

# Pharmacological Profile of (2*R*-*trans*)-4-[1-[3,5-bis(trifluoromethyl)benzoyl]-2-(phenylmethyl)-4-piperidinyl]-*N*-(2,6-dimethylphenyl)-1-acetamide (S)-Hydroxybutanedioate (R116301), an Orally and Centrally Active Neurokinin-1 Receptor Antagonist

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## ABSTRACT

In comparison with a series of reference compounds, (2*R*-*trans*)-4-[1-[3,5-bis(trifluoromethyl)benzoyl]-2-(phenylmethyl)-4-piperidinyl]-*N*-(2,6-dimethylphenyl)-1-acetamide (S)-Hydroxybutanedioate (R116301) was characterized as a specific, orally, and centrally active neurokinin-1 (NK<sub>1</sub>) receptor antagonist with subnanomolar affinity for the human NK<sub>1</sub> receptor ( $K_i$ : 0.45 nM) and over 200-fold selectivity toward NK<sub>2</sub> and NK<sub>3</sub> receptors. R116301 inhibited substance P (SP)-induced peripheral effects (skin reactions and plasma extravasation in guinea pigs) and a central effect (thumping in gerbils) at low doses (0.08–0.16 mg/kg, s.c. or i.p.), reflecting its high potency as an NK<sub>1</sub> receptor antagonist and excellent brain disposition. Higher doses blocked various emetic stimuli in ferrets, cats, and dogs (ED<sub>50</sub> values: 3.2 mg/kg, s.c.; 0.72–2.5 mg/kg, p.o.). Even higher doses (11–25 mg/kg, s.c.) were required in mice (capsaicin-induced ear edema) and rats (SP-induced ex-

travasation and salivation), consistent with lower affinity for the rodent NK<sub>1</sub> receptor and known species differences in NK<sub>1</sub> receptor interactions. R116301 inhibited the ocular discharge (0.034 mg/kg) but not the dyspnoea, lethality, or cough (>40 mg/kg, s.c.) induced by [ $\beta$ ALA<sup>8</sup>]-neurokinin A (NKA) (4–10) in guinea pigs, attesting to NK<sub>1</sub> over NK<sub>2</sub> selectivity. R116301 did not affect senktide-induced miosis (>5 mg/kg, s.c.) in rabbits, confirming the absence of an interaction with the NK<sub>3</sub> receptor. R116301 was inactive in guinea pigs against skin reactions induced by histamine, platelet-aggregating factor, bradykinin, or *Ascaris* allergens (>10 mg/kg, s.c.). In all species, R116301 showed excellent oral over parenteral activity (ratio, 0.22–2.7) and a relatively long duration (6.5–16 h, p.o.). The data attest to the specificity and sensitivity of the animal models and support a role of NK<sub>1</sub> receptors in various diseases.

Tachykinins belong to a family of short peptides that are widely distributed in the mammalian central and peripheral nervous system (Lundberg, 1995; Maggi, 1995; Bertrand and Geppetti, 1996). They share the common C-terminal sequence Phe-Xaa-Gly-Leu-Met-NH<sub>2</sub>. Tachykinins released

from peripheral sensory nerve endings are believed to be involved in neurogenic inflammation. In the spinal cord/central nervous system, tachykinins may play a role in pain transmission/perception and in some autonomic reflexes and behaviors. The three major tachykinins are substance P (SP), neurokinin (NK) A and NKB with preferential affinity for three distinct receptor subtypes, termed NK<sub>1</sub>, NK<sub>2</sub>, and NK<sub>3</sub>, respectively. However, functional studies on cloned receptors suggest strong functional cross-interaction between the three

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**ABBREVIATIONS:** SP, substance P; NK, neurokinin; NKA, neurokinin A; R116301, (2*R*-*trans*)-4-[1-[3,5-bis(trifluoromethyl)benzoyl]-2-(phenylmethyl)-4-piperidinyl]-*N*-(2,6-dimethylphenyl)-1-acetamide (S)-hydroxybutanedioate; CGP49823, (2*R*,4*S*)-2-benzyl-1-(3,5-dimethylbenzoyl)-4-(quinolin-4-ylmethylamino)piperidine; CP-96345, (2*S*,3*S*)-*cis*-2-(diphenylmethyl)-*N*-((2-methoxyphenyl)-methyl)-1-azabicyclo(2.2.2)-octan-3-amine; CP-99994, (2*S*,3*S*)-3-(2-methoxybenzylamino)-2-phenylpiperidine; GR-203040, (2*S*,3*S*)-2-methoxy-5-tetrazol-1-ylbenzyl(2-phenylpiperidin-3-yl)amine; MK-869 or L-754030, aprepitant; L-760,735, 2-(*R*)-(1-(*R*)-3,5-bis(trifluoromethyl)phenyl)ethoxy)-4-(5-(dimethylaminomethyl)-1,2,3-triazol-4-yl)methyl-3-(5-phenyl)morpholine; RP-67580, (3*aR*,7*aR*)-7,7-diphenyl-2-[1-imino-2-(2-methoxyphenyl)ethyl]perhydroisoindol-4-one; SDZ-NKT-343, 2-nitrophenylcarbamoyl-(S)-prolyl-(S)-3-(2-naphthyl)alanyl-*N*-benzyl-*N*-methylamide; SR-140333, nolpitantium; SR-48968, saredutant; SR-142801, osanetant; Y-24180, (±)-4-(2-chlorophenyl)-2-[2-(4-isobutylphenyl)ethyl]-6,9-dimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine; LY255582, (3*R*,4*R*)-3,4-dimethyl-1-[(3*S*)-3-hydroxy-3-cyclohexyl-propyl]-4-(3-hydroxyphenyl)piperidine; PAF, platelet-aggregating factor; 5-HT, serotonin; MDL-103392, 4-piperidinecarboxamide, 1-[2-[3-(3,4-dichlorophenyl)-1-(3,4,5-trimethoxybenzoyl)-3-pyrrolidinyl]ethyl]-4-phenyl; MDL-105212, (3*R*)-MDL-103392.

tachykinins and their corresponding receptors (Maggi and Schwartz, 1997). The tachykinins are involved in emesis, anxiety states (stress-related), smooth muscle contraction, inflammation, and nociception/pain perception, although clinically relevant involvement of SP, particularly for the last items, is questionable (Hill, 2000).

R116301 is a new NK<sub>1</sub> receptor antagonist (Fig. 1). The present study reports on its pharmacological profile in several NK receptor-related models in various species. Species differences in the structure of NK<sub>1</sub> receptors are responsible for species-related potency differences of NK<sub>1</sub> receptor antagonists (Maggi, 1995). The human NK<sub>1</sub> receptor closely resem-

bles the NK<sub>1</sub> receptor of guinea pigs and gerbils but differs markedly from the NK<sub>1</sub> receptor of rodents. Therefore, most tests are performed in guinea pigs and gerbils. Attention is paid to potency, selectivity, specificity, onset and duration of action, and species differences. R116301 is compared with the following available NK<sub>1</sub> receptor antagonists (Fig. 1 for chemical structures): CGP49823 (Vassout et al., 1994), CP-96345 (Snider et al., 1991), CP-99994 (Piedimonte et al., 1993), GR-203040 (Ward et al., 1995), MDL-103392 (racemate of the active enantiomer MDL-105212; Kudlacz et al., 1996), aprepitant (MK-869 or L-754030; Kramer et al., 1998; Rupniak and Kramer, 1999), L-760735 (McAllister et al.,

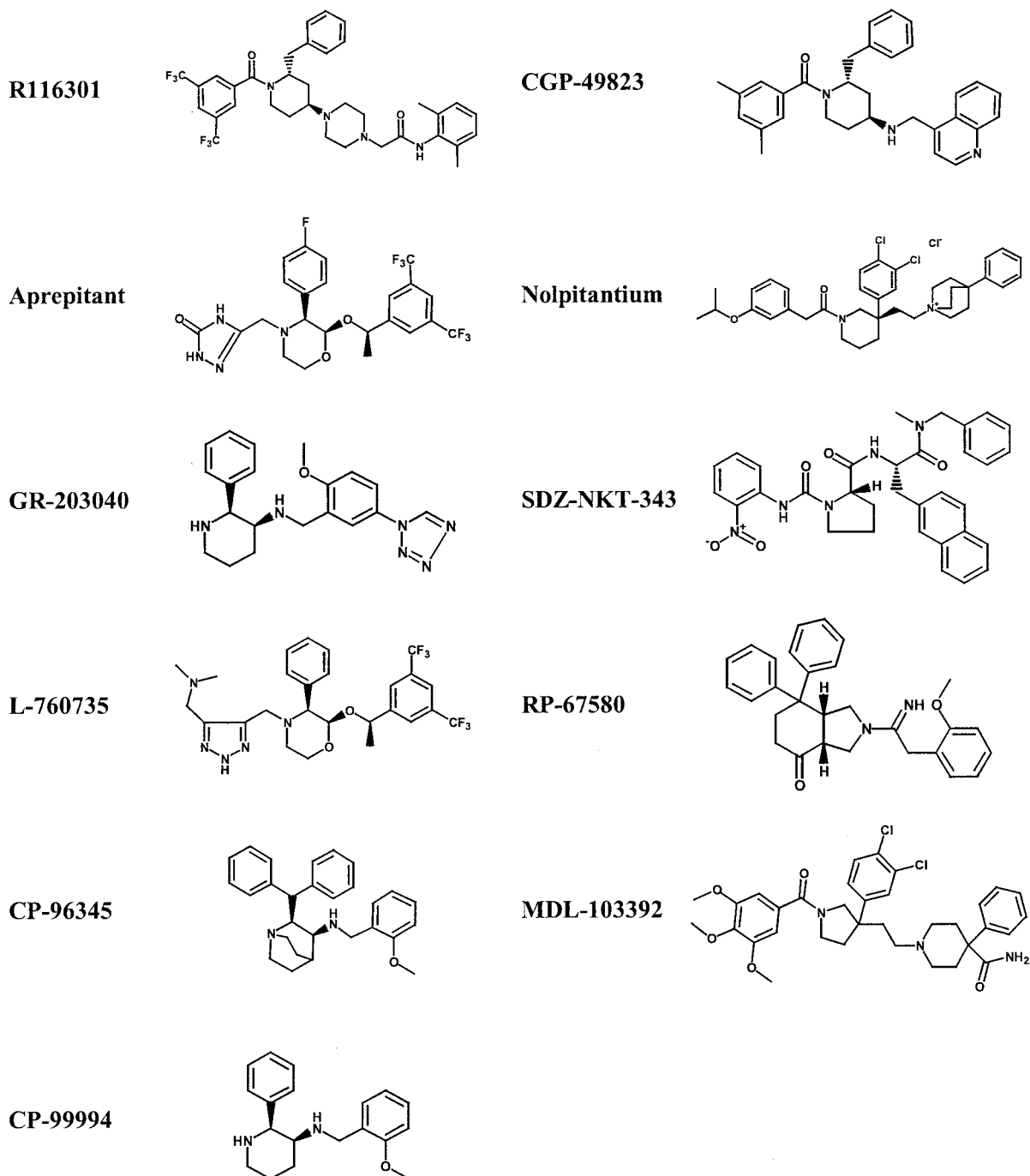


Fig. 1. Chemical structure of R116301 [(S)-hydroxybutanedioate salt] and the reference NK<sub>1</sub> receptor antagonists.

