

Anti-emetic effects of a novel NK-1 receptor antagonist HSP-117 in ferrets; (1998) 254 ESNETL 3 169-172

October 2, 1998

Section: Pgs. 169-172 Vol. 254 No. 3 ISSN: 0304-3940

Length: 2518 words

History: Received: July 3, 1998; Revised: August 20, 1998; Accepted: August 20, 1998

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Body

ABSTRACT

We have developed a non-peptide compound, HSP-117, antagonist of the tachykinin NK-1 receptor. Binding of ³H-substance P (SP) to the membranes of IM-9 cells was inhibited by the antagonists HSP-117 and CP-99,994, the inhibitory activity of HSP-117 being about 50-fold that of CP-99,994. The SP-induced firing responses of single neuron activity in slices of the nucleus tractus solitarius of ferrets were inhibited by 10 μ M HSP-117. Intracerebroventricular injection of HSP-117 significantly inhibited retching and vomiting induced by copper sulphate and morphine and the inhibitory effect of HSP-117 on emesis was greater than that of CP-99,994. These results indicate that (1) HSP-117 is a potent anti-emetic agent, blocking NK-1 receptors in the nucleus tractus solitarius and (2) NK-1 receptors in the nucleus tractus solitarius play an important role in emesis induced by broad-spectrum emetic stimuli.

FULL TEXT

Substance P (SP) is a family of tachykinin peptides with a variety of pharmacological effects, particularly it plays an important role in the transmission of sensory information on noxious stimuli from the periphery to central nervous system. Molecular biological and pharmacological studies have demonstrated the existence of three types of tachykinin receptor, NK-1, NK-2 and NK-3, which have highest affinities for SP, neurokinin A (NKA) and neurokinin B (NKB), respectively, (see reviews ⁶¹³¹⁴).

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Anti-emetic effects of a novel NK-1 receptor antagonist HSP-117 in ferrets

Recently, NK-1 receptor antagonists were reported to be effective for inhibiting emesis induced by central or peripheral emetogens in ferrets³¹⁸¹⁹²⁰. In addition, binding studies demonstrated that the NK-1 receptor is present at high density in the area postrema and the nucleus tractus solitarius, both of which are associated with the emetic reflex in ferrets. SP and SP-like immunoreactivity have also been found at significant levels in the area postrema and nucleus tractus solitarius of rats and cats⁹¹⁰¹¹¹². Moreover, capsaicin or its analog, resiniferatoxin (RTX), which are thought to cause depletion of SP, reduce emetic responses, although, their mechanism of action is unclear²⁵. These findings strongly suggest that SP is involved in emetic responses in the central nervous system.

Recently, our group have developed a non-peptide compound, (2S,3S)-3-[(5-isopropyl-2,3-dihydrobenzofuran-7-yl) methyl] amino-2-phenylpiperidine dihydrochloride (HSP-117), as a selective antagonist of the NK-1 receptor (Fig. 1A). In this study, we examined the effects of this novel NK-1 receptor antagonist on emesis in ferrets, and demonstrated the existence of NK-1 receptors in their nucleus tractus solitarius by an electrophysiological technique in ferrets.

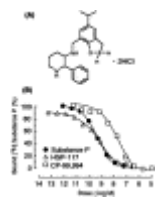


Fig. 1. (A) Structure of HSP-117 and (B) inhibitory effects of HSP-117 and CP-99,994 on ³H-substance P binding in the IM-9 cell.

Adult male ferrets (0.6–1.6 kg, Marshall Farms, USA) were housed individually in a room at 20–23°C with a 12:12 h light–dark cycle (light on at 0700 h) and were given free access to commercial food and tap water.

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¹⁸ 18. F.D. Tattersall, W. Rycroft, R.J. Hargreaves, R.G. Hill; The tachykinin NK1 receptor antagonist CP-99,994 attenuates cisplatin induced emesis in the ferret; *Eur. J. Pharmacol.*; Vol. 250, (1993), pp. R5-R6.

