Pharmacological Properties of FK886, a New, Centrally Active Neurokinin-1 Receptor Antagonist

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Received July 18, 2012; accepted October 24, 2012

The pharmacological properties of the novel neurokinin-1 (NK₁) receptor antagonist FK886, ([3,5-bis(trifluoromethyl)phenyl][2R]-2-(3-hydroxy-4-methylbenzyl)-4-{2-[(2S)-2-(methoxyethyl)morpholin-4-yl]-ethyl}piperazin-1-yl)methanone dihydrochloride, were studied. FK886 potently inhibited the binding of [125I]Bolton-Hunter-labeled substance P ([125I]BH-SP; 100 pM) to human NK₁ receptors expressed in Chinese hamster ovary (CHO) cells (IC₅₀=0.70 nM). It also possessed high affinities for dog, ferret, gerbil and guinea pig NK₁ receptors, but not for rat NK₁ receptor. FK886 was highly selective for the NK₁ receptor, with 250- and >20000-fold selectivity for human NK₁ over NK₂ and NK₃, respectively. Further, it did not inhibit radioligand binding at 54 different sites, including receptors, ion channels and transporters. FK886 inhibited substance P (3.2 nM)-induced inositol phosphate formation in human NK₁ receptor-expressing CHO cells (IC₅₀=1.4 nM) without stimulating NK₁ receptors. The antagonism exerted by FK886 against human NK₁ receptor was insurmountable in saturation binding experiments, with both the affinity and B₅₀ of [125I]BH-SP being significantly reduced. After intravenous administration, FK886 (0.01–0.1 mg/kg) dose-dependently inhibited the foot-tapping behavior induced by intracerebroventricular administration of a selective NK₁ receptor agonist, GR73632 (10 pmol), in gerbils, with significant inhibition being observed at doses of 0.032–0.1 mg/kg, indicating excellent brain penetration. The brain penetration of FK886 was further demonstrated by the cerebral distribution of radioactivity after intravenous injection of radiolabeled FK886. Taken together, these results demonstrate that FK886 is a potent, highly selective and centrally active, insurmountable antagonist of the NK₁ receptor, and suggest that FK886 antagonizes various NK₁ receptor-mediated biological effects in the central nervous system.

Key words FK886; neurokinin-1 antagonist; neurokinin receptor; substance P; tachykinin

Substance P (SP) belongs to a group of related neuropeptides named tachykinins, which include neurokinin A (NKA) and neurokinin B (NKB). These peptides are widely distributed in the central and peripheral nervous systems, where they act as neurotransmitters or neuromodulators. Tachykinins are involved in various pathological functions such as pain, anxiety/depression and emesis.1-3

Previously, Manabe et al. reported a potent non-peptide NK₁ receptor antagonist, FK355, 2-[(3R)-4-[(3,5-bis(trifluoromethyl)benzoyl]-3-((1H-indol-3-ylmethyl)piperazin-1-yl)-N-(4-methylpiperazin-1-yl)acetamide (2F)-but-2-enedioic acid (1:1) (Fig. 1A), which was developed by the chemical modification of a high affinity dipeptide NK₁ receptor antagonist, FK886.3,4 FK355 potently and selectively inhibited SP binding to NK₁ receptors in vitro and potently inhibited SP-induced plasma extravasation in guinea pig in vivo. Because FK355 and its derivatives displayed poor brain penetration, we investigated the development of alternative NK₁ receptor antagonists using FK355 as the template. Here, we report the pharmacological properties of a new compound, FK886, [3,5-bis(trifluoromethyl)phenyl][2R]-2-(3-hydroxy-4-methylbenzyl)-4-{2-[(2S)-2-(methoxyethyl)morpholin-4-yl]-ethyl}piperazin-1-yl)methanone dihydrochloride5 (Fig. 1B), a potent, highly selective and centrally active, insurmountable antagonist of the NK₁ receptor that can antagonize various NK₁ receptor-mediated biological effects in the central nervous system (CNS). We describe the in vitro and in vivo pharmacological properties of FK886 below.

