (Guard *et al.*, 1990). The binding affinity of GR203040 at a range of non-tachykinin receptors was measured by Battelle (7, route de Drize, 1227 Carouge, Geneva, Switzerland) and at Na^+, Ca^{2+} and K^+ channels by Cerep (le Bois l'Evêque BP, 186600 Celle l'Evescault, France).

Cell culture and membrane preparation Human NK_1 receptors were stably expressed in CHO cells by use of the calcium phosphate precipitation technique followed by antibiotic (G418) selection and methotrexate treatment (Chen & Okayama, 1988; Sasaki *et al.*, 1992). Human NK_2 receptors were stably expressed in CHO cells as previously described (Hagan *et al.*, 1993). U373 MG cells were purchased from the American tissue culture collection. All cell culturing was performed at 37°C in a humidified atmosphere (5% CO_2 in air). CHO and U373 MG cells were cultured in Dulbecco's minimum essential medium (DMEM), supplemented with 10% (v/v) foetal calf serum and glutamine (4 mM). Methotrexate (15 nM) was included in the medium used for CHO cells. When confluent, CHO and U373 MG cells were harvested with phosphate buffered saline containing 0.02% EDTA and centrifuged at 375 *g* for 7 min. Cells were then resuspended in 10 volumes of HEPES (50 mM) buffer (pH 7.4), containing leupeptin (0.1 mM), bacitracin (25 $\mu g\ ml^{-1}$), EDTA (1 mM), phenyl methyl sulphonyl fluoride (PMSF; 1 mM) and pepstatin A (2 μM), and homogenised with a Waring blender. To remove unbroken cells and nuclei, the suspension was centrifuged at 500 *g* for 20 min and the supernatant then centrifuged at 48,000 *g* for 30 min. The resultant pellet was resuspended in HEPES buffer (without the PMSF or pepstatin A) by vortexing for 5 s, and forced through 0.8 mm, and then 0.6 mm, bore syringe needles. Membrane suspensions were frozen at −80°C until required.

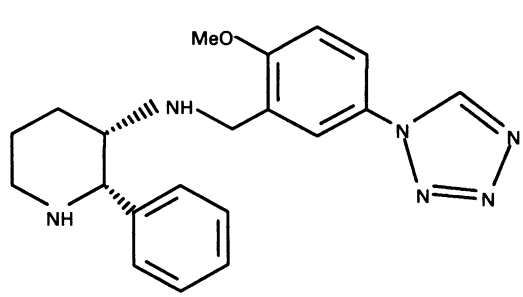

Figure 1 The structure of GR203040 ((2S, 3S)-2-methoxy-5-tetrazol-1-yl-benzyl-(2-phenyl-piperidin-3-yl)-amine).

Guinea-pig, ferret, rat and gerbil cerebral cortical membranes were freshly prepared for each experiment. Cerebral cortices were removed and placed in ice-cold buffer (Tris (50 mM), leupeptin (0.1 mM), bacitracin (25 $\mu g\ ml^{-1}$), EDTA (1 mM), PMSF (1 mM) and pepstatin A (2 μM), pH 7.4). The tissues were blotted and weighed, and then homogenised in 10 volumes of the ice-cold buffer with an Ultra-Turrax homogeniser. The homogenates were then centrifuged at 48,000 *g* for 25 min (4°C). The supernatants were discarded and the pellets resuspended in 10 volumes of the Tris buffer (PMSF and pepstatin A omitted) and centrifuged at 48,000 *g* for 20 min (4°C). The resultant pellets were resuspended in assay buffer (containing Tris (50 mM), bovine serum albumin (0.04%), bacitracin (80 $\mu g\ ml^{-1}$), leupeptin (8 $\mu g\ ml^{-1}$), phosphoramidon (2 μM) and $MnCl_2$ (3 mM), pH 7.4) to give a tissue concentration of 80 mg ml^{-1}.

Protein concentrations were determined by the method of Bradford (1976) with bovine serum albumin as the standard.

Binding protocols

CHO and U373 MG cell membranes In the NK_1 receptor binding assays, CHO and U373 MG cell membranes (3–5 μg and 25–35 μg protein respectively) were incubated (40 min at room temperature) in buffer (containing HEPES (50 mM) and $MnCl_2$ (3 mM), pH 7.4) or test compound, and [^{3}H]-substance P (0.7–1.0 nM final concentration). Non-specific binding was defined by the addition of CP-99,994 (1 μM). NK_2 receptor binding assays in CHO cell membranes were carried out essentially as described previously (Hagan *et al.*, 1993; Beresford *et al.*, 1995). The membrane suspension (5 μg protein) in assay buffer (Tris base (50 mM), $MnCl_2$ (3 mM), bovine serum albumin (0.05%), chymostatin (2 $\mu g\ ml^{-1}$) and leupeptin (4 $\mu g\ ml^{-1}$, pH 7.4)) were incubated for 90 min at room temperature with wash buffer (Tris base (50 mM), $MnCl_2$ (3 mM), lauryl sulphate (0.01%), pH 7.4) or test compound, and [^{3}H]-GR100679 (0.5 nM final concentration). Non-specific binding was defined by use of GR159897 (1 μM; Beresford *et al.*, 1995).

Cortical membranes NK_1 receptor binding assays in rat, ferret and gerbil cortex were performed essentially as described by Dam and Quirion (1986). Cerebral cortical membranes (8–15 mg wet weight) were incubated with [^{3}H]-substance P (0.6–1 nM) for 40 min at room temperature. Non-specific binding was defined in rat cortical membranes by use of RP67580 (1 μM), and in ferret and gerbil, with CP-99,994 (1 μM). The NK_3 receptor binding assays were carried out essentially as described by Guard *et al.* (1990). Guinea-pig cortical membranes (8 mg wet weight) were incubated at room temperature for 60 min with HEPES wash buffer or test compound and [^{3}H]-senktide (final concentration of 0.8–1.0 nM). Non-specific binding was defined by the addition of eledoisin (10 μM).

Reactions were terminated by rapid vacuum filtration through Whatman GF/B filters pre-soaked in either Triton-X (0.5%) containing polyethyleneimine (0.2%) (for NK_1 and NK_2 receptor binding) or bovine serum albumin (0.5%) (for NK_3 receptor binding). Filters were washed 3 times with wash buffer and radioactivity bound to filters was determined in a liquid scintillation counter.