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Anti-emetic effects of a novel NK-1 receptor antagonist HSP-117 in ferrets

Before microinjections into the lateral brain ventricle, ferrets were anesthetized with pentobarbital sodium (40 mg/kg, i.p.) and placed in a stereotaxic apparatus (Kopf Instruments). A guide cannula (23 gauge stainless steel; 13 mm length) was inserted into the right lateral brain ventricle (coordination sites: anterior, -2.5 mm; lateral, -3.5 mm from the cross-structure). The tip of the guide cannula was located vertically 10 mm below the skull surface and the cannula was fixed to the skull with dental cement and two holding screws. After surgery, penicillin (10 000 units i.m.) was given as an antibiotic. The ferrets were then allowed to recover for 36–96 h before experiments.

For experiments on anti-emetic responses, animals were given intracerebroventricularly either HSP-117 or CP-99,994, or artificial cerebrospinal fluid (ACSF) as a vehicle control for HSP-117 or saline as a vehicle control for CP-99,994, in a volume of 20 μ l over a 1 min period. Five minutes later, the animals were given copper sulphate (25.0 mg/kg intra-gastric) or morphine (0.5 mg/kg s.c.) as a emetogen and the numbers of emetic episodes occurring during the following 60 min were recorded. At the end of each experiment, malachite-green dye was injected to confirm the site of drug injection.

Electrophysiological experiments were performed as described previously⁷. Ferrets were anesthetized with ether, and their brains were quickly removed and placed in cold oxygenated Krebs–Ringer buffer. Coronal slices of the brainstem (400 μ m thickness) containing each side of nucleus tractus solitarius were cut with a vibratome. The slices were allowed to equilibrate in a chamber filled with Krebs–Ringer buffer at 34°C for at least 1.5 h before experiments. Then they were transferred to a submerged recording chamber and perfused with Krebs–Ringer buffer at a rate of about 3 ml/min at 34°C. The perfused Krebs–Ringer buffer was saturated with 95% O₂ and 5% CO₂. Drugs were applied by switching the normal perfusing Ringer buffer to a solution containing drug. Spontaneous single-unit discharges were recorded with a glass-electrode filled with Ringer's solution (10 M Ω) from the nucleus tractus solitarius.

³H-SP binding was carried out as described previously¹⁷. Human lymphoblasts (IM-9 cells) were grown in RPMI 1640 culture medium supplemented with 10% fetal calf serum at 37°C under 5% CO₂/95% air for a few days and then cells harvested by centrifugation at 1500 rpm for 5 min. Pelleted cells were washed by resuspension in 30 ml of buffer A (50 mM Tris–HCl pH 7.5, 150 mM NaCl, 0.02% BSA), collected by centrifugation and resuspended in assay buffer (buffer A, 40 μ g/ml bacitracin, 4 μ g/ml leupeptin, 4 μ g/ml chymostatin, 4 μ g/ml phosphoramidon). Cell suspensions (500 μ l) at a concentration of 1 \times 10⁷ cells/ml assay buffer were incubated with ³H-SP 10 μ l (final 250 pM) for 60 min at room temperature 1 min after addition of various concentrations of test compounds or non-radioactive SP. For removal of unbound ³H-SP, the suspensions were centrifugated at 1500 rpm for 5 min. Then the pelleted cells were solubilized in 10 ml of scintillation fluid. Radioactivity in the pellet was determined as the specific binding of ³H-SP to the IM-9 cells.

HSP-117 and CP-99,994 were synthesized at Hisamitsu Pharmaceutical (Tsukuba, Japan). IM-9 cells were purchased from the American Type Culture Collection (USA). SP was obtained from Bachem (UK). ³H-SP was purchased from Amersham (UK).

Data are expressed as the mean \pm SEM for each treatment group. The significance of differences between values for vehicle and treatment groups was examined by one-way analysis of variance (ANOVA) followed by Dunnet's test.

To determine the selectivity of the novel NK-1 antagonist HSP-117, we examined its inhibition and that of CP-99,994, and the specific binding of ³H-SP at 2.0 nM to membranes of IM-9 cells. As shown in Fig. 1B, ³H-SP binding to the membranes was inhibited by both antagonists, but the inhibitory activity of HSP-117 was more than about 50-fold that of CP-99,994.

