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

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The pharmacological properties of the novel neurokinin-1 (NK₁) receptor antagonist FK886, ([3,5-bis-(trifluoromethyl)phenyl][(2*R*)-2-(3-hydroxy-4-methylbenzyl)-4-{2-[(2*S*)-2-(methoxymethyl)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 B_{max} 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 exert their biological effect *via* the activation of three different G protein-coupled neurokinin (NK) receptors: NK₁, NK₂ and NK₃, which preferentially bind to SP, NKA and NKB, respectively.^{1,2)} Recent clinical and preclinical studies have noted that central NK₁ receptor activation is associated with various pathological functions such as pain, anxiety/depression and emesis.^{3–6)}

Previously, Manabe *et al.* reported a potent non-peptide NK₁ receptor antagonist, FK355, 2-[(3*R*)-4-[3,5-bis(trifluoromethyl)benzoyl]-3-(1*H*-indol-3-ylmethyl)piperazin-1-yl]-*N*-(4-methylpiperazin-1-yl)acetamide (2*E*)-but-2-enedioate (1:1) (Fig. 1A), which was developed by the chemical modification of a high affinity dipeptide NK₁ receptor antagonist, FK888.⁷⁾ 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][(2*R*)-2-(3-hydroxy-4-methylbenzyl)-4-{2-[(2*S*)-2-(methoxymethyl)morpholin-4-yl]-ethyl}piperazin-1-yl]methanone dihydrochloride⁸⁾ (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]MePhe⁷-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

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Fig. 1. Chemical Structures of (A) FK355 and (B) FK886

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.⁹ 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 methanesulfonyl fluoride (*p*-APMSF) and 0.25 M 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 1000×*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 8000×*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 pretreated with 0.1% polyethyleneimine. The filter was washed with 50 mM Tris-HCl buffer, pH 7.4, containing 5 mM MnCl₂ and dried. Radioactivity was counted with a γ-counter. Each binding assay was carried out three times in duplicate.

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 of 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.⁹ 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 preincubated with FK886 (0.32–100 nM) 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 nM 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 1 h 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 (AG1-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 glycerophosphoinositide, 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, Shi-
zuoka, 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 12:12h light/dark cycle (lights on 07:00–19:00h).
Food and water were available *ad libitum*. All animal experi-
mental 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 (10pmol in 5 μ L) 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 μ L) or vehicle
(5 μ L of saline) was administered intracerebroventricularly
1 min before GR73632 injection. The duration of repetitive and
persistent foot tapping was recorded from immediately after
agonist injection over a 60-min period. If the repetitive foot-
tapping behavior was separated by at least 30 s, the behavior
was considered discontinued.

**Tissue Distribution of [14 C]FK886 and [14 C]FK355 in
Rats** Rats were deprived of food but not water overnight
prior to injection. Injection of [14 C]FK886 (3.2 mg/1.13 MBq/
mL/kg) or [14 C]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 μ L) 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 $\pm$ S.E.M.
unless otherwise noted. IC₅₀ values were calculated by the lin-
ear 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 [125 I]BH-SP binding
to recombinant human NK₁ receptors in a concentration-
dependent manner that yielded an IC₅₀ value of 0.70 nM (Table
1), which is almost identical to the affinity of the values mea-
sured for FK888 and FK355. FK886 also possessed high affin-
ity for dog, ferret, gerbil, and guinea pig NK₁ receptors, but
30-fold lower affinity for rat NK₁ receptor (Table 2).

The IC₅₀ values of FK886 for the NK₂ and NK₃ receptors
were 250- and >20000-fold lower than that measured for the
human NK₁ receptor, respectively (Table 1). FK886 did not
inhibit (IC₅₀ > 1 μ M) radioligand binding at 54 different sites,
including receptors, ion channels and transporters (Table 3).

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

Compound	Receptor	IC ₅₀ (nM)	
		Mean	95% Confidence interval (nM)
FK886	NK ₁	0.70	0.62–0.78
	NK ₂	180	87–280
	NK ₃	>15000	
FK888	NK ₁	3.1	2.8–3.3
FK355	NK ₁	1.4	1.3–1.6

Values were obtained from three separate experiments performed in duplicate.

Table 2. Affinity of FK886 for NK₁ Receptors from Various Animal
Species

Source	IC ₅₀ (nM)	
	Mean	95% Confidence interval (nM)
Dog forebrain	1.0	1.0–1.0
Ferret forebrain	1.3	1.1–1.6
Gerbil forebrain	1.0	0.94–1.1
Guinea pig forebrain	1.3	1.1–1.6
Rat forebrain	23.0	20–25

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 μ M) was more than
1000-fold lower than that measured for human NK₁ receptors.