Data analysis Inhibition curves were analysed and pIC_{50} values calculated by use of the curve fitting program, ALLFIT (Glaxo, VAX library). pIC_{50} values were converted to inhibition constants (pK_i values) using the Cheng Prusoff equation

($K_i = IC_{50}/(1 + L/K_D)$, where L is the ligand concentration and K_D is the dissociation constant) (Cheng & Prusoff, 1973). The K_D and B_{max} (maximum number of binding sites mg^{-1} of tissue) values were determined from saturation curves, analysed by the curve fitting program RADLIG (Glaxo VAX library). Values are expressed as arithmetic means ± s.e.mean.

Ex vivo *binding*

GR203040 (s.c. or p.o.) was administered to male (Glaxo bred, Random Hooded; 100–175 g) rats and Mongolian gerbils of either sex (Bantam and Kingman; 40–60 g). At various times following administration (5 min–18 h), the animals were anaesthetized with a lethal dose of pentobarbitone (100 mg kg^{-1}, s.c.) and perfused transcardially with 50 ml of ice-cold saline to remove any blood from the brain. The forebrain from each animal was removed (a coronal section just rostral to the hypothalamus) with a scalpel blade. Tissues were weighed and placed in a volume of Tris buffer (Tris base (50 mM), $MnCl_2$ (3 mM), bacitracin (40 μg ml^{-1}), leupeptin (4 μg ml^{-1}), PMSF (0.01 μM) and phosphoramidon (1.0 μM), pH 7.4) at a concentration of 60 mg ml^{-1}, and homogenized with an Ultra Turrax homogeniser (two 4 s bursts at maximum setting). The homogenate was then filtered through a nylon monofilament mesh (400 μm internal diameter of interstices). Aliquots (250 μl) of membranes were incubated in the Tris buffer with [^{3}H]-substance P (0.6 nM) in a total assay volume of 500 μl. Non-specific binding was determined by the presence of CP-99,994 (1 μM). Assay tubes were incubated for 45 min at room temperature and reactions terminated by rapid filtration (Whatman GF/B filters). The filters were washed with ice-cold Tris buffer before being placed in liquid scintillant and bound radioactivity was measured in a scintillation counter. Data are expressed as a percentage of specific binding determined in vehicle-treated control rats. ED_{50} values (i.e. doses producing 50% inhibition of specific binding) were calculated (ALLFIT program).

Dog middle cerebral and basilar arteries Brains were removed from beagle dogs (either sex, 7–10 kg) killed by sodium pentobarbitone (100 mg kg^{-1}, i.v.). The middle cerebral and basilar arteries were dissected and stored overnight in modified Krebs solution (Apperley *et al.*, 1976). Arteries were cut into rings (4 mm in length) and suspended between two 200 μm parallel wires in 10 ml organ baths containing modified Krebs solution (37°C, gassed with 5% CO_2 in O_2). Changes in isometric tension were recorded. A resting tension of 0.3 g was applied and re-adjusted to 0.3 g every 10 min for 1 h, and tension was then increased to 1 g for the remainder of the experiment. Tissues were pre-contracted with a submaximal concentration of prostaglandin $F_{2\alpha}$ (typically 1–10 μM), which produced approximately 70–80% of its maximum contraction. A cumulative concentration-response curve was constructed to substance P methyl ester (SPOMe), a selective substance P analogue. Tissues were washed every 5 min for 30 min and then re-contracted 30 min later with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). GR203040, or its vehicle, was added to the organ bath 15 min before the addition of $PGF_{2\alpha}$, approximately 30 min before a second curve was constructed to SPOMe in each tissue. At the end of all experiments, papaverine (0.2 mM) was added to each tissue and responses to SPOMe were expressed as a percentage of this papaverine-induced relaxation.

Guinea-pig ileum Male Dunkin-Hartley guinea-pigs (Porcellus; 200–250 g) were killed by cervical dislocation. A segment of ileum (12–15 cm long) was removed and divided into 8 equal lengths. A 1 ml glass pipette was placed into the lumen of each segment of ileum and the longitudinal muscle layer carefully removed and mounted, under 0.5 g tension, in an organ bath (5 ml) containing modified Krebs solution (Apperley *et al.*, 1976), gassed with 5% CO_2 in O_2. Responses were recorded with an isotonic transducer (Bioscience T_3). Non-cumulative concentration-response curves to SPOMe were constructed and repeated at hourly intervals, until responses were reproducible. NK_1 receptor antagonists were left in contact with the tissues for 30 min before construction of an agonist concentration-response curve. Data are expressed as a percentage of the maximum response achieved in the control concentration-response curve (immediately preceding antagonist addition), and a control tissue receiving only SPOMe was included in each experiment.

Data analysis To measure NK_1 receptor antagonist affinity, concentration-ratios were calculated at EC_{50} levels (i.e. the concentration producing 50% of the maximum response). Concentration-ratios estimated from individual preparations were used to calculate the apparent dissociation constant (pK_B) for GR203040 (pK_B = log(dose-ratio − 1) − log(antagonist concentration)). Where the antagonist caused a suppression of the maximum agonist response, its affinity was calculated by the method of Kenakin (1984).

Measurement of Na^+ currents Whole cell voltage-clamp recordings were made from human neuroblastoma SH-SY5Y cells and rat superior cervical ganglion (SCG) neurones. Both types of cell were cultured on coverslips and maintained in medium consisting of DMEM supplemented with 10% (v/v) foetal calf serum, penicillin (25 u ml^{-1}) and streptomycin (25 μg ml^{-1}). Drugs were diluted in a superfusion medium consisting of NaCl (147 mM), glucose (12 mM), HEPES (10 mM), KCl (2 mM), $CaCl_2$ (2 mM) and $MgCl_2$ (1 mM). Patch pipettes were filled with caesium gluconate (120 mM), EGTA (10 mM), HEPES (10 mM) and NaCl (5 mM), at pH 7.3. Cells were voltage clamped at −60 mV using an Axopatch 200A amplifier (Axon Instruments), and Na^+ currents were measured after a depolarizing step to 0 mV was applied. NK_1 receptor antagonists were applied for 3 min before recording commenced. Data were analysed by use of pClamp6 software.