MATERIALS AND METHODS

Materials FK886 and FK355 were synthesized at Chemistry Research Laboratories, Astellas Pharma Inc. [14C]FK886 (1.06 MBq/mg) and [14C]FK355 (1.19 MBq/mg) were synthesized at GE Healthcare U.K. (Buckinghamshire, England) and Sekisui Medical (Tokyo, Japan), respectively. SP and NKA were obtained from Peptide Institute (Osaka, Japan). GR73632 was obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.). [125I]Bolton–Hunter–labeled substance P ([125I]BH-SP; 81.4 TBq/mmol), [125I]NKA (81.4 TBq/mmol), and [125I]MePhe²-NKB ([125I]NKB; 81.4 TBq/mmol) were purchased from PerkinElmer (Boston, MA, U.S.A.), and myo-[2-3H]inositol (666 GBq/mmol) was purchased from GE Healthcare U.K. (Buckinghamshire, England).

FK886 and FK355 were dissolved in dimethylsulfoxide (DMSO) and then diluted into respective assay buffers such
that the final DMSO concentration did not exceed 0.1% in any in vitro experiment. For in vivo studies, FK886, FK355 and GR73632 were dissolved in saline.

**NK₁ Receptor Binding** Human NK₁ receptors were cloned and stably expressed in Chinese hamster ovary (CHO) cells as described previously. All subsequent steps were performed at 4°C. Cells were harvested and homogenized in 25 mM Tris–HCl buffer, pH 7.4, containing 10 mM MgCl₂, 1 mM ethylenediaminetetraacetic acid (EDTA), 5 µg/mL p-amidinophenyl methansulfonyl fluoride (p-APMSF) and 0.25 mM sucrose. The homogenate was centrifuged at 500 × g for 10 min, and the supernatant was further centrifuged at 100000 × g for 60 min. The final pellet was resuspended in 25 mM Tris–HCl buffer, pH 7.4, containing 10 mM MgCl₂, 1 mM EDTA and 5 µg/mL p-APMSF. To prepare forebrain membranes of dog, ferret, gerbil, guinea pig, and rat, tissue samples were homogenized in 1 mM potassium phosphate buffer, pH 7.5, containing 0.1 mM EDTA and 0.32 M sucrose. The homogenate was centrifuged at 10000 × g for 10 min, and the supernatant was centrifuged at 20000 × g for 20 min. The pellet was suspended in purified water and kept on ice for 45 min, followed by re-homogenization and centrifugation at 80000 × g for 20 min. The supernatant was collected and centrifuged at 25000 × g for 20 min. The pellet was suspended with 5 mM Tris–HCl buffer, pH 7.5, and centrifuged at 25000 × g for 20 min. This washing process was repeated twice and the final pellet was suspended with 5 mM Tris–HCl buffer, pH 7.5. Binding of [125I]BH-SP to NK₁ receptors was evaluated by incubating the membrane preparation (6 µg protein for human and 90–150 µg protein for dog, ferret, gerbil, guinea pig and rat) with 100 pM [125I]BH-SP in 250 µL assay buffer (50 mM Tris–HCl buffer, pH 7.4, containing 5 mM MnCl₂, 0.2 mg/mL bovine serum albumin (BSA), 5 µg/mL p-APMSF, 4 µg/mL leupeptin, 40 µg/mL bacitracin and 20 µg/mL chymostatin) for 90 min (human) or 30 min (dog, ferret, gerbil, guinea pig, and rat) at room temperature. Nonspecific binding was determined in the presence of 3 µM non-radioactive SP. The reaction was terminated by rapid filtration through a glass fiber filter pre-washed with 10 ml of water followed by 5 ml of 5 mM sodium formate–60 mM sodium formate to remove free inositol and phosphate. For 1h on ice, the resulting extracts from each well were transferred to glass tubes. The extracts were washed twice with 2 mL water-saturated diethyl ether to remove trichloroacetic acid, mixed with 150 µL 0.2 M Tris-base and applied to anion exchange resin (AGI-X8 formate, Bio-Rad Laboratories, Hercules, CA, U.S.A.) columns. The columns were washed with 10 mL of water followed by 5 mL of 5 mM sodium tetraborate–60 mM sodium formate to remove free inositol and glycerophosphoinositate, respectively. The total inositol phosphate was eluted with 2.5 mL of 0.1 M formic acid–1 M ammonium formate. The radioactivity of the eluate was measured with a liquid scintillation counter. Assays were repeated three times.