1999), RP-67580 (Garret et al., 1991), SDZ-NKT-343 (Walpole et al., 1998), nelpitantium (SR-140333; Emonds-Alt et al., 1993). The testing of some compounds was restricted by limited availability. The sensitivity, reliability, and specificity of the various test models is evaluated based on the results obtained with the test compounds. A preliminary article on R116301 has been presented in poster format (Jurzak et al., 2000). A patent application has been filed (Janssens et al., 1997).

## Materials and Methods

### Animals

Swiss mice (Janssen Pharmaceutica, Beerse, Belgium), Wistar rats (Janssen Pharmaceutica), Mongolian CRW gerbils (*Meriones unguiculatus*; Charles River Breeding Laboratories, Inc., Sulzfeld, Germany), Dunkin-Hartley-Purbright guinea pigs (Janssen Pharmaceutica or Charles River Breeding Laboratories, Inc.), Fish ferrets (Harlan CPB, Horst, The Netherlands), New Zealand white rabbits (Broekman Institute, Someren, The Netherlands), cats (Broekman Institute), and Beagle dogs (Janssen Pharmaceutica or Harlan CPB) were used. They were fasted overnight (tap water remained available ad libitum) and housed under standard laboratory conditions ( $21 \pm 2^\circ\text{C}$ ;  $65 \pm 15\%$  relative humidity; light/dark cycle set at 12 h). During the test period, they were housed in individual cages. The ferrets, cats, and dogs were used more than once, with an intertrial interval of at least 1 week. All animal studies were approved by the local Ethical Committee in compliance with the Declaration of Helsinki.

### Chemicals and Test Compounds

R116301 was dissolved up to 2.5 mg/ml in 10% hydroxypropyl- $\beta$ -cyclodextrin. Higher doses were prepared as suspensions in 1% polysorbate 80 in distilled water. The preparations were stored at room temperature in closed containers protected from light. They were studied at various time intervals after subcutaneous (10 ml/kg for mice, rats, and guinea pigs; 1.0 ml/kg for rabbits; 0.5 ml/kg for cats and dogs), oral (10 ml/kg for mice, rats, and guinea pigs; 1.0 ml/kg for rabbits; 0.5 ml/kg for cats and dogs), or intravenous administration (10 ml/kg for mice; 2 ml/kg for rats; 0.5 ml/kg for rabbits, cats, and dogs). All doses were expressed in milligram base equivalents per kilogram body weight. With the exception of aprepitant (L-754030 or MK-869; synthesized by and obtained from Johnson & Johnson Pharmaceutical Research Institute and Development, Springhouse, NJ) and L-760735 (synthesized by our Department of Medicinal Chemistry), the reference compounds were kindly provided by the companies of origin: CGP49823 and SDZ-NKT-343 (Novartis, Basel, Switzerland); CP-96345 and CP-99994 (Pfizer, Sandwich, Kent, UK); GR-203040 (GlaxoSmithKline, Uxbridge, Middlesex, UK); MDL-103392 (Aventis, Strasbourg, France); RP-67580 (Aventis); saredutant/SR-48968, nelpitantium/SR-140333, and osanetant/SR-142801 (SANOFI Research Center, Montpellier, France). For all these compounds with exception of SR-140333 and SDZ-NKT-343, some batches were synthesized in our Department of Medicinal Chemistry. The origin of the other chemicals is indicated in parentheses after each name: substance P (Sigma-Aldrich, St. Louis, MO), [ $\beta$ ALA<sup>8</sup>]-neurokinin A (4–10) (NovaBiochem, Laufelfingen, Switzerland), capsaicin (Sigma-Aldrich), PAF (Sigma-Aldrich), bradykinin (Sigma-Aldrich), histamine (Sigma-Aldrich), and Evans blue dye (Sigma-Aldrich; Direct blue 53; dye content approximately 85%). *Ascaris suum* worms were obtained from freshly slaughtered pigs. A batch of about 20 ml of perierteric fluid was centrifuged; the clear supernatant was divided in 0.1-ml portions and stored at  $-18^\circ\text{C}$ . The injected solutions of *Ascaris coeloma* fluid were fresh 1:16 dilutions in 0.9% NaCl.

## Pharmacological Tests

**In Vitro Receptor Binding.** *Membrane preparation from cells.* Chinese hamster ovary cells expressing human (h)NK<sub>1</sub>, NK<sub>2</sub> or NK<sub>3</sub> receptors were grown in Dulbecco's modified Eagle's medium/HAM's F-12 medium containing 10% fetal calf serum and antibiotics. Twenty-four hours after stimulation with 5 mM Na-butyrate to enhance expression levels, the cells were collected from plates using a rubber scraper and suspended in 50 mM Tris-HCl, pH 7.4. They were centrifuged at 23,500g for 10 min in a Sorvall-RC 5B centrifuge (DuPont Instruments, Meyvis, Belgium). The pellets were homogenized in 5 mM Tris-HCl, pH 7.4, using an Ultra-Turrax homogenizer (Janke & Kunkel IKA Labortechnik, Staufen im Breisgau, Germany) and centrifuged at 30,000g for 20 min. The final pellet was suspended in 50 mM Tris-HCl, pH 7.4. The membranes were frozen in 1-ml aliquots  $-70^\circ\text{C}$ . Before use, vials were thawed and rehomogenized in incubation buffer B (50 mM Tris-HCl, pH 7.4, containing 2 mM MgCl<sub>2</sub>, 1 mM EGTA, and 0.1% bovine serum albumin). The protein concentration was determined after using a Bradford kit from Bio-Rad (Hercules, CA).

*Membrane preparation from brain tissue.* To prepare membranes for [<sup>3</sup>H]substance P binding from guinea pigs, gerbils, ferrets, and rats, animals were killed by decapitation, and the forebrains were dissected. The tissue was homogenized in 50 mM Tris-HCl, pH 7.4, using an Ultra-Turrax homogenizer. The homogenates were centrifuged at 23,500g for 10 min at  $0-4^\circ\text{C}$  in a Sorvall-RC 5B centrifuge. The pellets were washed twice in 50 mM Tris-HCl, pH 7.4, by resuspension with a dual-homogenizer (Kimble Kontes, Vineland, NJ) and centrifugation. After the last wash, the pellets were suspended in incubation buffer A [50 mM Tris-HCl, pH 7.4, containing 2 mM MgCl<sub>2</sub>, 1 mM EGTA, 1 mM *o*-phenantrolin, and 0.1% bovine serum albumin at a dilution of 25 volumes/original wet weight of tissue (v/w)]. The final pellet of rat forebrain was suspended in incubation buffer B at a dilution of 40 v/w. Guinea pig, gerbil, and rat forebrain membranes were always freshly prepared. For ferret forebrain, the membranes were prepared in 50 mM Tris-HCl, pH 7.4, and stored at a dilution of 5 v/w at  $-70^\circ\text{C}$ . Before use, these membranes were thawed and further diluted until dilution of 25 v/w in incubation buffer A.

*[<sup>3</sup>H]Substance P binding to NK<sub>1</sub> receptors and data analysis.* The membrane homogenates were incubated for 20 min at  $25^\circ\text{C}$  with 0.5 nM [<sup>3</sup>H]SP (for rat membranes 1 nM was used) in a total volume of 0.5 ml. Specific binding of the radioligand was distinguished from nonspecific binding by addition of unlabeled SP to a final concentration of  $10^{-7}$  M. The incubation was stopped by the addition of 5 ml of ice-cold 50 mM Tris-HCl buffer, pH 7.4, followed by rapid filtration over Whatman GF/B glass fiber filters (Maidstone, Kent, UK) (pre-soaked in 0.1% polyethylenimine for 1 h) using a 40-well filtration unit. The filters were washed twice with ice-cold buffer to remove nonbound radioactivity and placed in plastic miniature vials. After a 24-h incubation with 2 ml of Ultima Gold scintillation cocktail, vials were vigorously shaken, and the radioactivity was counted in a Packard Tri-Carb 1500 CA liquid scintillation analyser (Packard BioScience, Meriden, CT).

R116301 was also investigated in various other in vitro binding assays using membrane preparations of animal tissue or membranes of cell lines transfected with cloned human receptors. Brain, peripheral organs, blood of animal or human origin, or permanent cell lines were used as tissue sources. The procedures for membrane preparations and references to the various receptor binding models were described previously (Briejer et al., 2001).

**Substance P-Induced Plasma Protein Extravasation in Guinea Pigs.** SP (2  $\mu\text{g}/\text{kg}$ , i.v.) and Evans blue dye (30 mg/kg, i.v.) were injected simultaneously (one solution; 4 ml/kg) into the femoral artery of guinea pigs of both sexes (325–425 g) at predefined, logarithmically spaced, time intervals (1, 2, 4, 8, 16 or 32 h) after pretreatment with test compound or solvent. Up to 10 min after challenge, the animals were scored by visual inspection for blue

coloring (0, 1, 2, or 3) of the nose, the forepaws, and the conjunctiva. The criterion for drug-induced inhibition of plasma extravasation: score <2 (occurrence in 0.2, 0.0, and 0.0% for blue coloring of the nose, paws, and conjunctiva, respectively, in saline-treated controls;  $n = 500$ ).

**Capsaicin-Induced Ear Edema in Mice.** Ear edema was induced by local application of capsaicin (250  $\mu\text{g}$ ) on the ear of female mice (20–26 g) pretreated with test compound or solvent (modified after Inoue et al., 1996). The capsaicin was dissolved in acetone at a concentration of 12.5 mg/ml and applied in a volume of 10  $\mu\text{l}$  both on the ventral and dorsal side of the left ear. Pure acetone was applied in a volume of 10  $\mu\text{l}$  both on the ventral and dorsal side of the right ear, which served as a control measure. Immediately after the capsaicin challenge, Evans blue dye (7.5 mg/ml in 0.9% NaCl) was injected intravenously in a volume of 10 ml/kg, corresponding to a dose of 75 mg/kg. Thirty minutes after the capsaicin application, ear edema was scored (0, 1, 2, or 3) as the blue coloring of the left ear. Criterion for drug-induced inhibition: score <2 (never observed in controls;  $n = 62$ ).

**Substance P-Induced Salivation and Plasma Protein Extravasation in Rats.** Plasma protein extravasation and salivation were induced by injection of SP (2  $\mu\text{g}/4$  ml/kg, i.v.) in the tail vein of female rats (200–240 g) that were anesthetized with pentobarbital (40 mg/kg, i.p.; 10 min before the SP challenge) and placed on a heating pad to maintain body temperature [modified after Snider et al. (1991); Robineau et al. (1995)]. Evans blue dye (30 mg/kg, i.v.) was injected simultaneously with SP (one solution; 4 ml/kg). Test compound or solvent was administered at a predefined time interval before SP injection. From 0 through 4 min after the SP challenge, salivation was measured by placing cotton swabs in the rat's mouth at 2-min intervals and quantifying the amount of saliva secreted by the difference in the weight of the two cotton swabs before and after the collection period. Five minutes after challenge, the animals were scored for blue coloring (0, 1, 2, or 3) of the nose and the paws. Thirty minutes after challenge, the animals were sacrificed by  $\text{CO}_2$  asphyxiation and scored (0, 1, 2, or 3) for blue coloring of the trachea and urinary bladder. Criteria for drug-induced inhibition: <100 mg of saliva for inhibition of salivation (2.0% in controls;  $n > 100$ ); score <2 for inhibition of plasma extravasation (0, 0, 1.0, and 1.0 for blue coloring of the nose, paws, trachea, and urinary bladder, respectively, in controls).