Rat isolated hepatic portal vein Male rats (Glaxo bred, Lister Hooded, 250–400 g) were killed by stunning and exsanguination. The hepatic portal vein was removed, cut into rings, and placed in a Ca^{2+}-free, modified Krebs solution (Apperley *et al.*, 1976) gassed with 95% O_2/5%CO_2 and maintained at 37°C. The veins were placed in 10 ml organ baths under 0.5 g tension and left to equilibrate for 1 h before constructing a 'priming' cumulative concentration-response curve to $CaCl_2$ (0.1–30 mM). Changes in tension were measured with an isometric transducer. Tissues were then washed several times with Ca^{2+}-free Krebs solution and a second concentration-response curve to $CaCl_2$ was constructed 30 min later. Following further washing, NK_1 receptor antagonists or vehicle were added and allowed 15 min to equilibrate before a third concentration-response curve to $CaCl_2$ was constructed. The antagonist affinity (pK_B value) was estimated by calculating the concentration-ratios at the EC_{50} level (i.e. the concentrations producing 50% of the maximum response to $CaCl_2$ in the second concentration-response curve).

In vivo *studies*

Measurement of carotid arterial vascular resistance Adult male Albino rabbits (2.5–3.5 kg) of either sex were anaesthetized with sodium pentobarbitone (45 mg kg^{-1}), injected via the marginal ear vein. Anaesthesia was maintained during surgery by administration of additional bolus doses of pentobarbitone (up to 45 mg kg^{-1} i.v. in total), and during experiments (90 mg kg^{-1} pentobarbitone s.c. every 2 h). The trachea was cannulated to permit artificial respiration (60 strokes min^{-1}, 5 ml kg^{-1}) with room air supplemented by oxygen. The right femoral artery and vein were cannulated for the continuous measurement of arterial blood pressure and i.v. administration of drugs, respectively. The right lingual artery was cannulated to allow drug injection into the carotid arterial bed and carotid blood flow was measured by a Doppler flow probe (2 mm diameter; Bioengineering Department, University of

Iowa, model 545C-4), placed around the right common carotid artery. Responses were recorded as a d.c. voltage (0.5 V per kHz Doppler shift) and carotid blood flow (ml min^{-1}) was estimated by use of the Doppler equation, $V=(F_d \times C)/(2 \times F_o \times \text{cosineA})$, where V is the velocity of blood (mm s^{-1}), F_d is the Doppler shift frequency (kHz), C is the velocity of sound in blood (1.565 kms^{-1}), F_o is the transmitter frequency (20,000 kHz) and A is the angle between the sound beam and blood velocity vector (45°). Carotid vascular resistance, equivalent to the mean arterial blood pressure divided by the carotid blood flow, was monitored continuously by passing the pressure and flow signals to a peripheral resistance meter (Bioengineering Department, Glaxo Research and Development Ltd). The peak changes in blood pressure, carotid blood flow and vascular resistance were recorded. Rectal temperature was monitored with a thermistor (CFP 8185) and maintained at 37–38°C with a heated blanket.

After the completion of surgery, 30 min was allowed to lapse to achieve stable resting levels in blood pressure, carotid arterial flow and vascular resistance. Two consecutive, two-point dose-response curves were constructed to SPOMe (0.014, and 0.14 ng kg^{-1}), injected via the right lingual artery (15 min between each dose). Increasing doses of GR203040 (1–100 $\mu g\ kg^{-1}$) were then injected via the right femoral vein at hourly intervals and agonist dose-response curves repeated 15 min after each dose of GR203040. The potency of GR203040 was quantified by calculating a DR_{10} value (i.e. the dose of GR203040 which produced a 10 fold shift in the SPOMe dose-response curve). To determine the duration of NK_1 receptor antagonism, a dose of GR203040, 20 times greater than its DR_{10} was injected (i.v.) in a separate group of animals, which were re-challenged with SPOMe at hourly intervals after antagonist administration. Dose-ratios were calculated at each time-point.

Measurement of plasma protein extravasation in anaesthetized rats Plasma protein extravasation (PPE) was measured essentially as described by Buzzi & Moskowitz (1990). Male Sprague-Dawley rats (190–220 g) were anaesthetized with pentobarbitone (60 mg kg^{-1}, i.p.) and the left femoral vein cannulated for drug administration. Animals were placed in a stereotaxic frame (Lab Standard S1600) and the skull was exposed by a mid-sagittal incision. A 2 mm hole was then drilled on either side of the sagittal suture (3 mm posterior to bregma and 3 mm lateral to the sagittal suture). [^{125}I]-human serum albumin ([^{125}I]-HSA) was injected (370 kBq per animal, i.v.) together with Evans blue (50 mg kg^{-1}, i.v.), which aided verification of electrode placements post mortem. Bipolar electrodes (NEX-200, Rhodes) were lowered bilaterally into each trigeminal ganglion and, 5 min after [^{125}I]-HSA administration, biphasic electrical stimulation (5 ms, 5 Hz, 1.2 mA for 5 min) was delivered to one ganglion. GR203040 or vehicle was administered (i.v.) 15 min before electrical stimulation.

Immediately after electrical stimulation, animals were perfused (0.9% saline for 3 min) via the left cardiac ventricle at mean arterial blood pressure (100 mmHg). The right atrium was incised to allow outflow of perfusate. Conjunctiva, eyelid and lip were dissected out, the skull opened and the dura mater dissected from the cranium. Tissues from the stimulated and unstimulated sides were weighed and counted for radioactivity. Results are expressed as c.p.m. mg^{-1} tissue and the difference between unstimulated and stimulated sides was assessed by either Student's paired *t* test or ANOVA followed by a Dunnett's t test with significance levels set at $P<0.05$. Data were then expressed for each animal as a ratio of c.p.m. mg^{-1} tissue between stimulated and unstimulated sides.

Measurement of blood pressure and heart rate Male rats (Glaxo bred, Lister Hooded, 250–400 g) were anaesthetized with pentobarbitone sodium (60 mg kg^{-1}, i.p.). The right common carotid artery and left jugular vein were cannulated for, respectively, measurement of blood pressure and intravenous drug administration. Heart rate was derived from the blood pressure signal, and both parameters were recorded on a Modular Instruments Data Analysis System or Lectromed recorder. Vehicle, followed by increasing doses of NK_1 receptor antagonist (0.1–10 mg kg^{-1}, i.v.) were administered at 15 min intervals and peak changes in diastolic blood pressure and heart rate were measured.