**NK₂ and NK₃ Receptor Binding** Cell membranes were prepared from human NK₂ or NK₃ receptor-expressing CHO cells using the same procedure as that for the NK₁ membrane preparation. Binding assays for human NK₂ and NK₃ receptors were performed similarly to the NK₁ binding assay except for the following. For the NK₂ receptor, the membrane preparation (16 µg protein) was incubated with 100 pm [125I]NKA for 30 min and nonspecific binding was determined in the presence of 3 µM non-radioactive NKA. For the NK₃ receptor, the membrane preparation (10 µg protein) was incubated with 100 pm [125I]NKB for 60 min and nonspecific binding was determined in the presence of 3 µM non-radioactive NKB. Glass fiber filters for rapid filtration were pretreated with 0.2% BSA.

**Binding to Receptors, Ion Channels and Transporters Unrelated to Tachykinin** In order to determine the selectivity of FK886, binding assays for 55 binding sites of receptors, ion channels, and transporters were conducted at Daiichi Pure Chemicals (Tokyo, Japan) according to their established proprietary protocols. The respective assay conditions are summarized in Table 3.

**In Vitro Functional Assay** The antagonist activity of FK886 was determined by measuring the FK886 inhibition of SP-induced inositol phosphate formation in human NK₁ receptor-expressing CHO cells. The cells were seeded at 1×10⁵ cells/well in 12-well plates in alpha-modified minimal essential medium supplemented with 10% fetal calf serum and cultured for one day. Cells were cultured for a further 18–24 h in the labeling medium supplemented with 37 KBq/mL myo-[2-³H]inositol. Prior to the assays, cells were washed twice with phosphate-buffered saline (PBS) containing 0.2% BSA and incubated in the same buffer for 30 min at 37°C. The cells were pre-incubated with FK886 (0.32–100 mM) in assay buffer (PBS containing 0.2% BSA and 10 mM LiCl to prevent phosphatidylinositol breakdown) for 30 min at 37°C. Agonist stimulation was started by replacement of assay buffer with fresh assay buffer that included 3.2 mM SP and FK886. After 30 min incubation at 37°C, the assay was terminated by removing the assay buffer and the addition of 1 mL of 5% (w/v) trichloroacetic acid. After 1h on ice, the resulting extracts from each well were transferred to glass tubes. The extracts were washed twice with 2 mL water-saturated diethyl ether to remove trichloroacetic acid, mixed with 150 µL 0.2 M Tris-base and applied to anion exchange resin (AGI-X8 formate, Bio-Rad Laboratories, Hercules, CA, U.S.A.) columns. The columns were washed with 10 mL of water followed by 5 mL of 5 mM sodium tetraborate–60 mM sodium formate to remove free inositol and glycerophosphoinositate, respectively. The total inositol phosphate was eluted with 2.5 mL of 0.1 M formic acid–1 M ammonium formate. The radioactivity of the eluate was measured with a liquid scintillation counter. Assays were repeated three times. In order to pool data across individual experiments, the radioactivity of inositol phosphate in the presence of SP was expressed as the fold increase in radioactivity compared to the
corresponding control (in the absence of SP).

**Animals** Seven-week-old male gerbils (Japan SLC, Shizuoka, Japan) and 7-week-old male Sprague-Dawley rats (CLEA Japan, Tokyo, Japan) were used. The animals were acclimated for one week prior to the experiments. The animals were maintained in a temperature- and humidity-controlled room with a 12:12 h light/dark cycle (lights on 07:00–19:00 h). Food and water were available ad libitum. All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Astellas Pharma Inc.

**GR73632-Induced Foot Tapping in Gerbils** Under light ether anesthesia, the jugular vein was exposed and an incision was made in the midline of the scalp to expose the skull. GR73632 (10 pmol in 5 µL) was injected directly into the lateral ventricle (i.c.v.) via a cuffed microsyringe vertically inserted 4.5 mm below the bregma. FK886 (0.01–0.1 mg/kg), FK355 (0.32–3.2 mg/kg) or vehicle (saline) was administered via the jugular vein (10 mL/kg) 1 min prior to the GR73632 injection. Otherwise, FK355 (1–100 nmol in 5 µL) or vehicle (5 µL of saline) was administered intracerebroventriculally 1 min before GR73632 injection. The duration of repetitive and persistent foot tapping was recorded from immediately 1 min before GR73632 injection. The duration of repetitive and persistent foot tapping was considered discontinued.