Scatchard analysis of the data for saturation binding of
[125 I]BH-SP to human NK₁ receptors in the absence and
presence of increasing concentrations (0.2–0.6 nM) of FK886
demonstrated that the antagonism exerted by FK886 was in-
surmountable.^{10,11} FK886 produced a concentration-dependent
and significant increase in the K_d value (*i.e.*, reduction in the
affinity) of the radio-labeled SP, with a concomitant and sig-
nificant 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 [3 H]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 μ M
(data not shown).

GR73632-Induced Foot Tapping in Gerbils The ability
of FK886 to block central NK₁ receptor activation was evalu-
ated 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. Di-
rect 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

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 nM)	>1
Adenosine A ₂	Rat striatum	[³ H]CGS-21680 (6 nM)	>1
Adenosine A ₃	Rat recombinant	[¹²⁵ I]AB-MECA (1 nM)	>1
α ₁ -Adrenergic (non-selective)	Rat brain	[³ H]Prazosin (0.5 nM)	>1
α ₂ -Adrenergic (non-selective)	Rat cerebral cortex	[³ H]RX821002 (1 nM)	>1
β-Adrenergic (non-selective)	Rat brain	[³ H]DHA (0.5 nM)	>1
Angiotensin II (type 1)	Human recombinant	[¹²⁵ I]Angiotensin II (0.15 nM)	>1
Angiotensin II (type 2)	Human recombinant	[¹²⁵ I]CGP 42112A (0.02 nM)	>1
Bradykinin B ₂	Human recombinant	[³ H]Bradykinin (0.2 nM)	>1
Ca channel (L, benzothiazepine)	Rat cerebral cortex	[³ H]Diltiazem (2.5 nM)	>1
Ca channel (L, benzothiazepine)	Guinea pig heart	[³ H]Diltiazem (2.5 nM)	>1
Ca channel (N)	Rat cerebral cortex	[¹²⁵ I]ω-Conotoxin (0.005 nM)	>1
Ca channel (N)	Guinea pig heart	[¹²⁵ I]ω-Conotoxin (0.005 nM)	>1
Cholecystokinin A	Human recombinant	[³ H]L-364718 (0.5 nM)	>1
Dopamine D ₁	Rat striatum	[³ H]SCH-23390 (0.5 nM)	>1
Dopamine D ₂	Rat striatum	[³ H]Spiperone (2 nM)	>1
Dopamine transporter	Human recombinant	[³ H]WIN35428 (2.5 nM)	>1
Estrogen	Rat uterine	[³ H]β-Estradiol (1 nM)	>1
Endothelin A	Human recombinant	[¹²⁵ I]Endothelin-1 (0.1 nM)	>1
Endothelin B	Human recombinant	[¹²⁵ I]Endothelin-1 (0.01 nM)	>1
GABA _A (agonist site)	Rat cerebellum	[³ H]Muscimol (10 nM)	>1
GABA _A (benzodiazepine central)	Rat brain	[³ H]Flunitrazepam (1 nM)	>1
GABA _B	Rat cerebral cortex	[³ H]GABA (15 nM) + Isoguvacine (1 μM)	>1
Galanin	Rat brain	[¹²⁵ I]Galanin (0.01 nM)	>1
Glutamate (AMPA)	Rat cerebral cortex	[³ H]AMPA (10 nM)	>1
Glutamate (kainate)	Rat brain	[³ H]Kainic acid (5 nM)	>1
Glutamate (NMDA agonist site)	Rat cerebral cortex	[³ H]CGP-39653 (5 nM)	>1
Glutamate (NMDA glycine site)	Rat cerebral cortex	[³ H]MDL105519 (1 nM)	>1
Glycine (strychnine-sensitive)	Rat spinal cord	[³ H]Strychnine (5 nM)	>1
Histamine H ₁ (central)	Guinea pig cerebellum	[³ H]Pyrilamine (2.5 nM)	>1
Histamine H ₂	Rat cerebral cortex	[³ H]Cimetidine (35 nM)	>1
Histamine H ₃	Rat brain	[³ H]N-methyl-histamine (0.8 nM)	>1
Leukotriene B ₄	Guinea pig lung	[³ H]Leukotriene B ₄ (0.1 nM)	>1
Leukotriene D ₄	Guinea pig lung	[³ H]Leukotriene D ₄ (0.07 nM)	>1
Muscarinic (non-selective)	Rat cerebral cortex	[³ H]QNB (0.5 nM)	>1
Muscarinic M ₁	Human recombinant	[³ H]N-Methylscopolamine (0.2 nM)	>1
Muscarinic M ₂	Human recombinant	[³ H]N-Methylscopolamine (0.2 nM)	>1
NE transporter	Human recombinant	[³ H]Nisoxetine (0.5 nM)	>1
Nicotinic	Rat brain	[³ H]L-Nicotine (2.5 nM)	>1
Opiate (non-selective)	Rat cerebral cortex	[³ H]Naloxone (1.5 nM)	>1
Oxytocin	Rat uterine	[¹²⁵ I]OVTA (0.005 nM)	>1
PAF	Rabbit platelet	[³ H]PAF (1 nM)	>1
K channel (K _A)	Rat cerebral cortex	[¹²⁵ I]Dendrotoxin (0.005 nM)	>1
K channel (K _{ATP})	Rat brain	[³ H]Glibenclamide (0.5 nM)	>1
K channel (K _V)	Rat brain	[¹²⁵ I]Charybdotoxin (0.01 nM)	>1
K channel (SK _{Ca})	Rat brain	[¹²⁵ I]Apamin (0.02 nM)	>1
Serotonin (non-selective)	Rat striatum	[³ H]Serotonin (2.5 nM)	>1
Serotonin transporter	Human recombinant	[³ H]Imipramine (1.5 nM)	>1
Sigma (non-selective)	Guinea pig brain	[³ H]DTG (3 nM)	>1
Sodium channel site 2	Rat brain	[³ H]Batrachotoxinin (9 nM)	>1
Sodium channel site 2	Guinea pig heart	[³ H]Batrachotoxinin (9 nM)	0.87
Testosterone	Rat prostate gland	[³ H]R1881 (1 nM)	>1
Thromboxane A ₂	Rabbit platelet	[³ H]SQ-29548 (2.5 nM)	>1
Vasopressin V ₁	Rat liver	[³ H]Arg-Vasopressin (1 nM)	>1
VIP	Human recombinant	[³ H]VIP (0.15 nM)	>1