Drugs and solutions

The following compounds were used in the study: bacitracin, chymostatin, Evans blue, leupeptin, papaverine, pepstatin A, phosphoramidon (all from Sigma), eledoisin (Peninsula), penicillin (Gibco), prostaglandin $F_{2\alpha}$ (dinoprost tromethamine; Upjohn), streptomycin (Gibco) and substance P methyl ester (SPOMe; Cambridge Research Biochemicals). GR203040 (hydrochloride salt), GR205608 ((2**R**, 3**R**)-2-methoxy-5-tetrazol-1-yl-benzyl-(2-phenyl-piperidin-3-yl)-amine), CP-96,345 ((2S,3S)-*cis*-2 (diphenylmethyl)-N-[(2-methoxyphenyl)-methyl] -1-azabicyclo [2,2,2] octan-3-amine), CP-99,994 ((+)-(2S,3S)-3-(2-methoxybenzyl-amino)-2-phenylpiperidine) and RP67580 ((3a**R**,7a**R**)-7,7-diphenyl-2-[imino-2-(2-methoxyphenyl)ethyl] perhydroisoindol- 4-one) were synthesized by the Medicinal Chemistry Department, Glaxo Research and Development Ltd, U.K. [^{125}I]-HSA (1.85 MBq ml^{-1}) and [^{3}H]-GR100679 (3.3 TBq $mmol^{-1}$) were purchased from Amersham, U.K., while [^{3}H]-substance P (4.4 TBq $mmol^{-1}$) and [^{3}H]-senktide (3.1 TBq $mmol^{-1}$) were purchased from Cambridge Research Biochemicals, and NEN Du Pont respectively. Drugs were dissolved and diluted in distilled water or 0.9% saline.

Results

Receptor and ion channel binding affinity

The affinity of GR203040 was assessed at a variety of tachykinin and non-tachykinin receptors (Table 1), and ion channels. In all binding assays, the slopes were not significantly different from unity, consistent with a competitive receptor interaction. GR203040 potently inhibited [^{3}H]-substance P binding to human NK_1 receptors expressed in CHO (Figure 2) and U373 MG cells (pK_i values of 10.3 ± 0.1, $n=5$ and 10.5 ± 0.2, $n=4$ respectively). CP-99,994, by comparison, had pK_i values of 9.6 ± 0.1 ($n=20$) and 9.7 ± 0.1 ($n=20$) in CHO (Figure 2) and U373 MG cells respectively. GR205608 (the 2**R**, 3**R** enantiomer of GR203040) had 10,000 fold lower affinity at the human NK_1 receptor expressed in CHO cells ($pK_i = 6.3 \pm 0.1$, $n=4$). GR203040 also demonstrated high affinity for NK_1 receptors in ferret and gerbil cortex (pK_i values of 10.1 ± 0.1, $n=4$ and 10.1 ± 0.2, $n=4$ respectively). GR203040 displaced [^{3}H]-substance P from NK_1 receptors in rat cortex with lower affinity ($pK_i = 8.6 \pm 0.2$; $n=3$). However, GR203040 failed to inhibit [^{3}H]-GR100679 binding to human NK_2 receptors (expressed in CHO cells) ($pK_i < 5.0$) and, in guinea-pig cortex, [^{3}H]-senktide binding to NK_3 receptors ($pK_i < 6.0$).

GR203040 had sub-micromolar affinity at the histamine H_1 receptor ($pIC_{50} = 7.5$) and micromolar affinity at 5-HT_{1D}, muscarinic M_1 and histamine H_2 receptors. GR203040 had negligible affinity at 34 other receptors ($pIC_{50} \leqslant 5$, Table 1). In terms of its affinity for ion channels, GR203040 (10 μM) inhibited [^{3}H]-batrachotoxin binding in rat cortex (Na^+ channel, site 2; 79% inhibition), but had no effect at Na^+ (site 1), Ca^{2+} (T, L and N types) or K^+ (ATP, voltage and Ca^{2+}-dependent) channels (assay details from Cerep, le Bois l'Evêque BP, 186600 Celle l'Evescault, France).

Ex vivo *binding*

In the gerbil *ex vivo* binding experiments, GR203040 (0.01–1 mg kg^{-1} s.c., 30 min pretreatment or 0.1–10 mg kg^{-1} p.o., 45 min pretreatment) produced a dose-dependent inhibition of

Table 1 Affinity values (pK_i or pIC_{50}) for GR203040 at neurokinin (NK) and non-NK receptors using radioligand binding

Receptor	*Tissue*	*Radioligand*	*pK_i or $*pIC_{50}$*
NK_1	Human recombinant receptor in CHO cells	[^{3}H]-substance P	10.3
NK_1	Human receptor in U373 MG cells	[^{3}H]-substance P	10.5
NK_1	Gerbil cortex	[^{3}H]-substance P	10.1
NK_1	Ferret cortex	[^{3}H]-substance P	10.1
NK_1	Rat cortex	[^{3}H]-substance P	8.6
NK_2	Human recombinant receptor in CHO cells	[^{3}H]-GR100679	<5.0
NK_3	Guinea-pig cortex	[^{3}H]-senktide	<6.0
5-HT_{1D}	Bovine striatum	[^{3}H]-5-HT	*5.2
Muscarinic M_1	Rat brain	[^{3}H]-pirenzepine	*<6.0
Histamine H_1	Rat cortex	[^{3}H]-pyrilamine	*7.5
Histamine H_2	Guinea-pig cortex	[^{3}H]-tiotidine	*<6.0

None of the slope factors differed significantly from unity.
$pIC_{50} \leq 5.0$ at adenosine A_1 and A_2, α_1- and α_2-adrenoceptors, angiotensin AT_1 and AT_2, β_1- and β_2-adrenoceptors, bradykinin, CGRP, dopamine D_1, D_2 and D_4, endothelin, $GABA_A$ and $GABA_B$, glycine (strychnine-sensitive), histamine H_3, 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_3, muscarinic M_2 and M_3, nicotinic, NMDA, opioid μ, δ and κ, and somatostatin receptors (assay details from Battelle, 7, route de Drize, 1227 Carouge, Geneva, Switzerland).