**Tissue Distribution of [14C]FK886 and [14C]FK355 in Rats** Rats were deprived of food but not water overnight prior to injection. Injection of [14C]FK886 (3.2 mg/1.19 MBq/mL/kg) or [14C]FK355 (1 mg/1.19 MBq/mL/kg) was made via the tail vein, and blood samples were collected from the aorta under ether anesthesia 5 min after the injection. The cerebrum was removed from each animal, weighed and prepared as 10% (w/v) aqueous homogenate. Aliquots (100 µL) of each plasma and cerebrum homogenate were solubilized in 1 mL Soluene-350 (PerkinElmer, Boston, MA, U.S.A.) and mixed with 10 mL Econofluor (PerkinElmer). The radioactivity of the samples was counted with a liquid scintillation counter. The background was obtained from the samples of non-treated animals. Twice the mean background was assumed to be the lower detection limit.

**Data Analysis** Results are expressed as the mean±S.E.M. unless otherwise noted. IC50 values were calculated by the linear regression of log-logit-transformed concentration response curves over the range of 5–95% maximal effect. Significance was assessed by one-way analysis of variance followed by Dunnett’s multiple comparison test.

**RESULTS**

**Binding Studies** FK886 inhibited [125I]BH-SP binding to recombinant human NK1 receptors in a concentration-dependent manner that yielded an IC50 value of 0.70 ns (Table 1), which is almost identical to the affinity of the values measured for FK886 and FK355. FK886 also possessed high affinity for dog, ferret, gerbil, and guinea pig NK1 receptors, but 30-fold lower affinity for rat NK1 receptor (Table 2).

| Compound | Receptor | IC50 (nm) |
|----------|----------|----------|
| FK886    | NK1      | 0.70     |
|          | NK2      | 180      |
|          | NK3      | >15000   |
| FK888    | NK1      | 3.1      |
| FK355    | NK1      | 1.4      |

Table 1. Affinities of FK Compounds for Human Recombinant NK1, NK2 and NK3 Receptors

Values were obtained from three separate experiments performed in duplicate.

Although FK886 interacted weakly with sodium channel site 2 in guinea pig heart, affinity (IC50=0.87 µM) was more than 1000-fold lower than that measured for human NK1 receptors.

Scatchard analysis of the data for saturation binding of [125I]BH-SP to human NK1 receptors in the absence and presence of increasing concentrations (0.2–6 nm) of FK886 demonstrated that the antagonism exerted by FK886 was insurmountable. FK886 produced a concentration-dependent significant increase in the Kd value (i.e., reduction in the affinity) of the radio-labeled SP, with a concomitant and significant reduction in maximal binding (B_max) (Table 4).

**In Vitro Functional Assay** The functional activity of FK886 at the human NK1 receptor was assessed by its ability to inhibit SP-induced inositol phosphate formation in human NK1 receptor-expressing CHO cells. SP (3.2 nm) provoked a 25-fold increase in intracellular [3H]inositol phosphate in these cells and was inhibited by FK886 in a concentration-dependent manner (Fig. 2). The IC50 value was 1.4 nm. FK886 itself had no stimulatory activity at concentrations up to 10 µM (data not shown).

**GR73632-Induced Foot Tapping in Gerbils** The ability of FK886 to block central NK1 receptor activation was evaluated by measuring FK886 activity toward the foot-tapping behavior induced by the central administration of a potent and selective NK1 agonist, GR73632, in gerbils. To observe the duration of action of the compounds on central NK1 receptors, we set the observation period to 60 min, which is much longer than that of previous studies. As for the dose of GR73632, 10 pmol was chosen to induce long-lasting foot tapping. Direct injection of GR73632 to the brain induced constant foot-tapping behavior, with a 45–50 min tapping time during the 60-min observation period. Intravenously administered FK886 (0.01–0.1 mg/kg) decreased the tapping time dose-dependently, with a significant decrease observed at doses of 0.032 mg/kg or higher (Fig. 3A). Furthermore, foot tapping was nearly
completely inhibited at a dose of 0.1 mg/kg. Although FK355 did not decrease tapping time at doses of 0.32–3.2 mg/kg (Fig. 3B), intracerebroventricularly administered FK355 (1–100 nmol/head) induced a dose-dependent decrease in tapping time (Fig. 3C).