**Skin Reaction Test in Guinea Pigs: SP, Histamine, PAF, Bradykinin, and Ascaris Allergens.** Five dorsal skin sites of guinea pigs of both sexes (300–500 g) were injected intradermally (0.05 ml) with saline containing SP (0.05  $\mu\text{g}$ ), histamine (0.25  $\mu\text{g}$ ), PAF (0.00625  $\mu\text{g}$ ), bradykinin (0.1  $\mu\text{g}$ ), and *Ascaris* allergens (1/16 diluted with saline). Immediately thereafter, the animals were challenged i.v. with Evans blue dye (30 mg/kg; 7.5 mg/ml, 4 ml/kg) and sacrificed 30 min later. The intensity of the blue-colored skin reactions was scored by two independent observers in comparison with standard reactions. The scoring system was maximal (4), pronounced (3), moderate (2), slight (1), and no difference (0) with surrounding skin. The scores of the two observers were summed for further evaluation. Test compound or solvent was administered at a predefined interval before induction of the skin reactions. Criteria for drug-induced effects were: 1) SP reactions: score <7 for inhibition, score <5 for pronounced inhibition, and score <3 for blockade (occurring in 3.6, 0.0, and 0.0%, respectively, of solvent-treated control guinea pigs;  $n = 57$ ); 2) histamine reactions: score <7 for inhibition and score <3 for blockade (occurring in 3.6% and 0.0%, respectively, of the controls); 3) PAF reactions: score <7 for inhibition and score <3 for blockade (both occurring in 0.0% of the controls); and 4) bradykinin reactions: score <7 for inhibition and score <3 for blockade (both occurring in 0.0% of the controls); and 5) *Ascaris* allergens reactions: score <3 for blockade (occurring in 5.4% of the controls).

**SP-Induced Thumping in Gerbils.** Male gerbils (50–70 g) were prepared for intracerebral injection under anesthesia (exposure to 4% isoflurane in 70%  $\text{N}_2\text{O}$  + 30%  $\text{O}_2$  during 1 min) by making an

incision in the skin above the cranium (modified after Bristow and Young, 1994 and Rupniak et al., 1997). SP (400 ng in 2  $\mu\text{l}$ ) was rapidly injected into the cerebral ventricles after advancing a Hamilton needle 3.5 mm below a point 2 mm anterior to bregma and 1 mm lateral to the midline; the needle was removed 10 s later. The animals were observed for thumping behavior during the following 5 min. Thumping was defined as a sharp downward movement of the hind-paws producing a characteristic rhythmic tapping sound. The onset of the SP-induced thumping, the total number of thumps, and the total thumping time (seconds) were registered. The animals were then immediately euthanized with  $\text{CO}_2$ . For the present purpose, absence of thumps during the 5-min observation period was adopted as an all-or-nothing criterion for inhibition of the SP-induced thumping (1.2% false positive controls;  $n = 625$ ).

**[ $\beta\text{ALA}^8$ ]-NKA (4–10)-Induced Lethality in Guinea Pigs.** The following phenomena were scored or noted after injection of [ $\beta\text{ALA}^8$ ]-neurokinin A (4–10) (50  $\mu\text{g}/4$  ml/kg; i.v.) into the vena saphena of female guinea pigs (300–500 g) pretreated with test compound or solvent: cough (intensity scores: 0, 1, or 2), white ocular discharge (scores: 0, 1, or 2), dyspnoea (score 1 = cyanosis, score 2 = dyspnoea, score 3 = loss of righting reflex and/or clonic convulsions), and lethality (the survival time up to 60 min after [ $\beta\text{ALA}^8$ ]-NKA (4–10) challenge is noted) (modified after Robineau et al., 1995). [ $\beta\text{ALA}^8$ ]-neurokinin A (4–10) is a metabolism-resistant analog of neurokinin-A with a high degree of selectivity for the  $\text{NK}_2$  receptor (Rovero et al., 1989). The various phenomena generally occurred within 3 to 5 min after the [ $\beta\text{ALA}^8$ ]-NKA (4–10) challenge. The following all-or-nothing criteria were adopted for the determination of the  $\text{ED}_{50}$  values of the test compounds: score = 0 for antagonism of cough (occurrence: 0.0% false positives in the control population;  $n = 96$ ), score = 0 for antagonism of ocular discharge (2.1% false positives), score <2 for protection from dyspnoea (1.0% false positives), and survival time >60 min for protection from lethality (5.2% false positives).

**Emesis in Ferrets, Cats, and Dogs.** Vomiting or retching was induced by the peripherally selective opioid loperamide (0.31 mg/kg, s.c.), by ipecac syrup (1 ml/kg, p.o.; from a commercial source and containing ether-soluble alkaloids of ipecac in a concentration of 1.4 mg/ml), by the dopamine agonist apomorphine (0.31 mg/kg, s.c.), or by the  $\alpha_2$ -adrenoceptor agonist xylazine (1.25 mg/kg, s.c.) in male ferrets (1–3 kg), cats (3–7 kg), or dogs (5–20 kg) pretreated with test compound or solvent. The number of retches or vomits was counted over a 1 h-period or (in the case of ipecac syrup-induced emesis in cats) a 3 h-period starting immediately after the emetic challenge.

**Loperamide-induced retching in ferrets.** In control animals pretreated with solvent, loperamide (0.31 mg/kg, s.c.) induced pronounced retching (mean  $\pm$  S.D.: 95  $\pm$  39 counts;  $n = 529$ ) and, to a lesser extent, vomiting (5  $\pm$  4). All-or-nothing criteria for drug-induced effects on retching: <20 retches for inhibition (2.0% false positives) and 0 retches for blockade (0% false positives).

**Ipecac syrup-induced retching in ferrets.** Oral administration of ipecac syrup induced pronounced retching (mean  $\pm$  S.D.: 76  $\pm$  36 counts) and, to a lesser extent, vomiting (mean  $\pm$  S.E.: 5.6  $\pm$  2.3 counts;  $n = 98$ ) in control animals. Absence of retching or vomiting never occurred in these control animals and was used as criterion for antiemetic activity.

**Apomorphine-induced retching in ferrets.** Injection of apomorphine resulted in pronounced retching in control ferrets (mean  $\pm$  S.D.: 63  $\pm$  29 counts;  $n = 92$ ) and, to a lesser extent, vomiting (mean  $\pm$  S.D.: 3.1  $\pm$  2.0 counts). Absence of retching never occurred in these control animals and was used as criterion for antiemetic activity. Vomiting was absent in 13% of the control animals.

**Apomorphine-induced vomiting in dogs.** Apomorphine induced consistent vomiting in all control dogs (mean  $\pm$  S.D.: 10  $\pm$  5 vomits;  $n = 195$ ). Criteria for drug-induced protection: inhibition of emesis: <2 emetic bouts (occurring in 0.12% of controls); blockade of emesis: complete absence of emesis (not observed in controls).

**Xylazine-induced retching in cats.** Control cats ( $n = 113$ ) retched

(mean  $\pm$  S.D.:  $41 \pm 18$  counts) and vomited (mean  $\pm$  S.D.:  $2.9 \pm 1.8$  counts) after injection of xylazine. Absence of retching or vomiting never occurred and was used as criterion for antiemetic activity.

**Ipecac syrup-induced vomiting in cats.** Oral administration of ipecac syrup induced vomiting and retching during a 3-h observation period in control cats pretreated with distilled water ( $n = 30$ ). For practical reasons, only the number of emetic bouts was counted, which remained relatively low (mean  $\pm$  S.E.:  $4.5 \pm 0.6$  counts). Absence of vomiting never occurred and was used as the criterion for antiemetic activity.

**Senktide-Induced Miosis in Rabbits.** Senktide (12.5  $\mu$ g/0.5 ml/kg, i.v.)-induced miosis was evaluated just before and 15 min after challenge in male New Zealand white rabbits (approximately 1 kg) pretreated with test compound or solvent (1.0 ml/kg) (modified after Medhurst et al., 1997). The senktide was injected into an ear vein. The left pupil of each rabbit was measured under normal ambient lighting with a comparing reticule (scaled to 0.5 mm). In solvent-pretreated control rabbits ( $n = 73$ ), pupil diameter declined from  $6.0 \pm 0.5$  mm immediately before to  $2.7 \pm 0.7$  mm 15 min after the senktide challenge. At this 15-min time interval after senktide injection, only two of the control rabbits (2.7%) showed a pupil diameter  $\geq 5.0$  mm, which was adopted as all-or-nothing criterion for significant inhibition of the senktide-induced miosis by test compounds.

## General Procedure and Statistics

**In Vitro Binding Studies.** Sigmoidal inhibition curves were calculated by computerized nonlinear regression analyses according to Oestreicher and Pinto.  $pIC_{50}$  values ( $-\log$  concentration producing 50% inhibition of specific [ $^3$ H]SP binding) were derived. Inhibition equilibrium constants ( $K_i$  values) were calculated according to  $K_i$  (nanomolar) =  $IC_{50}/(1 + C/K_d)$ , where  $C$  is the concentration and  $K_d$  the equilibrium dissociation constant of the [ $^3$ H]SP.

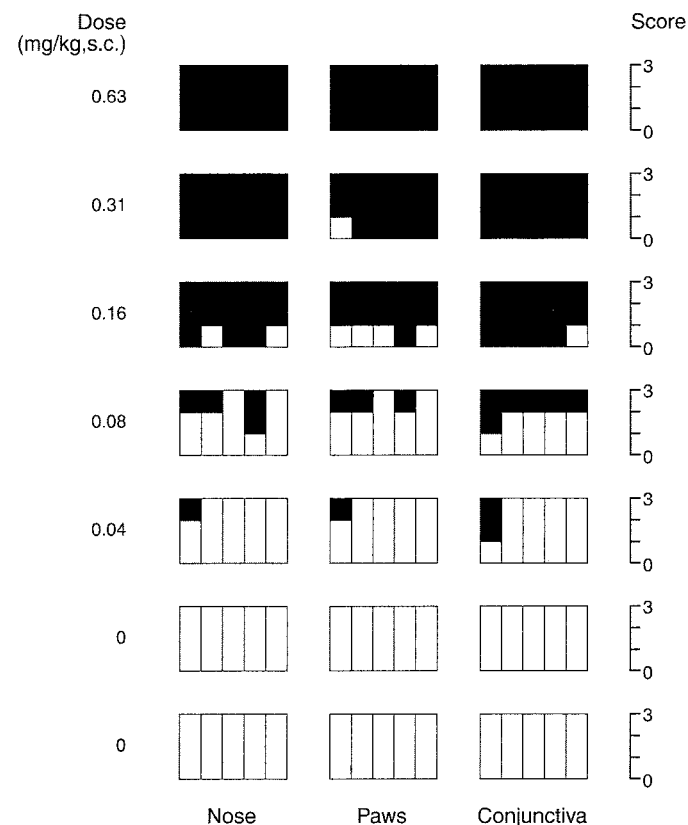
**In Vivo Functional Studies.** All experiments were performed by unbiased trained technicians using coded solutions. Doses were selected from the geometrical series 0.00063–0.00125–0.0025. . . 40.0–80.0–160 mg/kg in such a way that at least three doses covered the 0 to 100% effect range of the dose-response curve. Each dose group consisted of five animals, which were tested in separate daily experimental sessions (including solvent-treated control animals) to account for day-to-day variability and to minimize systematic errors. Control injections of solvent were included in each experimental session. Based on an analysis of a frequency distribution of a large series of the historical control data, all-or-nothing criteria for stimulation or inhibition were defined as a change of the measured or scored variable to values that never or almost never (in less than 5%) occurred in the control population. With the help of the thus defined all-or-nothing criteria, graded data were transformed to categorical data which generated per dose level the number of animals in which the intensity of a particular phenomenon was higher (stimulation) or lower (inhibition or blockade) than in the control animals. On the basis of the thus obtained dose-response relations,  $ED_{50}$  values and corresponding 95% confidence limits were calculated by probit analysis according to the method of Finney (1962) for categorical data.