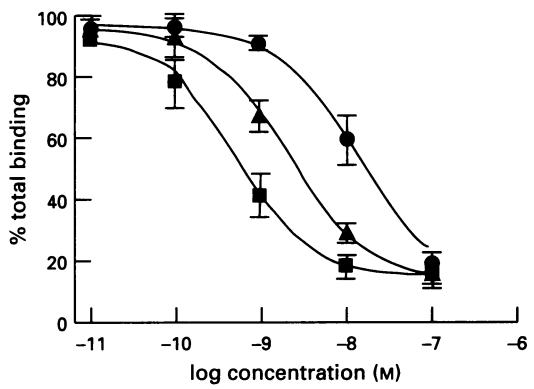

Figure 2 Inhibition of [^{3}H]-substance P binding by GR203040 (■), CP-99,994 (▲) and substance P (●) at human NK_1 receptors stably expressed in Chinese hamster ovary (CHO) cells. The results are expressed as % total [^{3}H]-substance P binding against the log concentration of each compound and values are means ± s.e.mean ($n=3$). These data were taken from 3 experiments in which GR203040, CP-99,994 and substance P were tested in the same assay.

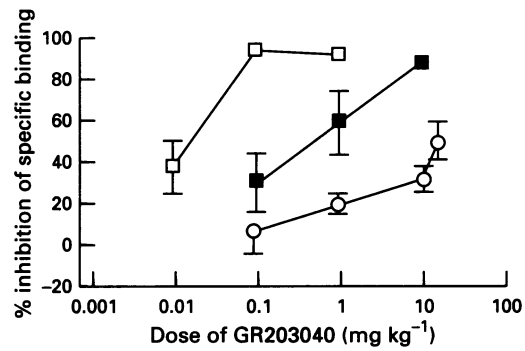

Figure 3 Inhibition of the *ex vivo* binding of [^{3}H]-substance P to gerbil and rat cerebral cortical membranes by GR203040, administered by the s.c. (□) or p.o. (■) routes in the gerbil and by the s.c. route (○) in the rat. *Ex vivo* measurements were made 30 or 45 min following s.c. and p.o. administration, respectively. Results are expressed as % inhibition of specific [^{3}H]-substance P binding measured in vehicle-treated animals and values are means ± s.e.mean ($n=3-6$).

the binding of [^{3}H]-substance P to cerebral cortical membranes (Figure 3). The mean ED_{50} values were 15 μg kg^{-1} s.c. ($n=3$) and 0.42 mg kg^{-1} p.o. ($n=6$). At 10 μg kg^{-1} s.c., inhibition was evident within 5 min of administration and maintained for >6 h. By 18 h, no significant inhibition (9.5 ± 12.0%; $n=3$) was observed.

In the rat *ex vivo* binding experiments, GR203040 (0.1–15 mg kg^{-1} s.c., 30 min pretreatment) produced a dose-dependent inhibition of the binding of [^{3}H]-substance P to cerebral cortex. In contrast to the results in the gerbil only 53 ± 9% inhibition was observed at 15 mg kg^{-1} s.c. (Figure 3). The mean ED_{50} value was 15.4 mg kg^{-1} s.c. ($n=6$). In a subsequent study, measuring the duration of NK_1 receptor occupancy, GR203040 (10 mg kg^{-1}, s.c.; $n=3$) produced 62 ± 4 and 68 ± 2% inhibition, 1 and 2 h after administration, respectively, but this effect was markedly reduced by 6 h (27 ± 3% inhibition).

NK_1 receptor antagonism in dog middle cerebral and basilar arteries

SPOMe (0.1–100 nM) caused a concentration-dependent relaxation of prostaglandin $F_{2\alpha}$-induced tone of the dog middle cerebral and basilar arteries. Consecutive concentration-response curves were reproducible in control tissues (<2 fold shift) (Figure 4). GR203040 (0.03 nM) produced a rightward displacement of the SPOMe concentration-effect curve with apparent pK_B values of 11.2 ± 0.2 and 11.1 ± 0.3 (both $n=3$) in the middle cerebral (Figure 4) and basilar arteries, respectively. At a higher concentration (0.1 nM), GR203040 caused a suppression of the maximum agonist response (Figure 4).

NK_1 receptor antagonism in the guinea-pig ileum

In the guinea-pig ileum, GR203040 (0.01–3 nM) had no spasmogenic activity but antagonized contractions induced by the NK_1 receptor agonist, SPOMe. GR203040 caused non-parallel rightward displacement of SPOMe concentration-response curves and suppression of the maximum contractile response indicating insurmountable antagonism. An apparent pK_B estimate of 11.9 ± 0.3 ($n=3$) was calculated, according to the method of Kenakin (1984). For comparison, CP-99,994 had a pK_B of 10.3 ± 0.2 ($n=3$).

Influence of Na^+ currents

Saline vehicle had no effect on Na^+ currents in SH-SY5Y and SCG cells, measured by whole cell patch-clamp recording.

GR203040 (10–400 μM) produced a concentration-dependent, but very weak inhibition of Na^+ current in both cell types (mean pIC_{50} values of 3.8 and 3.9 respectively; $n=5$). Similar effects were observed with CP-99,994 (mean pIC_{50} values of 4.2 and 3.8 respectively; $n=5$).

Influence on Ca^{2+}-induced contraction of the rat hepatic portal vein

$CaCl_2$ (0.1–30 mM) evoked concentration-dependent contractions of rat isolated portal vein. Saline vehicle had no effect on the $CaCl_2$ concentration-response curve, while GR203040 (0.1 mM) produced a small, parallel, rightward shift (Figure 5). A pK_B of 4.1 ± 0.1 ($n=5$) was calculated for GR203040. CP-96,345 (10 μM) and CP-99,994 (0.1 mM) were more potent than GR203040 at antagonizing the $CaCl_2$-induced contractions (pK_B values of 6.2 ± 0.1 ($n=3$) and 4.9 ± 0.2 ($n=3$) respectively; Figure 5). None of the compounds had any effect on basal tension.