### Table 3. Affinity of FK886 for Various Receptors, Ion Channels and Transporters Unrelated to Tachykinin Receptors

| Binding site | Source | Radio ligand | IC$_{50}$ (µM) |
|--------------|--------|--------------|----------------|
| Adenosine A$_1$ | Rat brain | [${}^3$H]CCPA (7 µM) | >1 |
| Adenosine A$_2$ | Rat striatum | [${}^3$H]CGS-21680 (6 µM) | >1 |
| Adenosine A$_3$ | Rat recombinant | [125I]AB-MECA (1 µM) | >1 |
| α$_2$-Adrenergic (non-selective) | Rat brain | [${}^3$H]Prazosin (0.5 µM) | >1 |
| α$_2$-Adrenergic (non-selective) | Rat cerebral cortex | [${}^3$H]RX821002 (1 µM) | >1 |
| β$_2$-Adrenergic (non-selective) | Rat brain | [${}^3$H]DHA (0.5 µM) | >1 |
| Angiotensin II (type 1) | Human recombinant | [125I]Angiotensin II (0.15 µM) | >1 |
| Angiotensin II (type 2) | Human recombinant | [125I]CGRP 42112A (0.02 µM) | >1 |
| Bradykinin B$_2$ | Human recombinant | [${}^3$H]Bradykinin (0.2 µM) | >1 |
| Ca channel (L, benzothiazepine) | Rat cerebral cortex | [${}^3$H]Diltiazem (2.5 µM) | >1 |
| Ca channel (L, benzothiazepine) | Guinea pig heart | [${}^3$H]Diltiazem (2.5 µM) | >1 |
| Ca channel (N) | Rat cerebral cortex | [125I]Iodo-Coctoxin (0.005 µM) | >1 |
| Ca channel (N) | Guinea pig heart | [125I]Iodo-Conotoxin (0.005 µM) | >1 |
| Cholecystokinin A | Human recombinant | [125I]Ethyl-1 (0.5 µM) | >1 |
| Dopamine D$_1$ | Rat striatum | [${}^3$H]SCH-23390 (0.5 µM) | >1 |
| Dopamine D$_2$ | Rat striatum | [${}^3$H]Speromine (2 µM) | >1 |
| Dopamine transporter | Human recombinant | [${}^3$I]WIN35428 (2.5 µM) | >1 |
| Estrogen | Rat uterine | [${}^3$H]β-Estradiol (1 µM) | >1 |
| Endothelin A | Human recombinant | [125I]Endothelin-1 (0.1 µM) | >1 |
| Endothelin B | Human recombinant | [125I]Endothelin-1 (0.01 µM) | >1 |
| GABA$_A$ (agonist site) | Rat cerebellum | [125I]Flumitrazepam (1 µM) | >1 |
| GABA$_B$ (benzodiazepine central) | Rat brain | [${}^3$H]Speromine (2 µM) | >1 |
| GABA$_B$ | Rat cerebellum | [${}^3$H]Speromine (2 µM) | >1 |
| Galanin | Rat brain | [125I]Galanin (0.01 µM) | >1 |
| Glutamate (AMPA) | Rat cerebral cortex | [${}^3$H]AMPA (10 µM) | >1 |
| Glutamate (kainate) | Rat brain | [${}^3$H]Kainic acid (5 µM) | >1 |
| Glutamate (NMDA agonist site) | Rat cerebral cortex | [${}^3$H]CGP-39653 (5 µM) | >1 |
| Glutamate (NMDA glycine site) | Rat cerebral cortex | [125I]MDL105519 (1 µM) | >1 |
| Glycine (strychnine-sensitive) | Rat spinal cord | [${}^3$H]Strymchine (5 µM) | >1 |
| Histamine H$_1$ (central) | Guinea pig cerebellum | [${}^3$H]Pyrilamine (2.5 µM) | >1 |
| Histamine H$_3$ | Rat cerebral cortex | [${}^3$H]Cimetidine (35 µM) | >1 |
| Histamine H$_3$ | Rat brain | [125]V-methyl-histamine (0.8 µM) | >1 |
| Leukotriene B$_4$ | Guinea pig lung | [${}^3$H]Leukotriene B$_4$ (0.1 µM) | >1 |
| Leukotriene D$_4$ | Guinea pig lung | [${}^3$H]Leukotriene D$_4$ (0.07 µM) | >1 |
| Muscarinic (non-selective) | Rat cerebral cortex | [${}^3$H]QNB (0.5 µM) | >1 |
| Muscarinic M$_1$ | Human recombinant | [${}^3$H]N-Methylscopolamine (0.2 µM) | >1 |
| Muscarinic M$_2$ | Human recombinant | [${}^3$H]N-Methylscopolamine (0.2 µM) | >1 |
| NE transporter | Human recombinant | [${}^3$H]Nisoxetine (0.5 µM) | >1 |
| Nicotinic | Rat brain | [${}^3$H]Nicotine (2.5 µM) | >1 |
| Opiate (non-selective) | Rat cerebral cortex | [125I]Naloxone (1.5 µM) | >1 |
| Oxytocin | Rat uterine | [125I]OVT (0.005 µM) | >1 |
| PAF | Rabbit platelet | [${}^3$H]PAF (1 µM) | >1 |
| K channel (K$_A$) | Rat cerebral cortex | [125I]Dendrotoxin (0.005 µM) | >1 |
| K channel (K$_ATP$) | Rat brain | [${}^3$H]Glibenclamide (0.5 µM) | >1 |
| K channel (K$_V$) | Rat brain | [125I]Charybotoxin (0.01 µM) | >1 |
| K channel (S$_K$) | Rat brain | [125I]Apanin (0.02 µM) | >1 |
| Serotonin (non-selective) | Rat striatum | [${}^3$H]Serotonin (2.5 µM) | >1 |
| Serotonin transporter | Human recombinant | [${}^3$H]Imipramine (1.5 µM) | >1 |
| Sigma (non-selective) | Guinea pig brain | [${}^3$H]DTG (3 µM) | >1 |
| Sodium channel site 2 | Rat brain | [${}^3$H]Baclophan (9 µM) | >1 |
| Sodium channel site 2 | Guinea pig heart | [${}^3$H]Baclophan (9 µM) | 0.87 |
| Testosterone | Rat prostate gland | [125I]R1881 (1 µM) | >1 |
| Thromboxane A$_2$ | Rabbit platelet | [${}^3$H]SO-29548 (2.5 µM) | >1 |
| Vasopressin V$_1$ | Rat liver | [${}^3$H]Arg-Vasopressin (1 µM) | >1 |
| VIP | Human recombinant | [${}^3$H]VIP (0.15 µM) | >1 |