## Results

### In Vitro Receptor Binding Profile

R116301 showed subnanomolar affinity for the human  $NK_1$  receptor ( $pIC_{50} \pm$  S.D.:  $8.53 \pm 0.06$ ;  $K_i$ : 0.45 nM;  $n = 4$  replicates), with 230- and 1600-fold selectivity regarding binding to the human  $NK_3$  ( $pIC_{50} \pm$  S.D.:  $6.22 \pm 0.14$ ;  $K_i$ : 104 nM;  $n = 3$ ) and  $NK_2$  ( $pIC_{50} \pm$  S.D.:  $5.87 \pm 0.36$ ;  $K_i$ : 711 nM;  $n = 4$ ) receptors, respectively. R116301 showed very low affinity for human serotonin 5-HT $_{2B}$  ( $pIC_{50} \pm$  S.D.:  $5.68 \pm 0.15$ ;  $K_i$ : 928 nM;  $n = 2$ ) and 5-ht $_{5a}$  ( $pIC_{50} \pm$  S.D.:  $5.16 \pm 0.04$ ;

$K_i$ : 3544 nM;  $n = 2$ ) receptors and for rat  $Ca^{2+}$  ligand binding sites ( $pIC_{50} \pm$  S.D.:  $5.45 \pm 0.15$ ;  $K_i$ : 2454 nM;  $n = 3$ ) and  $Na^+$  channels ( $pIC_{50} \pm$  S.D.:  $5.60 \pm 0.17$ ;  $K_i$ : 2526 nM;  $n = 3$ ). Up to 10  $\mu$ M, the compound had no affinity for a wide range of other binding sites, including adrenergic receptors (human  $\alpha_{1A}$ ,  $\alpha_{2A}$ ,  $\alpha_{2B}$ ,  $\alpha_{2C}$ ,  $\beta_1$ ,  $\beta_2$ ,  $\beta_3$ ), dopaminergic (rat  $D_1$ ; human  $D_{2L}$ ,  $D_3$ , and  $D_4$ ), serotonergic (human 5-HT $_{1A}$ , 5-HT $_{1B}$ , 5-HT $_{1D}$ , 5-ht $_{1e}$ , 5-ht $_{1f}$ , 5-HT $_{2A}$ , 5-HT $_{2C}$ , 5-HT $_{3}$ , 5-HT $_{4b}$ , and 5-HT $_{7}$ ), human histamine  $H_1$ , rat cholinergic muscarinic, transporters (rat DA, NE; human 5-HT, GlyT $_1$ , and GlyT $_2$ ), aspartate (rat NMDA-MK801, NMDA-glycine, and AMPA), opioid (human  $\mu$ ,  $\delta$ ; guinea pig  $\kappa$ ), human haloperidol-sensitive  $\sigma_1$ , peptide receptors (rat CCK $_A$ ; human CCK $_B$ , bradykinin-B $_2$ , VIP). Since tachykinin receptors are known to exhibit strong species dependence in pharmacology, the  $NK_1$  affinity of R116301 was investigated on tissue membrane preparations from forebrains of guinea pigs, gerbils, ferrets, and rats. Relative to its affinity for the human  $NK_1$  receptor, R116301 showed about 10 times lower affinity for the gerbil, ferret, and guinea pig  $NK_1$  receptors ( $K_i$ : 6.4, 8.3, and 13 nM, respectively) and 200 times lower affinity for the rat  $NK_1$  receptor ( $K_i$ : 98 nM). These results show a high affinity, selectivity, and specificity of R116301 for the human  $NK_1$  receptor and a species preference as generally observed for other  $NK_1$  receptor antagonists as well.



**Fig. 2.** Individual scores (ranging from 0 to 3) for SP-induced blue coloring of the nose, forepaws, and conjunctiva of guinea pigs 1 h after s.c. pretreatment with saline ( $n = 10$ ) or various doses of R116301 ( $n = 5$ /dose group). The individual scores are represented by small, white, vertical bars (5/dose group) on a black background. In this way, the degree of inhibition can be readily estimated from the visible area of black background.

TABLE 1

ED<sub>50</sub> values (95% CL; milligrams per kilogram) of R116301 and reference NK<sub>1</sub> receptor antagonists for inhibition of SP-induced extravasation in guinea-pigs

Compound	Route	Time	ED <sub>50</sub> (95% Confidence Limits)		
			Nose	Paws	Conjunctiva
		<i>h</i>		<i>mg/kg</i>	
R116301	s.c.	1	0.097 (0.072–0.13)	0.11 (0.091–0.14)	0.085 (0.057–0.13)
	p.o.	1	0.26 (0.17–0.38)	0.26 (0.17–0.38)	0.29 (0.22–0.40)
		16	0.68 (0.42–1.1)	0.68 (0.42–1.1)	1.5 (1.0–2.3)
	i.v.	5 min	0.11 (0.075–0.17)	0.074 (0.055–0.10)	0.042 (0.031–0.058)
GR-203040	s.c.	1	0.0031 (0.0023–0.0042)	0.0041 (0.0030–0.0055)	0.0024 (0.0017–0.0032)
	p.o.	1	0.59 (0.43–0.79)	0.67 (0.45–1.0)	0.59 (0.43–0.79)
Nolpitantium	s.c.	1	0.050 (0.023–0.0042) <sup>a</sup>	0.050 (0.023–0.11) <sup>a</sup>	0.050 (0.023–0.11) <sup>a</sup>
	s.c.	4	>0.16	>0.16	>0.16
	p.o.	1	>2.5	>2.5	>2.5
Aprepitant	s.c.	1	0.028 (0.023–0.035)	0.032 (0.024–0.044)	0.028 (0.023–0.035)
	p.o.	1	0.085 (0.063–0.11)	0.13 (0.086–0.19)	0.064 (0.048–0.087)
L-760735	s.c.	1	0.056 (0.046–0.070)	0.065 (0.048–0.088)	0.065 (0.048–0.088)
CP-99994	s.c.	1	0.17 (0.12–0.23)	0.17 (0.12–0.23)	0.17 (0.12–0.23)
	p.o.	1	21 (16–29)	25 (18–33)	25 (18–33)
CGP-49823	s.c.	1	0.31 (---) <sup>a</sup>	0.31 (---) <sup>a</sup>	0.31 (---) <sup>a</sup>
CP-96345	s.c.	1	0.22 (0.18–0.28)	0.19 (0.14–0.26)	0.59 (0.43–0.80)
	p.o.	1	2.7 (1.7–4.3)	2.3 (1.4–3.8)	3.6 (2.2–5.7)
	p.o.	4	>10	>10	>10
	p.o.	16	>40	>40	>40
SDZ-NKT-343	i.p.	1	2.5 (---) <sup>a</sup>	2.5 (---) <sup>a</sup>	2.5 (---) <sup>a</sup>
	p.o.	1	>10	>10	>10
MDL-103392	s.c.	1	5.4 (3.6–8.0)	>10	8.1 (5.4–12)
RP-67580	s.c.	1	10 (---) <sup>a</sup>	10 (---) <sup>a</sup>	≥10

<sup>a</sup> ED<sub>50</sub> estimated with only two to three instead of five animals per dose level.

### Inhibition of SP-Induced Plasma Extravasation in Guinea Pigs

Figure 2 shows individual scores (ranging from 0 to 3) for the SP-induced plasma extravasation in the nose, forepaws, and conjunctiva of guinea pigs 1 h after s.c. pretreatment with saline ( $n = 10$ ) or various doses of R116301 ( $n = 5$ /dose group). R116301 reduced the scores dose dependently for blue coloring in the three organs. The ED<sub>50</sub> values for reducing the scores below 2 were 0.097, 0.11, and 0.085 mg/kg for the nose, forepaws, and conjunctiva, respectively (Table 1).

Table 1 compares R116301 with other NK<sub>1</sub> receptor antagonists at the indicated time interval after s.c., p.o., or i.v. administration. Regarding inhibition of extravasation in the nose after s.c. injection, R116301 (0.097 mg/kg) was 31 times less potent than GR-203040 (0.0031 mg/kg) but about equipotent with aprepitant (0.028 mg/kg), nolpitantium (0.050 mg/kg), CP-99994 (0.17 mg/kg), and CP-96345 (0.22 mg/kg). The p.o. over s.c. ED<sub>50</sub> ratio was much better for R116301 (2.7) and aprepitant (3.0) than for CP-96345 (12), CP-99994 (124), GR-203040 (190), and the quaternary compound nolpitantium (>50).

At 4 times the peak-effect dose (graphical estimated: 0.27 mg/kg), R116301 showed a rapid onset (<1 h) and a relatively long duration of action (16 h; Fig. 3). In contrast, activity had already appreciably declined 4 h after s.c. injection of nolpitantium or p.o. administration of CP-96345 (Table 1). The rat-selective NK<sub>1</sub> receptor antagonist RP-67580 hardly inhibited extravasation in guinea pigs at the dose of 10 mg/kg, s.c. After an s.c. dose of 40 mg/kg, the NK<sub>2</sub> receptor antagonist saredutant (40 mg/kg, s.c.), the NK<sub>3</sub> receptor antagonist osanetant (>40 mg/kg, s.c.), and the histamine H<sub>1</sub> receptor antagonists oxatamide and levocabastine did not at all affect the SP-induced plasma extravasation at doses far above the dose required for their primary activity.