NK_1 receptor antagonism in the rabbit carotid arterial bed

Rabbits had a mean resting arterial blood pressure of 81 ± 4 mmHg ($n=14$) and right carotid arterial blood flow of 32.5 ± 4.8 ml min^{-1} ($n=14$). Saline (0.01 ml kg^{-1}), injected via the right lingual artery, had no effect on blood pressure or carotid blood flow and vascular resistance. SPOMe (0.014 and 0.14 ng kg^{-1}, i.a.), however, produced a dose-dependent, transient (duration approximately 90 s) increase in carotid blood flow and decrease in carotid vascular resistance with little change in blood pressure (% changes from resting levels of 82.9 ± 19.1, -40.5 ± 4.9 and -0.5 ± 1.0% respectively ($n=12$) at 0.14 ng kg^{-1}, i.a.). Responses produced by SPOMe were reproducible for several hours when a minimum of 30 min was allowed between successive dose-response curves.

GR203040 (1–100 μg kg^{-1}, i.v., 15 min pretreatment) but not its vehicle, saline (0.5 ml kg^{-1} i.v.) which was inactive, antagonized SPOMe-induced reductions in carotid vascular resistance ($DR_{10}=1.1$ μg kg^{-1}, 95% confidence limits: 0.9–1.4, slope of 1.5 ± 0.2; $n=3$). In a separate group of animals, at a dose 20 fold greater than its DR_{10} value (i.e. 22 μg kg^{-1}, i.v.), the respective dose-ratios (with ranges) for GR203040 were 203 (164–266), 96 (75–123) and 56 (7–113) at 15, 75 and 135 min ($n=3$) (Figure 6 for 100 μg kg^{-1} GR203040).

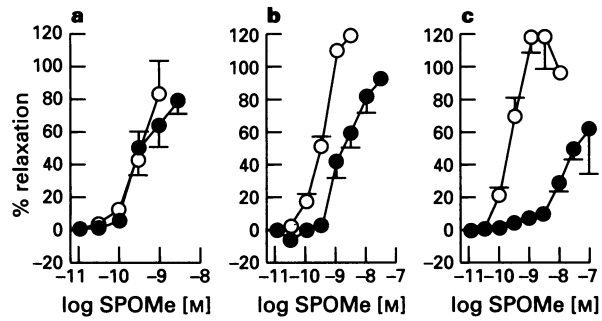

Figure 4 The effect of GR203040 (0.03 and 0.1 nM) on substance P methyl ester (SPOMe)-induced relaxation of the dog isolated middle cerebral artery. Two reproducible, control SPOMe concentration-response curves (a) and concentration-response curves to SPOMe, prior to and following addition of GR203040 (0.03 (b) and 0.1 nM (c), respectively). The first concentration-response curve in each tissue is represented by (○) and the second, by (●). Responses are expressed as a percentage of the maximum relaxation produced by papaverine (0.2 mM). Values are means ± s.e.mean ($n=3$).

Effect of GR203040 on plasma protein extravasation

Electrical stimulation (1.2 mA, 5 Hz for 5 min, 5 ms pulse width) of the trigeminal ganglion increased the leakage of [^{125}I]-HSA within rat dura mater (32.9 ± 3.6 and 19.3 ± 2.1 c.p.m. mg^{-1} tissue on ipsilateral and contralateral sides to stimulation, $P=0.0001$, $n=13$). Extravasation also occurred in extracranial tissues; leakage of [^{125}I]-HSA in stimulated and unstimulated sides was 799.6 ± 91.7 and 129.5 ± 22.1 c.p.m. mg^{-1} tissue (conjunctiva), 663.4 ± 39.0 and 219.9 ± 35.2 c.p.m. mg^{-1} tissue (eyelid) and 973.5 ± 70.9 and 172.1 ± 19.6 c.p.m. mg^{-1} tissue (lip), respectively ($n=6$). The extravasation ratios, calculated from the c.p.m. mg^{-1} tissue in the stimulated and unstimulated sides, were 1.8 ± 0.1 (dura mater), 5.7 ± 0.9 (conjunctiva), 3.9 ± 0.8 (eyelid) and 6.1 ± 0.5 (lip) ($n=13$).

Pretreatment of rats with GR203040 (1, 3 and 10 mg kg^{-1}, i.v.) produced a dose-dependent inhibition of PPE in the dura mater (Figure 7). The ID_{50} value for GR203040 (i.e. the dose producing 50% inhibition of PPE) was 3.0 mg kg^{-1}. Extravasation in the conjunctiva, eyelid and lip was also inhibited by GR203040 (3 and 10 mg kg^{-1}, i.v.) (Figure 7, for lip).

Effects on blood pressure and heart rate in anaesthetized rats

Rats had a mean resting diastolic blood pressure and heart rate of 91 ± 6 mmHg ($n=12$) and 329 ± 9 beats min^{-1} respectively ($n=12$). GR203040 (0.3–10 mg kg^{-1}, i.v.), but not its vehicle (0.5 ml kg^{-1}, i.v.), which was inactive, caused a dose-related, transient (duration approximately 2 min) increase in diastolic

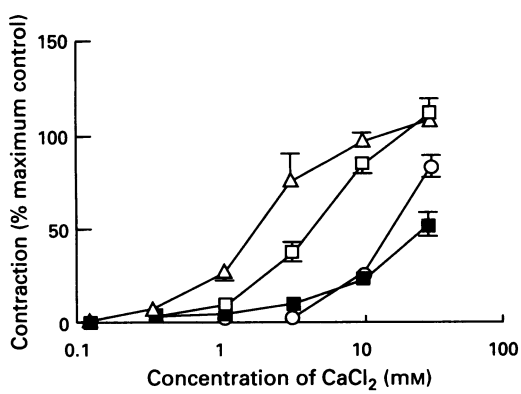

Figure 5 The effect of vehicle (△), GR203040 (0.1 mM; □), CP-96,345 (10 μM; ■) and CP-99,994 (0.1 mM; ○) on Ca^{2+}-induced contractions in rat isolated hepatic portal vein. Values are means ± s.e.mean ($n=3$–5), expressed as a percentage of an original maximum contraction to Ca^{2+}.