### Tissue Distribution in Rats

To confirm penetration of FK886 and FK355 into the brain, the distribution of radiolabeled FK886 and FK355 was studied. In cerebrum collected 5 min after [14C]FK886 (3.2 mg/kg, intravenously (i.v.)) administration, the radioactivity was 1412 ng equivalents g/tissue,
which was 0.83-fold that in plasma (Table 5). On the other hand, radioactivity was not detected in rat cerebrum 5 min after [14C]FK355 (1 mg/kg, i.v.) administration.

**DISCUSSION**

In this study, we evaluated the pharmacological properties of FK886, a highly potent and specific antagonist of the NK
\[\text{1} \]
receptor. In receptor binding studies, FK886 potently inhibited the binding of radioactive SP to human NK\[\text{1}\] receptors with an IC\[50\] value in the subnanomolar range, a value almost identical to those measured for FK888 and FK355. FK886 had a similar and comparable affinity for dog, ferret, gerbil, and guinea pig NK\[\text{1}\] receptors to that for the human receptor, whereas affinity was 30 times lower for the rat NK\[\text{1}\] receptor. This may be explained by the fact that the primary sequence of the transmembrane regions of the NK\[\text{1}\] receptor protein have seven amino acids that are identical in human and guinea pig, but differ in rat.\[12,13\] While these variations do not affect the potency or efficacy of agonists, they do severely influence the potency of antagonists in different species. The clustering of species-related heterogeneity in the amino acid sequence of the transmembrane regions exactly matches the clustering of species-related differences in the affinities of NK\[\text{1}\] antagonists.\[14\] Accordingly, NK\[\text{1}\] antagonists have been classified into two types: CP-96,345 (human/guinea pig preference) and RP67580 (rat/mouse preference).\[15,16\] In this regard, FK886 could be classified as of the human/guinea pig preference type.

FK886 is highly specific for the NK\[\text{1}\] receptor, with 250- and >20000-fold lower affinities for recombinant human NK\[\text{2}\] and NK\[\text{3}\] receptors, respectively, than for NK\[\text{1}\] receptor, as well as negligible binding affinity (IC\[50\]>1 µM) at 54 different binding sites, including receptors, ion channels, and transporters.