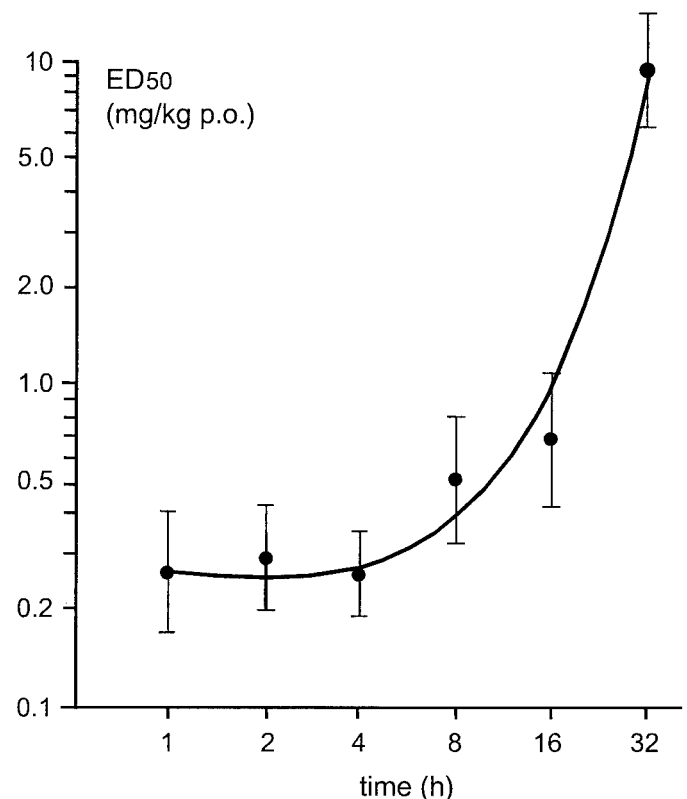
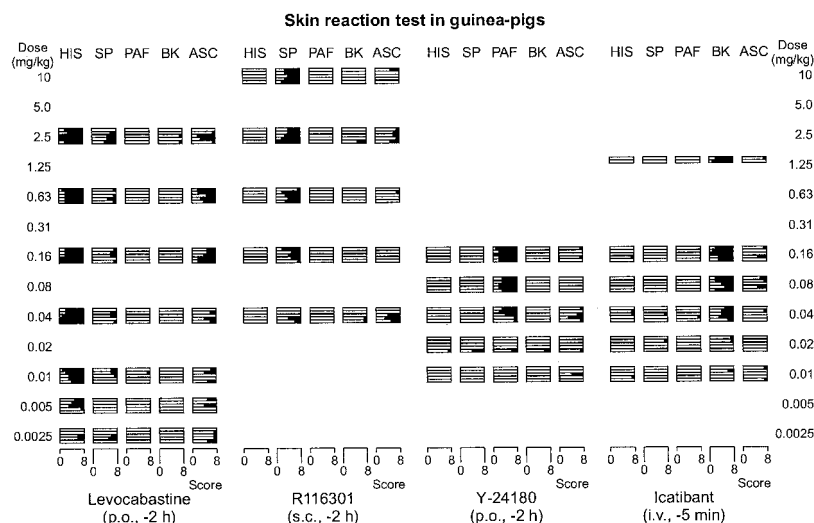


Fig. 3. ED<sub>50</sub> (milligrams per kilogram) of R116301 for inhibition of SP-induced extravasation in the nose plotted versus time interval after oral administration. Vertical bars represent the 95% confidence limits to the ED<sub>50</sub> values.

**Skin Reaction Test in Guinea Pigs: SP, Histamine, PAF, and Bradykinin.** R116301 (s.c., –2 h) was compared with the histamine H<sub>1</sub> receptor antagonist levocabastine



**Fig. 4.** Individual scores (ranging from 0 to 8;  $n = 5$ /dose group) for skin reactions induced by histamine, SP, PAF, bradykinin, and *Ascaris* allergens obtained in guinea pigs after pretreatment with the  $H_1$  receptor antagonist levocabastine (p.o., -2 h), the  $NK_1$  receptor antagonist R116301 (s.c., -2 h), the PAF antagonist Y-24180 (p.o., -2 h), or the  $BK_2$  antagonist icatibant (i.v., -5 min). The individual scores are represented by small, white, horizontal bars (5 per dose group) on a black background. In this way, the degree of inhibition can be readily estimated from the visible area of black background.

(p.o., -2 h), the PAF antagonist Y-24180 (p.o., -2 h), and the  $BK_2$  antagonist icatibant (i.v., -5 min) for its ability to antagonize histamine-, SP-, bradykinin-, PAF-, and *Ascaris* allergen-induced skin reactions in guinea pigs. Individual scores (ranging from 0 through 8) for the skin reactions are represented by horizontal bars ( $n = 5$ /dose group) in Fig. 4.  $ED_{50}$  (and 95% confidence limits) of the test compounds for various levels of inhibition are mentioned below.

R116301 was able to antagonize the SP-induced skin reactions from a median score of 8 in solvent-pretreated control animals to a score  $<7$  [ $ED_{50}$ : 0.080 (0.031–0.021) mg/kg] and, at slightly higher doses, to a score  $<5$  [ $ED_{50}$ : 0.18 (0.070–0.47) mg/kg]. Up to 10 mg/kg, however, R116301 was not able to block the SP reactions completely (score  $<3$ ) or to affect the reactions induced by histamine, PAF, bradykinin, or *Ascaris* allergens.

The histamine  $H_1$  receptor antagonist levocabastine reduced the histamine-induced skin reactions from a median score of 8 in solvent-pretreated control animals to a score  $<7$  [ $ED_{50}$ : 0.0036 (0.0024–0.0053) mg/kg] and, at 3 times that dose, to a score  $<3$  ( $ED_{50}$ : 0.011 (0.0062–0.021) mg/kg). At 40 times the lowest antihistamine dose, levocabastine was also able to inhibit the SP-induced skin reactions to a score  $<7$

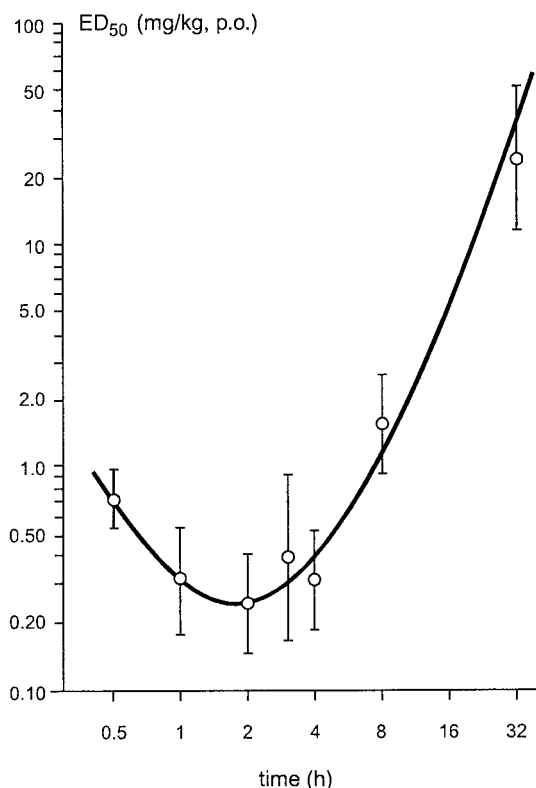
( $ED_{50}$ : 0.14 (0.038–0.50) mg/kg). In contrast to R116301, however, levocabastine (up to 2.5 mg/kg) was not able to reduce scores to below 5. Levocabastine (up to 2.5 mg/kg) did not affect the PAF-, bradykinin-, or *Ascaris* allergen-induced reactions. The PAF antagonist Y-24180 reduced the scores for the PAF reactions from a median value of 8 in solvent-pretreated control animals to a score  $<7$  ( $ED_{50}$ : 0.032 (0.022–0.049) mg/kg) and  $<3$  ( $ED_{50}$ : 0.074 (0.050–0.11) mg/kg) but did not affect the other types of reactions ( $ED_{50}$ :  $>0.16$  mg/kg). The bradykinin antagonist icatibant reduced the scores for the bradykinin reactions from a median value of 8 in the solvent-pretreated control animals to a score  $<7$  ( $ED_{50}$ : 0.028 (0.021–0.038) mg/kg) and  $<3$  ( $ED_{50}$ : 0.098 (0.066–0.15) mg/kg) but did not affect the other types of reactions ( $ED_{50}$ :  $>1.25$  mg/kg).

**SP-Induced Thumping in Gerbils.** Table 2 lists  $ED_{50}$  values (95% confidence limits; milligrams per kilogram) of R116301 and some other  $NK_1$  antagonists for inhibition of SP-induced thumping in gerbils. Together with GR-203040 ( $ED_{50}$ : 0.16 mg/kg, i.p.) and aprepitant (0.12 mg/kg, i.p.), R116301 was the most potent antagonist of the SP-induced thumping (0.16 mg/kg, i.p.). Moreover, R116301 was only 2 times less potent after oral rather than after intraperitoneal

TABLE 2

$ED_{50}$  values (95% confidence limits; milligrams per kilogram) of R116301 and reference  $NK_1$  receptor antagonists for inhibition of SP-induced thumping in gerbils

Compound	Route	Time	$ED_{50}$ (95% Confidence Limits)
			mg/kg
R116301	i.p.	0.5	0.16 (0.076–0.33)
	p.o.	1	0.32 (0.18–0.56)
Aprepitant	i.p.	0.5	0.12 (0.060–0.26)
	p.o.	1	0.20 (0.092–0.44)
GR-203040	i.p.	0.5	0.16 (0.092–0.28)
	p.o.	1	1.3 (0.60–2.6)
L-760735	i.p.	1	1.0 (0.48–2.1)
	p.o.	1	2.5 (1.2–5.3)
CP-96345	i.p.	0.5	5.0 (2.1–12)
	p.o.	1	$\geq 40$
CP-99994	i.p.	0.5	6.3 (3.0–13)
	p.o.	1	$>40$
CGP-49823	i.p.	0.5	13 (7.2–22)
	p.o.	1	$>40$
MDL-103392	i.p.	0.5	$>10$
	p.o.	1	$>40$
Nolpitantium	i.p.	0.5	$>40$



**Fig. 5.** ED<sub>50</sub> values (milligram per kilogram, p.o.) of R116301 for inhibition SP-induced thumping in gerbils plotted as function of time after oral administration. Vertical bars represent the 95% confidence limits to the ED<sub>50</sub> values.

administration (ED<sub>50</sub>: 0.32 and 0.16 mg/kg, respectively). Except for aprepitant (1.7) and L-760735 (2.5), the corresponding ratio was higher for the other NK<sub>1</sub> receptor antagonists: GR-203040 (8.1), CGP-49823 (>3.1), CP-99994 (>6.3), and CP-96345 (≥8). In line with their known selectivity, the NK<sub>2</sub> receptor antagonist saredutant (ED<sub>50</sub>: ≥40 mg/kg, i.p.; >40 mg/kg, p.o.) and the NK<sub>3</sub> receptor antagonist osanetant (ED<sub>50</sub>: >10 mg/kg, s.c.; >10 mg/kg, p.o.) did not affect the SP-induced thumping, confirming the selectivity of the assay. Graphically estimated at 4 times the peak-effect dose (graphical estimate, 0.25 mg/kg at 2 h), R116301 showed a rapid onset (<30 min) and a duration of action of 7.0 h after oral administration (Fig. 5).

**TABLE 3**

ED<sub>50</sub> values (milligrams per kilogram) for inhibition and blockade of loperamide (0.31 mg/kg, s.c.)-induced retching in ferrets at 1 h after subcutaneous or 2 h after oral administration

Compound	Route	Time	ED <sub>50</sub> (95% Confidence Limits)	
			Inhibition (<20 Retches)	Blockade (0 Retches)
		<i>h</i>	<i>mg/kg</i>	
R116301	s.c.	1	1.3 (0.60–2.6)	3.2 (1.5–6.6)
	p.o.	2	1.6 (0.91–2.8)	2.5 (1.4–4.4)
GR-203,040	s.c.	1	0.020 (0.014–0.030)	0.064 (0.037–0.11)
	p.o.	2	0.10 (0.058–0.17)	0.20 (0.12–0.35)
L-760735	s.c.	1	0.24 (- - -) <sup>a</sup>	0.31 (- - -) <sup>a</sup>
Aprepitant	p.o.	2	0.67 (0.45–1.0)	3.1 (1.9–5.0)
CP-96,345	s.c.	1	5.0 (- - -) <sup>a</sup>	>10.0
CP-99,994	s.c.	1	0.25 (0.12–0.53)	0.63 (0.36–1.1)
	p.o.	2	6.3 (3.6–11)	>10.0
SDZ-NKT-343	p.o.	2	>2.5	>2.5

<sup>a</sup> ED<sub>50</sub> estimated based on a limited number of animals tested per dose group.