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).

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,

Table 4. Effects of FK886 on the Binding Parameters of [125 I]Bolton-Hunter-Labeled Substance P to Recombinant Human NK₁ Receptor

Concentration (nM)	K_d (pM)	B_{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.

which was 0.83-fold that in plasma (Table 5). On the other hand, radioactivity was not detected in rat cerebrum 5 min after [14 C]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 NK₁ receptor. In receptor binding studies, FK886 potently inhibited the binding of radioactive SP to human NK₁ receptors with an IC₅₀ 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 NK₁ receptors to that for the human receptor, whereas affinity was 30 times lower for the rat NK₁ receptor. This may be explained by the fact that the primary sequence of the transmembrane regions of the NK₁ 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 NK₁ antagonists.¹⁴ Accordingly, NK₁ antagonists have been classified into two types: CP-96,345 (human/guinea pig preference) and

Fig. 2. Antagonist Activity of FK886 on Substance P-Induced Inositol Phosphate Formation in Human NK₁ Receptor-Expressing CHO Cells

Results are expressed as the fold increase compared to non-stimulated controls using the mean±S.E.M. of three separate experiments. ** p <0.01, *** p <0.001 vs. control by Dunnett's multiple comparison test.

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 NK₁ receptor, with 250- and >20000-fold lower affinities for recombinant human NK₂ and NK₃ receptors, respectively, than for NK₁ receptor, as well as negligible binding affinity (IC₅₀>1 μM) at 54 different binding sites, including receptors, ion channels, and transporters.

The potent antagonism of FK886 against the NK₁ receptor was further demonstrated by functional assay. While FK886 exhibited no stimulatory effects, it did block NK₁ receptor-mediated inositol phosphate formation in human NK₁ receptor-expressing CHO cells with an inhibiting potency comparable to that obtained from the binding study.

Because the brain NK₁ 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

Fig. 3. Effect of Intravenous Administration of FK886 (A), FK355 (B) and Intracerebroventricular Administration of FK355 (C) on Foot Tapping Behavior Induced by GR73632 (10 pmol, i.c.v.) in Gerbils

Drugs were administered 1 min before GR73632 injection. Values shown are tapping time during the 60-min observation period (mean±S.E.M., n =5). * p <0.05, ** p <0.01 vs. vehicle group by Dunnett's multiple comparison test.

Table 5. Tissue Concentrations of Radioactivity 5 min after Intravenous Administration of [14 C]FK886 and [14 C]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,^{17,18)} could be elicited by direct stimulation of either central NK₁^{19,20)} or NK₃²¹⁾ 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, GR73632,²²⁾ 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.²³⁾ Furthermore, Duffy *et al.* revealed that 40–90% receptor occupancy by NK₁ antagonists may maximally inhibit foot tapping.²⁴⁾ 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.

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