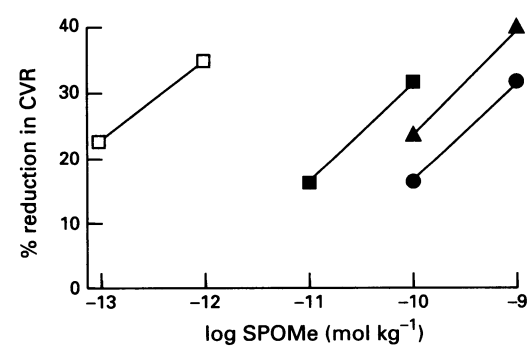

Figure 6 A typical experiment showing the effect of GR203040 (100 μg kg^{-1}, i.v.) on substance P methyl ester (SPOMe)-induced reductions in carotid vascular resistance (CVR) in the anaesthetized rabbit. Two-point SPOMe dose-response curves were constructed before (□), and 15 (●), 75 (▲) and 135 min (■) following, administration of GR203040.

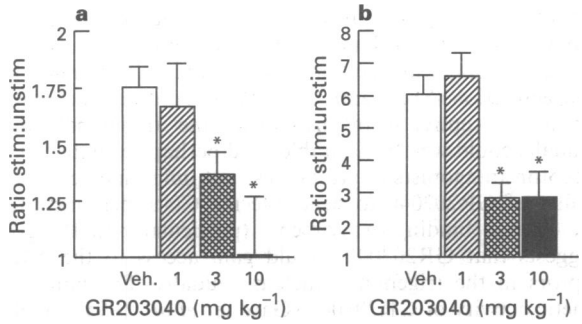

Figure 7 The effect of GR203040 (1 (hatched columns; $n=7$), 3 (cross-hatched columns; $n=6$) and 10 mg kg^{-1} (solid columns; $n=5$), i.v.) or vehicle (open columns; $n=13$) on plasma protein extravasation in (a) the dura and (b) the lip of anaesthetized rats, in response to electrical stimulation of the trigeminal ganglion. Values are mean ± s.e.mean ratios of extravasation on the stimulated, compared to unstimulated sides (see Methods). *$P<0.05$ (Student's t test), compared to vehicle.

blood pressure (of 47 ± 7% at 3 mg kg^{-1}; $n=7$) and a more prolonged (duration of around 5 min) reduction in heart rate (of 18 ± 2% at 3 mg kg^{-1}; $n=4$) (data not shown). CP-99,994 (0.3–3 mg kg^{-1}, i.v.) produced variable effects on blood pressure; at 3 mg kg^{-1}, reductions of diastolic blood pressure of up to 50% were observed, followed on occasion by pressor responses. CP-99,994 (0.3–3 mg kg^{-1}) produced a bradycardia similar in magnitude and potency to GR203040. When infused over 3 min, GR203040 (1 and 3 mg kg^{-1}, i.v.) had no effect on blood pressure and produced a much smaller bradycardia (7 ± 2% reduction at 3 mg kg^{-1}; $n=4$). Following subcutaneous administration, GR203040 (3 and 10 mg kg^{-1}) had no effect on either blood pressure or heart rate.

Discussion

GR203040 is a novel, highly potent and selective NK_1 receptor antagonist, possessing subnanomolar affinity in a variety of species, including man. GR203040 has higher affinity at human NK_1 receptors than the structurally-related NK_1 receptor antagonists, CP-96,345 and CP-99,994 (pK_i values of 10.5 and 10.3 for GR203040 in U373 MG and CHO cells respectively, compared to 9.6 for both CP-96,345 and CP-99,994 in IM-9 cells; McLean *et al.*, 1993). Consistent with the published data, in the present study CP-99,994 had pK_i values of 9.6 and 9.7 in CHO and U373 MG cells respectively. Similarly, GR203040 has higher affinity than the structurally-unrelated NK_1 receptor antagonist, SR140333, in the U373 MG cell line (pK_i of 9.2; Emonds-Alt *et al.*, 1993).

At rat NK_1 receptors, the affinity of GR203040 was approximately 50 fold less than that at the human receptor. This species difference is similar to that observed with CP-96,345 and CP-99,994 (Gitter *et al* 1991; McLean *et al.*, 1993), consistent with the structural similarities of these compounds. Conversely, SR140333 has similar affinity for the NK_1 receptors in a variety of species (Emonds-Alt *et al.*, 1993), while another antagonist, RP67580, shows the opposite profile, possessing a high degree of selectivity for rat and mouse NK_1 receptors (Garret *et al.*, 1991; Beaujouan *et al.*, 1993).

The ability of GR203040 to bind to NK_1 receptors in the CNS, following peripheral administration, was investigated by the technique of *ex vivo* binding. The results clearly showed that in gerbils, GR203040 gains rapid access to the CNS and has a reasonable duration of occupancy (>6 h) following subcutaneous administration. After oral administration, the potency was reduced by 28 fold. In rats, the potency of GR203040 (s.c.) was lower, as was its duration of NK_1 receptor occupancy. This reduced potency is likely to reflect, at least in part, the reduced affinity of GR203040 for the rat NK_1 receptor.

The radioligand binding experiments demonstrated not only that GR203040 possesses high affinity at NK_1 receptors, but also illustrated its high degree of selectivity (Table 1). Thus, GR203040 had greater than 10,000 fold higher affinity for NK_1 receptors over human NK_2 receptors expressed in CHO cells and NK_3 receptors in guinea-pig cortex. GR203040 had some affinity for histamine H_1 receptors ($pIC_{50}=7.5$), a finding that was consistent with functional data in the guinea-pig ileum, where GR203040 inhibits histamine-induced contractions with a pK_B of 7.2 (unpublished observation). However, there is still a good separation (≥1000 fold) between activity at NK_1 and H_1 receptors. At all other non-NK_1 receptors examined, GR203040 possessed, at most, only micromolar affinity (Table 1). NK_1 receptor binding affinity is enantiomer-specific; GR205608 had 10,000 fold lower affinity at NK_1 receptors expressed in CHO cells.