### Protection Against Loperamide-Induced Retching in Ferrets.

In control animals pretreated with solvent, loperamide (0.31 mg/kg, s.c.; *n* = 529) induced pronounced retching (mean ± S.D.: 95 ± 39 counts) and, to a lesser extent, vomiting (5 ± 4). R116301, GR-203,040, L-760735, aprepitant, CP-96,345, and CP-99,994, but not SDZ-NKT-343, antagonized the loperamide-induced emesis dose dependently. Table 3 lists the ED<sub>50</sub> values (milligrams per kilogram) for inhibition (<20 retches) and blockade (0 retches) of the loperamide-induced retching obtained 1 h after subcutaneous or 2 h after oral administration. Blockade of retching was generally obtained at 2 to 3 times the dose required for inhibition and always accompanied by complete blockade of vomiting. For the studies, R116301 was the only compound antagonizing the retching at comparable doses after both routes of administration. GR-203040 was 5 times and CP-99994 25 times less potent after p.o. than after s.c. dosing. GR-203040 was the most potent compound but induced behavioral side effects, such as muscular hypotonia, ataxia, and tremors. Slight tremors were also observed with aprepitant at the highest dose of 5 mg/kg, p.o. R116301 did not affect overt behavior up to the highest dose tested (10 mg/kg, s.c.; 40 mg/kg, p.o.). As might be expected, the opioid antagonists naloxone [ED<sub>50</sub> (and 95% confidence limits): 0.24 (0.13–0.44) mg/kg], naltrexone [0.016 (0.012–0.022) mg/kg, s.c.], methylnaltrexone [3.8 (2.1–7.0) mg/kg], and LY255582 [0.0054 (0.0033–0.0087) mg/kg] were also able to block loperamide-induced emesis at 1 h after s.c. injection. The peripheral dopamine antagonist domperidone (10 mg/kg, s.c., -1 h), the 5-HT<sub>3</sub> antagonist granisetron (2.5 mg/kg, p.o., -2 h), and the NK<sub>3</sub> receptor antagonist osanetant did not affect loperamide-induced emesis.

**Antiemetic Spectrum.** Table 4 lists the ED<sub>50</sub> values of R116301 for complete blockade of retching or vomiting induced by various emetics in ferrets, cats, and dogs.

**Ferrets.** The effects against loperamide have been discussed in detail above. R116301 also completely blocked ipecac syrup-induced retching (in controls: 76 ± 36 counts; mean ± S.D., *n* = 98) and vomiting (in controls: 5.6 ± 2.3 counts; mean ± S.D.) with an ED<sub>50</sub> of 1.2 (0.85–1.8) mg/kg for both effects. The serotonin 5HT<sub>3</sub> antagonist granisetron (2 h, p.o.) was also able to completely block emesis [ED<sub>50</sub> and 95% confidence limits: 0.065(0.038–0.11) mg/kg]. Neither naloxone (10 mg/kg, s.c., 1 h) nor domperidone (10 mg/kg, s.c., 1 h) affected ipecac syrup emesis in ferrets.

TABLE 4

ED<sub>50</sub> values (milligrams per kilogram) of R116301 for blockade of retching or vomiting induced by various emetic stimuli in ferrets, cats, and dogs at the indicated time interval after oral administration

Species	Emetic Stimulus	Response	ED <sub>50</sub> (95% CL)	Time
			mg/kg	h
Ferret	Loperamide	Retching	2.5 (1.4–4.4)	2
	Ipecac syrup	Retching	1.2 (0.85–1.8)	2
	Apomorphine	Retching	1.2 (0.82–1.9)	2
Dog	Apomorphine	Vomiting	1.8 (1.2–2.9)	2
Cat	Xylazine	Retching	0.72 (0.39–1.3)	1
Cat	Ipecac syrup	Vomiting	1.2 (0.85–1.9)	2

R116301 (p.o., 2 h) completely blocked apomorphine-induced retching (in controls:  $63 \pm 29$  counts; mean  $\pm$  S.D.,  $n = 92$ ) in ferrets with an ED<sub>50</sub> (and 95% confidence limits) of 1.2 (0.82–1.9) mg/kg. The corresponding ED<sub>50</sub> of the peripheral dopamine antagonist domperidone (s.c., 2 h) was 0.035 (0.013–0.091) mg/kg. Blockade of retching was always accompanied by complete blockade of vomiting. The opiate antagonist naloxone (s.c., –1 h) potentiated rather than inhibited apomorphine-induced emesis, as indicated by the mean ( $\pm$ S.E.M.) number of retches obtained at the various doses:  $63 \pm 3$  retches in controls ( $n = 92$ ),  $66 \pm 9$  retches at 0.04 mg/kg ( $n = 6$ ),  $105 \pm 6$  retches at 0.16 mg/kg ( $n = 6$ ),  $133 \pm 16$  retches at 0.63 mg/kg ( $n = 6$ ),  $189 \pm 18$  retches at 2.5 mg/kg ( $n = 6$ ), and  $188 \pm 21$  retches at 10 mg/kg ( $n = 6$ ). The ED<sub>50</sub> (and 95% confidence limits) of naloxone for inducing more than 125 retches (occurrence in only 2% of the control animals) was 0.40 (0.19–0.83) mg/kg. A similar potentiation was also observed to a lesser extent with another, very potent opiate antagonist LY255582 (ED<sub>50</sub> against loperamide-induced retching: 0.0054 mg/kg, s.c.; see above):  $63 \pm 9$  retches at 0.02 mg/kg,  $99 \pm 9$  retches at 0.04 mg/kg ( $n = 6$ ), and  $102 \pm 10$  retches at 0.16 mg/kg ( $n = 6$ ).

**Dogs.** R116301 blocked apomorphine-induced vomiting dose dependently (in controls:  $10 \pm 5$  counts; mean  $\pm$  S.D.,  $n = 195$ ) 2 h after oral administration. The ED<sub>50</sub> for complete blockade was 1.8 (1.2–2.9) mg/kg. The corresponding ED<sub>50</sub> (and 95% confidence limits) of the peripheral dopamine antagonist domperidone obtained 2 h after oral administration was 0.031 (0.021–0.045) mg/kg.

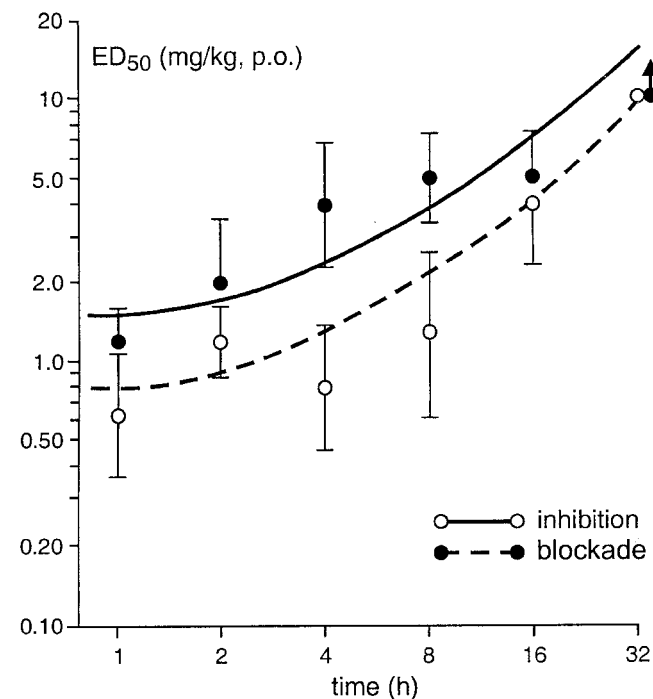
**Cats.** R116301 completely blocked xylazine-induced retching (in controls:  $41 \pm 18$  counts; mean  $\pm$  S.D.,  $n = 113$ ) and vomiting (in controls:  $2.9 \pm 1.8$  counts; mean  $\pm$  S.D.) 1 h after oral administration with ED<sub>50</sub> values of 0.72 (0.39–1.3) mg/kg and 0.55 (0.30–1.0) mg/kg, respectively. R116301 did not affect the xylazine-induced sedation that was observed in all cats or overt behavior up to 10 mg/kg, p.o. In contrast, both the retching and the sedation was antagonized by  $\alpha_2$ -adrenoceptor blockers, such as idazoxan [ED<sub>50</sub>: 0.11 (0.075–0.17) mg/kg and 0.098 (0.073–0.13) mg/kg, respectively], mirazapine [7.1 (4.7–11) mg/kg and 4.1 (3.0–5.5) mg/kg, respectively], and mianserin [9.3 (–) mg/kg and 5.4 (3.6–8.0) mg/kg, respectively].

R116301 antagonized ipecac syrup-induced vomiting (in controls:  $4.5 \pm 0.6$  counts;  $n = 30$ ) in cats. The ED<sub>50</sub> for complete suppression was 1.2 (0.85–1.9) mg/kg. In controls, the vomiting was always preceded by plaintive meowing. This meowing was completely suppressed by R116301, suggesting that the com-

pound not only antagonized emesis but nausea as well. It is also worthwhile to mention that R116301 (up to 10 mg/kg) did not affect the incidence of diarrhea that occurred in all cats within 2 h after the ipecac syrup. R116301 was also devoid of effects on overt behavior (up to 10 mg/kg).

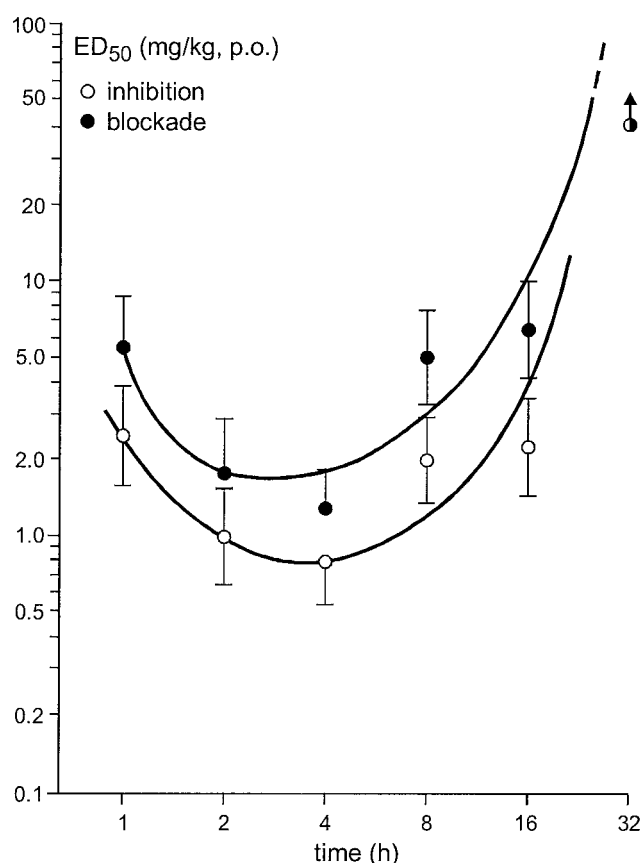
**Time Course of Antiemetic Activity in Ferrets and Dogs.** The ED<sub>50</sub> values for antagonism of loperamide-induced retching in ferrets obtained at several time intervals after oral administration of R116301 have been plotted as a function of time in Fig. 6. R116301 showed its peak effect already within 1 h after gavage (graphically estimated ED<sub>50</sub> for complete suppression of retching: 1.5 mg/kg). At 4 times this peak-effect dose, R116301 showed a (graphically estimated) duration of antiemetic action of approximately 12 h.