The potent antagonism of FK886 against the NK\[\text{1}\] receptor was further demonstrated by functional assay. While FK886 exhibited no stimulatory effects, it did block NK\[\text{1}\] receptor-mediated inositol phosphate formation in human NK\[\text{1}\] receptor-expressing CHO cells with an inhibiting potency comparable to that obtained from the binding study.

Because the brain NK\[\text{1}\] receptor is likely to be involved in symptoms of various CNS-related diseases, we examined the effects of FK886 on GR73632-induced foot tapping in gerbils to assess its in vivo antagonistic activity in the CNS. It has

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**Table 4. Effects of FK886 on the Binding Parameters of [\[125I\]Bolton–Hunter-Labeled Substance P to Recombinant Human NK\[\text{1}\] Receptor**

| Concentration (nM) | \(K_d\) (pM) | \(B_{\text{max}}\) (pmol/mg protein) |
|-------------------|-------------|------------------|
| 0                 | 91±5.4      | 4.2±0.25         |
| 0.2               | 100±6.6     | 3.6±0.34*        |
| 0.4               | 110±2.5*    | 2.6±0.14**       |
| 0.6               | 170±5.1*    | 2.5±0.16**       |

Values are mean±S.E.M. obtained from three separate experiments performed in duplicate. *\(p<0.05\), **\(p<0.01\) vs. control value as calculated by Dunnett’s multiple comparison test.

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**Table 5. Tissue Concentrations of Radioactivity 5 min after Intravenous Administration of [\[14C\]FK886 and [\[14C\]FK355 in Rats**

| Compound | Dose (mg/kg) | Plasma (ng eq/mL) | Cerebrum (ng eq/g tissue) | Cerebrum/plasma ratio |
|----------|--------------|-------------------|---------------------------|-----------------------|
| FK886    | 3.2          | 1666±82           | 1412±153                  | 0.83                  |
| FK355    | 1.0          | 740±87            | n.d.                      | —                     |

Values are mean±S.E.M. of 3 rats per group. n.d., not detected.
been reported that foot tapping behavior, which is thought to reflect a fear-related response in gerbils, could be elicited by direct stimulation of either central NK₁ or NK₂ receptors. Because direct injection of SP to the brain may stimulate not only NK₁ but also other subtypes of tachykinin receptors, a potent and selective NK₁ agonist, GR73632, was used to determine the NK₁ receptor specific foot tapping response.

Intravenous FK886 inhibited the GR73632-induced foot tapping in a dose-dependent manner. The action was potent and long-lasting; even an intravenous bolus injection of 0.1 mg/kg of FK886 completely inhibited the foot tapping for 60 min. Although we have not determined the brain concentration of FK886 in gerbils, good brain penetration could be confirmed by observing the cerebral distribution of radiolabeled FK886 in rats. Murakami et al. measured the brain receptor occupancy of FK886 in the striatum using ¹¹C-labeled FK886 and positron emission tomography, and reported that the FK886 dose in which occupancy in the striatum reached 90% was about 0.1 mg/kg in guinea pig and dog. Furthermore, Duffy et al. revealed that 40–90% receptor occupancy by NK₁ antagonists may maximally inhibit foot tapping. Taken together, it is reasonable to assume that the inhibition of foot tapping was a result of FK886 penetrating the blood–brain barrier. In contrast, intravenously administered FK355 did not inhibit foot tapping at doses up to 3.2 mg/kg, a result consistent with radiolabeled FK355 not being detected in the cerebrum after intravenous injection. On the other hand, intracerebroventricularly administered FK355 significantly inhibited the foot tapping in a dose-dependent manner. These results indicate that FK886, but not FK355, penetrates the CNS and potently antagonizes the central activation of NK₁ receptors when administered peripherally.

In conclusion, FK886 is a potent and centrally active, insurmountable antagonist of the NK₁ receptor that can antagonize various NK₁ receptor-mediated biological effects in the CNS.

Acknowledgements The authors wish to thank Dr. Yoshiteru Eikyu for his helpful advice, Dr. Toshifumi Shiraga, Dr. Eriko Fujita and Mr. Koji Takeshita for the pharmacokinetics data, and Dr. Kenji Tabata and Dr. Toshio Teramura for their patience and encouragement.

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