The potential interaction of GR203040 with Na^+ and Ca^{2+} channels was also investigated in the present study. Several non-peptide NK_1 receptor antagonists, for example CP-96,345 and RP67580, have affinity for Na^+ and L-type Ca^{2+} channels (Schmidt *et al.*, 1992; Caeser *et al.*, 1993; Guard *et al.*, 1993; Constantine *et al.*, 1994; Wang *et al.*, 1994). However, GR203040 was found to have only a weak effect on ion channels. GR203040, at a very high concentration (10 μM), inhibited [^{3}H]-batrachotoxin binding (Na^+ channel, site 2; 79% inhibition in rat cortex), but had no effect at Na^+ (site 1), Ca^{2+} (T, L and N types) or K^+ (ATP, voltage and Ca^{2+}-dependent) channels. In the rat portal vein, GR203040 had only weak antagonist activity against Ca^{2+}-induced contractions ($pK_B=4.1$), less than that observed with CP-99,994 ($pK_B=4.9$) and CP-96,345 ($pK_B=6.2$). GR203040 had a similar Na^+ channel antagonist potency in rat SCG neurones and human SH-SY5Y neuroblastoma cells (pIC_{50} of approximately 4.0).

While the ion channel antagonist activity was clearly exceptionally weak, it is possible that such a mechanism may be at least partly responsible for the bradycardia produced by NK_1 receptor antagonists, such as GR203040 and CP-99,994, in anaesthetized rats following high bolus intravenous dosing. It is difficult, however, to reconcile Na^+ channel antagonism as the mechanism of the GR203040-induced, transient vasopressor effects, that were observed in anaesthetized rats, albeit at doses more than 100 fold higher than those producing significant NK_1 receptor antagonism in the rabbit cranial circulation. While the pressor response produced by GR203040 is unlikely to involve an interaction with NK_1 receptors, the mechanism responsible remains unclear. It is likely that the pressor effects occur as a consequence of a transient, high plasma concentration of GR203040 being achieved following rapid intravenous dosing. The pressor response was markedly reduced upon intravenous infusion (over 3 min) and following subcutaneous administration, when a high dose of GR203040 (10 mg kg^{-1}) had no direct cardiovascular effects. Interestingly, other NK_1 receptor antagonists, such as CP-96,345 and CP-99,994, routinely reduced blood pressure following intravenous dosing in anaesthetized rats, although occasionally vasopressor responses were also observed.

The high NK_1 receptor antagonist affinity of GR203040 was demonstrated functionally *in vitro* in the guinea-pig ileum and dog middle cerebral and basilar arteries. In each tissue GR203040 antagonized powerfully SPOMe-induced responses in an insurmountable manner. Although the mechanism of its insurmountable antagonism is unclear, and requires further investigation, it is possible that GR203040 dissociates slowly from the NK_1 receptor. Such a profile was also observed with SR140333 (Emonds-Alt *et al.*, 1993).

The marked NK_1 receptor antagonist activity of GR203040 was evident *in vivo*. In anaesthetized rabbits, GR203040 antagonized SPOMe-induced reductions in carotid arterial vascular resistance (DR_{10} of 1.1 μg kg^{-1}, i.v.). At a higher dose (22 μg kg^{-1}, i.v.), blockade was still evident 2 h after administration. In comparison, CP-99,994 was much less active in this model (DR_{10} of approximately 10 μg kg^{-1} i.v.; M.J. Per-

ren, personal communication). Preliminary experiments have indicated that GR203040 is also highly effective in this model when administered intraduodenally, suggesting that the compound is orally active (data not shown; Ward *et al.*, 1995). The reduction in carotid vascular resistance, produced by local administration of SPOMe, is thought to represent craniovascular dilatation via NK_1 receptor activation (Perren *et al.*, 1995). The effectiveness of GR203040 in this model is consistent with its ability to antagonize NK_1 receptors in the cranial circulation.

GR203040 also inhibited plasma protein extravasation (PPE) in the dura, conjunctiva, eyelid and lip evoked by electrical stimulation of the trigeminal ganglion. A similar effect has been observed with other NK_1 receptor antagonists, for example RP67580 and GR82334 (Moussaoui *et al.*, 1993; Shepheard *et al.*, 1993; Beattie *et al.*, 1995). The reduced activity of GR203040 at inhibiting PPE, in comparison with its potency at antagonizing SPOMe-induced reductions in carotid vascular resistance in anaesthetized rabbits, is consistent with its relatively low affinity at the rat NK_1 receptor.

The ability of GR203040 to antagonize NK_1 receptor-mediated cranial vasodilatation *in vitro* (i.e. in dog cerebral arteries) and *in vivo* (in the rabbit cranial circulation), and dural PPE suggests that the compound may possess anti-migraine activity. There is good evidence implicating substance P in the pathogenesis of migraine headache. Substance P is stored in, and under certain conditions, can be released from, trigeminal sensory nerve terminals innervating cerebral and dural blood vessels (Edvinsson *et al.*, 1983; Goadsby *et al.*, 1988). Following its neuronal release, substance P activates endothelial NK_1 receptors to produce dilatation within the cranial vasculature and oedema formation in the meninges (Edvinsson *et al.*, 1981; Markowitz *et al.*, 1987; Beattie *et al.*, 1993). Furthermore, substance P may also be an important neurotransmitter or neuromodulator in the nociceptive pathway within the CNS. Thus, CP-99,994 inhibits c-fos expression in the trigeminal nucleus caudalis evoked by trigeminal ganglion activation (Shepheard *et al.*, 1995). Interference with afferent nociceptive transmission in the trigeminal nucleus caudalis could well be desirable (and perhaps essential) if NK_1 receptor antagonists are to possess anti-migraine activity. The ability of GR203040 to penetrate the CNS, demonstrated in the *ex vivo* binding experiments (particularly in the gerbil), suggests that GR203040 should gain access to the NK_1 receptors in the trigeminal nucleus caudalis. The potent anti-emetic activity of GR203040 (Gardner *et al.*, 1995) could also be useful in the treatment of migraine. This is likely to reflect a central action of GR203040 in the vomiting centre. The clinical effectiveness of GR203040 and other NK_1 receptor antagonists in migraine therapy will only be revealed when results from clinical studies are available. The potential offered by compounds such as GR203040 certainly merits their clinical investigation.

In conclusion, GR203040 is one of the most potent and selective NK_1 receptor antagonists yet described. It should offer considerable potential as a drug tool to investigate the physiological and pathological roles of substance P and NK_1 receptors. Moreover, GR203040 may prove to be a useful therapeutic agent in the clinical management of migraine headache. Additionally, GR203040 may provide a novel treatment for emesis, pain and inflammation, conditions in which substance P is also implicated.

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