Figure 7 shows the ED<sub>50</sub> values (and 95% confidence limits; milligrams per kilogram) obtained for inhibition and blockade of apomorphine-induced emesis in dogs at several time intervals after p.o. administration of R116301. R116301 showed its peak effect already within 3 to 4 h after gavage (graphically estimated ED<sub>50</sub> at time of peak effect: 0.80 and



**Fig. 6.** ED<sub>50</sub> (milligrams per kilogram, p.o.) of R116301 for inhibition (<20 retches) and blockade (0 retches) of loperamide-induced retching in ferrets plotted as function of time after oral administration. Vertical bars represent the 95% confidence limits to the ED<sub>50</sub> values.

<sup>1</sup> No confidence limits. The ED<sub>50</sub> was estimated based on blockade of retching in three of five animals at the highest tested dose of 10 mg/kg.



**Fig. 7.** ED<sub>50</sub> (milligrams per kilogram, p.o.) of R116301 for inhibition (<2 emetic bouts) and blockade (absence of emesis) of apomorphine-induced emesis in dogs plotted as a function of time after oral administration of the NK<sub>1</sub> receptor antagonist. Vertical bars represent the 95% confidence limits to the ED<sub>50</sub> values.

**TABLE 5**

ED<sub>50</sub> values (95% confidence limits; milligrams per kilogram) for inhibition of capsaicin-induced ear oedema in mice obtained at several time intervals after s.c. or p.o. administration of the test compounds

Test compound	Route	Time	ED <sub>50</sub> (95% CL)
			mg/kg
R116301	s.c.	1	19.0 (12–30)
		2	28.0 (18–46)
		4	11.0 (6.7–17)
		8	28.0 (19–42)
		16	32.0 (22–49)
		32	>40.0
		1	21.0 (14–32)
GR-203040	p.o.	1	1.8 (1.2–2.7)
	s.c.	1	3.1 (2.1–4.6)
	p.o.	1	3.1 (2.1–4.6)

1.7 mg/kg for inhibition and blockade, respectively). At 4 times this peak-effect dose, R116301 showed a rapid onset (<1.0 h) and a duration of action of 13 to 15 h (graphically estimated values). Up to the highest dose tested (40 mg/kg, p.o.), R116301 did not affect overt behavior of the dogs.

### Species Differences

**Capsaicin-Induced Ear Edema in Mice.** Both R116301 and GR-203040 inhibited the capsaicin-induced ear edema dose dependently. Table 5 lists the ED<sub>50</sub> values (and 95% confidence limits; milligrams per kilogram) obtained at several time intervals after subcutaneous or oral administration. R116301 was equipotent along the subcutaneous and

oral route of administration (ED<sub>50</sub>: 19 and 21 mg/kg, respectively) and maintained its activity from 1 till 16 h after subcutaneous injection. Compared with GR-203040, R116301 was 10 times less potent after subcutaneous administration and 7 times less potent oral administration.

**SP-Induced Salivation and Plasma Protein Extravasation in Rats.** SP induces a pronounced increase of salivation over the first 4 min after injection from  $9 \pm 11$  mg (mean  $\pm$  S.D.) in solvent-pretreated rats not challenged with SP to  $186 \pm 57$  mg in solvent-pretreated rats challenged with SP (Table 6). Table 6 also illustrates the dose-dependent inhibition of the SP-induced salivation by R116301 and some other NK<sub>1</sub> receptor antagonists. ED<sub>50</sub> values for the effects on the SP-induced salivation and the blue coloring of the nose, paws, trachea, and bladder have been listed in Table 7. It is remarkable that R116301 was about 4 times more potent when given orally than when injected subcutaneously (ED<sub>50</sub> for inhibition of salivation: 3.1 versus 14 mg/kg). Moreover, the SP-induced salivation was somewhat more sensitive than the plasma extravasation to the inhibitory effects of the NK<sub>1</sub> receptor antagonist. Considering the other NK<sub>1</sub> receptor antagonists, the rat-selective NK<sub>1</sub> receptor antagonist nelpitantium (tested on only 2 rats/dose level) was by far the most potent compound in rats (estimated ED<sub>50</sub> values: 0.02–0.08 mg/kg).

### NK<sub>1</sub> over NK<sub>2</sub> and NK<sub>3</sub> Selectivity

**[ $\beta$ ALA<sup>8</sup>]-NKA (4–10)-Induced Lethality in Guinea Pigs.** ED<sub>50</sub> values (and 95% confidence limits) of R116301 and some other NK<sub>1</sub> receptor antagonists for inhibition of the [ $\beta$ ALA<sup>8</sup>]-NKA (4–10)-induced phenomena have been listed in Table 8. GR-203040 and R116301 inhibited the ocular discharge dose dependently (ED<sub>50</sub>: 0.0018 and 0.034 mg/kg, respectively) without consistently affecting lethality, dyspnoea, or cough up to the highest dose tested (0.16 and 40 mg/kg, respectively). GR-203040 was 19 times more potent than R116301. The NK<sub>2</sub> receptor antagonist saredutant was able to protect the guinea pigs against both the [ $\beta$ ALA<sup>8</sup>]-NKA (4–10)-induced lethality (ED<sub>50</sub>: 0.098 mg/kg) and the dyspnoea (ED<sub>50</sub>: 0.58 mg/kg) but did not affect the ocular discharge and cough (ED<sub>50</sub>: >10 mg/kg). On the other hand, the mixed NK<sub>1</sub>/NK<sub>2</sub> receptor antagonist MDL-103392 dose dependently antagonized both the [ $\beta$ ALA<sup>8</sup>]-NKA (4–10)-induced ocular discharge (ED<sub>50</sub>: 0.95 mg/kg), the lethality (ED<sub>50</sub>: 3.8 mg/kg) and the dyspnoea (ED<sub>50</sub>: 26 mg/kg). Codeine dose dependently inhibited the [ $\beta$ ALA<sup>8</sup>]-NKA (4–10)-induced cough (ED<sub>50</sub>: 25 mg/kg) but did not consistently affect the other phenomena within the (limited) dose range tested (up to 40 mg/kg). At the highest dose of 40 mg/kg, two of the five tested animals displayed muscular hyper-tonia, a well known expression of central opioid receptor stimulation.

**Senktide-Induced Miosis in Rabbits.** R116301 (5 mg/kg, s.c.; –1 h) affected neither pupil diameter per se (i.e., before senktide) nor the senktide-induced miosis. On the other hand, the NK<sub>3</sub> receptor antagonist osanetant (Emonds-Alt et al., 1995) antagonized the senktide-induced miosis dose dependently (ED<sub>50</sub>: 0.34 mg/kg, s.c.) without affecting pupil diameter per se (up to 5 mg/kg). The NK<sub>1</sub> receptor antagonists CP-99994 and GR-203040 and the NK<sub>2</sub> receptor antagonist saredutant were inactive 1 h after s.c. injection of a dose of 5 mg/kg, s.c.

TABLE 6

Dose-dependent inhibition of SP-induced salivation in rats obtained 1 h after s.c., p.o., or i.p. administration of the test compounds  
The fraction of animals producing less than 100 mg of saliva is listed in the last column.

Test Compound	SP	Route	Dose	<i>n</i>	Amount of Saliva (Mean ± SD)	<i>n</i> <100 mg/ <i>n</i> tested
			<i>mg/kg</i>		<i>mg</i>	
Control (-SP)	N	— <sup>a</sup>	0	35	9 ± 11	35/35
Control (+SP)	Y	— <sup>a</sup>	0	141	186 ± 57	3/141
R116301	Y	p.o.	1.25	5	158 ± 34	1/5
			2.5	5	129 ± 55	2/5
			5	5	84 ± 14	4/5
			10	5	57 ± 24	5/5
			20	5	34 ± 17	5/5
			40	5	6 ± 2	5/5
R116301	Y	s.c.	2.5	5	150 ± 38	1/5
			5	5	127 ± 42	2/5
			10	5	132 ± 36	1/5
			20	5	102 ± 36	3/5
			40	5	97 ± 35	3/5
RP-67580	Y	s.c.	0.16	2	146 ± 7	0/2
			0.63	5	120 ± 23	1/5
			2.5	5	90 ± 22	3/5
			10	2	74 ± 30	2/2
			40	2	23 ± 9	2/2
CP-96345	Y	s.c.	1.25	5	178 ± 14	0/5
			2.5	5	97 ± 41	2/5
			5	5	66 ± 26	4/5
			10	5	37 ± 18	5/5
			40	2	18 ± 2	2/2
Nolpitantium	Y	i.p.	0.01	2	206 ± 27	0/2
			0.04	2	178 ± 37	0/2
			0.16	2	60 ± 3	2/2
			0.63	2	28 ± 2	2/2
			2.5	2	9 ± 7	2/2
			10	2	5 ± 2	2/2

Y, yes; N, no.

<sup>a</sup> Cumulative data obtained for the three different routes.

TABLE 7

ED<sub>50</sub> values (95% C.L.; milligrams per kilogram) for inhibition of SP-induced salivation and plasma protein extravasation in nose, paws, trachea, and urinary bladder of rats obtained 1 h after s.c., p.o., or i.p. administration of the test compounds

Test compound	Route	Time	ED <sub>50</sub> (95% Confidence Limits)				
			Inhibition of Salivation	Inhibition of Plasma Protein Extravasation in the			
				Nose	Paws	Trachea	Bladder
		<i>h</i>		<i>mg/kg</i>			
R116301	s.c.	1	14 (7.8–26)	>40	25 (15–40)	≥40	>40
R116301	p.o.	1	3.1 (2.1–4.6)	21 (16–29)	12 (8.3–18)	6.2 (3.8–10)	14 (9.5–21)
RP-67580	s.c.	1	1.6 (0.74–3.7)	≥10	0.96 (0.43–2.1)	1.6 (0.74–3.7)	1.6 (0.74–3.7)
CP-96345	s.c.	1	3.1 (2.1–4.6)	8.1 (5.0–13)	3.1 (2.1–4.6)	6.2 (4.6–8.3)	5.4 (4.0–7.3)
Nolpitantium	i.p.	1	(0.08) <sup>a</sup>	(0.08) <sup>a</sup>	(0.08) <sup>a</sup>	(0.04) <sup>a</sup>	(0.02) <sup>a</sup>

<sup>a</sup> Estimated ED<sub>50</sub> values based on only two instead of five rats per dose level.

TABLE 8

ED<sub>50</sub> values (and 95% confidence limits) for antagonism of [BALA<sup>8</sup>]-NKA (4–10)-induced cough, ocular discharge, dyspnoea, and lethality in guinea pigs obtained 1 h after s.c. injection of the test compounds

Test compounds	ED <sub>50</sub> Values (95% confidence limits)			
	Cough	Ocular Discharge	Dyspnoea	Lethality
			<i>mg/kg</i>	
GR-203040	>0.16	0.0018 (0.0012–0.0026)	>0.16	>0.16
R116301	>40.0	0.034 (0.019–0.064)	>40.0	>40.0
Saredutant	>10.0	>10.0	0.58 (0.36–0.94)	0.098 (0.073–0.13)
MDL-103392	>40.0	0.95 (0.42–2.1)	26.0 (14–48)	3.8 (2.1–7.0)
Codeine	25 (16–37)	≥40.0	>40.0	>40.0

## Discussion

R116301 shows high affinity and selectivity for hNK<sub>1</sub> receptors. The approximately 10-fold lower affinity found for guinea pig, gerbil, and ferret NK<sub>1</sub> receptors might be partly due to the very different preparations. The use of

membrane preparations from brain tissue results in higher protein concentrations in the assay, which could give rise to a higher nonspecific binding and, hence, lower free-drug concentrations. Although the *K<sub>i</sub>* values were derived after careful establishment of the assays and saturation analy-

sis, we cannot exclude that the shift in affinity is partly related to higher nonspecific binding.

The considerably lower affinity for rat NK<sub>1</sub> receptors agrees with the species differences generally observed with NK antagonists (see Introduction). R116301 shows a high potency (ED<sub>50</sub> values: 0.08–0.16 mg/kg) against SP-induced effects *in vivo*, both peripheral effects (skin reactions and plasma extravasation in guinea pigs) and a central effect (thumping in gerbils). It is considerably less potent (11–25 mg/kg, *s.c.*) in mice (capsaicin ear edema) and rats (SP-induced extravasation and salivation), confirming the species-differences in NK<sub>1</sub> receptor interaction. R116301 shows excellent oral versus parenteral activity in all tested species and a relatively long duration after oral administration: 16 h in mice (capsaicin ear edema) and guinea pigs (extravasation test), 6.5 h in gerbils (SP thumping), 12 h in ferrets (loperamide emesis), and 13–15 h in dogs (apomorphine emesis).

R116301 inhibited plasma extravasation induced by exogenous SP but also edema induced by endogenous SP after capsaicin challenge. This primary pungent ingredient of red peppers increases vascular permeability by releasing tachykinins from sensory nerves. Therefore, the activity of NK<sub>1</sub> receptor antagonists in this model supports a therapeutic role for these agents in neurogenic inflammation (Inoue et al., 1996). NK<sub>1</sub> receptor antagonists are also active against inflammation induced by cytotoxic drugs (Alfieri and Gardner, 1998) or hypertonic saline (Piedimonte et al., 1993). Being partially reduced by the histamine H<sub>1</sub> receptor antagonist levocabastine and not completely blocked by R116301, the SP-induced skin reactions in guinea pigs seem to be partially mediated via H<sub>1</sub> receptors, most likely due to histamine release after mast cell activation (Fewtrell et al., 1982). The inability of histamine antagonists to affect extravasation after *i.v.* injection of SP shows that histamine release plays a minor role in this system.

The metabolism-resistant NKA analog [βALA<sup>8</sup>]-neurokinin A (4–10) has a high degree of selectivity for the NK<sub>2</sub> receptor (Rovero et al., 1989). Indeed, the [βALA<sup>8</sup>]-NKA (4–10)-induced lethality and dyspnoea were dose dependently inhibited by NK<sub>2</sub> (saredutant and MDL-103392) and not by NK<sub>1</sub> receptor antagonists (R116301 and GR-203040). The [βALA<sup>8</sup>]-NKA (4–10)-induced lethality was about 5 to 7 times more sensitive than the dyspnoea to the inhibitory effects of the NK<sub>2</sub> receptor antagonists. On the other hand, the ocular discharge induced by [βALA<sup>8</sup>]-NKA (4–10) is dose dependently inhibited by NK<sub>1</sub> receptor antagonists (R116301, GR-203040, and MDL-103392) but not by NK<sub>2</sub> receptor antagonists (saredutant and MDL-103392). [βALA<sup>8</sup>]-NKA (4–10) apparently also stimulates NK<sub>1</sub> receptors at the tested dose. In fact, the [βALA<sup>8</sup>]-NKA (4–10)-induced ocular discharge is rather sensitive to NK<sub>1</sub> receptor antagonists, being inhibited by R116301 and GR-203040 at lower doses (0.034 and 0.0018 mg/kg, respectively) than required for inhibition of the SP-induced extravasation in the same species (ED<sub>50</sub>: 0.11 and 0.0041 mg/kg, respectively). Disregarding color, the [βALA<sup>8</sup>]-NKA (4–10)-induced ocular discharge in guinea pigs might be related to NK<sub>1</sub> agonist-induced chromodacryorrhea in gerbils (Bristow and Young, 1994). The NKA test is thus a rapid, reliable, sensitive and noninvasive procedure for evaluating both NK<sub>1</sub> (ocular discharge) and NK<sub>2</sub> (lethality and dyspnoea) antagonism in the same, unanesthetized animal. This is of interest because NK<sub>1</sub> and NK<sub>2</sub> antago-

nism have been postulated as potential therapies in the treatment of asthma, targeting two distinct symptoms of the disease, namely edema and bronchoconstriction, respectively.

[βALA<sup>8</sup>]-NKA (4–10) also induced cough. This cough did not respond to NK<sub>1</sub> receptor antagonists (R116301 and GR-203040), a NK<sub>2</sub> receptor antagonist (saredutant), or a mixed NK<sub>1</sub>/NK<sub>2</sub> receptor antagonist (MDL-103392). This might be surprising since NK<sub>1</sub> receptor antagonists have been found effective against cough induced by SP and other stimuli (Ujiiie et al., 1993; Fahy et al., 1995; Sekizawa et al., 1996). In agreement with its well known antitussive effect (Adcock et al., 1988), the opioid codeine was able to block the [βALA<sup>8</sup>]-NKA (4–10)-induced cough.

The ability of R116301 to inhibit the ocular discharge but not the dyspnoea, lethality, or cough induced by [βALA<sup>8</sup>]-NKA (4–10) in guinea pigs reflects NK<sub>1</sub> over NK<sub>2</sub> selectivity. Furthermore, in contrast to the NK<sub>3</sub> receptor antagonist osanetant (SR-142801; Emonds-Alt et al., 1995), R116301 did not affect senktide-induced miosis in rabbits, suggesting absence of interaction with the NK<sub>3</sub> receptor. Further attesting to its specificity, R116301 did not affect histamine-, PAF-, bradykinin-, or *Ascaris* allergen-induced skin reactions in guinea pigs, ipecac syrup-induced diarrhea in cats, xylazine-induced sedation in cats, or overt behavior in the various species. R116301 is thus a relatively selective and specific NK<sub>1</sub> receptor antagonist.

SP-induced thumping in gerbils is mediated via central NK<sub>1</sub> receptors (Bristow and Young, 1994; Rupniak et al., 1997). The present results thus indicate excellent brain penetration for R116301. The dissociation between inhibition of thumping in gerbils (*i.p.* ED<sub>50</sub>) and inhibition of extravasation in guinea pigs (*s.c.* ED<sub>50</sub>) indicates the following order of central nervous system penetration (ED<sub>50</sub> ratio in parentheses): R116301 (1.6), aprepitant (4.3), L-760735 (18), CP-96345 (23), CP-99994 (37), CGP-49823 (42), GR-203040 (52), nelpitantium (>800). Despite its high potency in the extravasation test, nelpitantium failed to affect SP-induced thumping in gerbils up to 40 mg/kg, its quaternary amine preventing access to the brain.

Several findings indicate involvement of SP in stress-related anxiety states. Central injection of SP induces a cardiovascular response resembling the classical “fight or flight” reaction characterized physiologically by vascular dilatation in skeletal muscles and decrease of mesenteric and renal blood flow. This cardiovascular reaction is accompanied by a behavioral response observed in rodents after noxious stimuli or stress (Culman and Unger, 1995). Stress can reduce SP content and down-regulate NK<sub>1</sub> receptors in the brain, suggesting SP release in stressful situations (Culman and Unger, 1995). In mice, centrally administered NK<sub>1</sub> agonists and antagonists are anxiogenic and anxiolytic, respectively (Teixeira et al., 1996). The NK<sub>1</sub> receptor antagonist aprepitant has been shown to improve depression and anxiety rating scales in depressed patients (Kramer et al., 1998; Rupniak and Kramer, 1999). The ability of NK<sub>1</sub> receptor antagonists to inhibit thumping induced by SP (or by electric shock; Ballard et al., 2001) might correspond to this antidepressant/anxiolytic activity because in gerbils thumping plays a role as an alerting or warning signal to conspecifics.

Central NK<sub>1</sub> receptors are also involved in the regulation of emesis. NK<sub>1</sub> receptor antagonists are active against various emetic stimuli (Watson et al., 1995; Tattersall et al.,

1996). The compounds are supposed to act by blocking central NK<sub>1</sub>-receptors in the nucleus tractus solitarius (Watson et al., 1995; Tattersall et al., 1996). The antiemetic activity of R116301 and the other NK<sub>1</sub> receptor antagonists is thus another index of central NK<sub>1</sub> antagonism. R116301 completely blocks emesis induced by four different stimuli (the opioid loperamide, ipecac syrup, the dopamine agonist apomorphine and the  $\alpha_2$ -adrenoceptor agonist xylazine) in three different species (ferrets, dogs, and cats). The oral dose required for blockade of emesis is very similar across stimuli and species, ranging from 0.72 to 2.5 mg/kg. The dissociation between antiemetic activity (inhibition of loperamide-induced retching in the ferret; s.c.) and inhibition of thumping in gerbils (i.p.) is quite high for R116301 relative to other NK<sub>1</sub> receptor antagonists (ED<sub>50</sub> ratio in parentheses): R116301 (8.1) versus CP-96345 (1.0), CP-99994 (0.25), L-760735 (0.24), and GR-203040 (0.020). To a lesser extent also aprepitant showed a lower potency against loperamide retching in the ferret (ED<sub>50</sub>: 0.67 mg/kg, p.o.) than against SP thumping in the gerbil (0.12 mg/kg, i.p.; 0.20 mg/kg, p.o.). Species differences in interaction with the NK<sub>1</sub> receptor seem not to be responsible since R116301 shows comparable affinity for the gerbil and ferret NK<sub>1</sub> receptors ( $K_i$ : 6.4 and 8.3, respectively). Species differences in brain penetration, metabolic profile, and/or plasma protein binding might also contribute.

Regarding its broad antiemetic profile, R116301 differs from domperidone and naloxone that specifically inhibit apomorphine- and loperamide-induced emesis, respectively. Moreover, domperidone and naloxone antagonize the emetic stimuli by direct competition at the level of dopamine D<sub>2</sub> receptors and  $\mu$ -opioid receptors, respectively, in the chemoreceptor trigger zone, whereas R116301 is considered to act indirectly. Despite their indirect action, central NK<sub>1</sub> receptor antagonists seem to be efficient antiemetics. R116301 not only completely suppressed emesis induced by various stimuli but also ipecac-induced plaintive meowing in cats, suggesting complete relief of nausea. Such plaintive meowing was not observed after xylazine in cats, presumably due to the sedation induced by this  $\alpha_2$ -adrenoceptor agonist.

The present results indicate that R116301 is a centrally acting and orally active NK<sub>1</sub> receptor antagonist and support the potential therapeutic application of NK<sub>1</sub> receptor antagonists in allergy, inflammation, asthma, disturbed excretion processes, emesis, and stress-related anxiety.

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