⁷ T. Iwase, N. Hori, T. Morioka, D.O. Carpenter; Low power laser irradiation reduces ischemic damage in hippocampal slices in vitro; *Lasers Surg. Med.*; Vol. 19, (1996), pp. 465-470.

¹⁷ Y. Takano, A.D. Loewy; ³H-substance P binding in the intermediolateral cell column and striatum of the rat; *Brain Res.*; Vol. 311, (1984), pp. 144-147.

Anti-emetic effects of a novel NK-1 receptor antagonist HSP-117 in ferrets

Next, we examined the inhibitory effect of HSP-117 on NK-1 receptors by an electrophysiological technique. SP (1 μM) caused an increase in spontaneous firings in slices of the nucleus tractus solitarius of ferrets. HSP-117 at 10 μM concentration inhibited the SP-induced firing responses of a single neuron (Fig. 2), although it had no effect on the basal spontaneous firing activity.

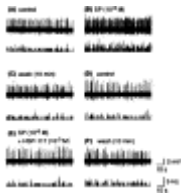


Fig. 2. Effects of SP and HSP-117 on spontaneous firing in slices of the brainstem containing nucleus tractus solitarius in ferrets. Typical recordings of the firing (upper) and its rate (spikes/s; lower) are shown.

Oral administration of copper sulphate (25.0 mg/kg, i.g.) or subcutaneous injection of morphine (0.5 mg/kg, s.c.) causes retching and vomiting in ferrets. Intracerebroventricular (i.c.v.) injection of HSP-117 (10, 100 μg) dose-dependently inhibited the retching and vomiting induced by copper sulphate and morphine (Fig. 3). The inhibitory effect of HSP-117 on emesis was greater than that of CP-99,994.

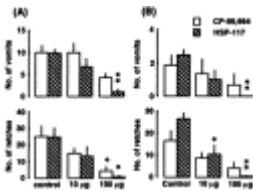


Fig. 3. Effects of i.c.v. injections of HSP-117 and CP-99,994 on the number of vomits and retches induced by copper sulphate (A) and morphine (B). Values are the mean \pm SEM $n=4-6$. * $P<0.05$, ** $P<0.01$ compared with each of the controls.

The physiological roles of SP have been demonstrated and recently the involvements of SP in various clinical disorders, such as inflammation, emesis, pain, asthma and cardiovascular disorders have become of interest. In the present study, we developed a selective piperidine NK-1 receptor antagonist, HSP-117. Binding experiments showed that HSP-117 has about 50-fold of magnitude greater affinity for NK-1 receptors in membranes of IM-9 cells than CP-99,994 (Fig. 1B). In IM-9 cells, human NK-1 receptors are predominant and so HSP-117 may be clinically useful. Moreover, HSP-117 antagonized the firing responses induced by SP in brain slices of ferrets including the nucleus tractus solitarius in vitro (Fig. 2). These findings indicate that HSP-117 is highly selective for NK-1 receptors not only in cell membranes but also in organs of the whole body, e.g. the nucleus tractus solitarius, suggesting that it should be useful for studies on brain NK-1 receptors.

In ferrets, emesis is known to be evoked by a wide variety of stimuli⁸. There are many reports that systemic administration of NK-1 antagonists inhibits emesis³⁴¹⁸¹⁹²⁰, but there are a few reports on the central effects of NK-1 antagonists¹⁵. In this study, central injection of HSP-117 (100 μg , i.c.v.) almost completely inhibited emesis induced by copper sulphate or morphine and was more effective than the same dose of CP-99,994 (Fig. 3).

⁸ 8. A.P. Knox, N.L. Strominger, A.H. Battles, D.O. Carpenter; Behavioral studies of sensitivity in the ferret; Brain Res. Bull.; Vol. 31, (1993), pp. 477-484.

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The site of the vomiting center in the brain is of interest. Our results indicate that NK-1 antagonists have a central site of action. Moreover, high densities of ³H-SP binding sites have been found in the nucleus tractus solitarius and the area postrema of rats ¹⁶, although little is known about the distribution of tachykinin peptides in the ferret brain. However, the present data do not indicate whether the action of NK-1 receptors in the anti-emetic response is direct or involves an other neuronal system, such as serotonergic neurons in the brain, and in fact, SP has been shown to be co-localized with serotonin in the peripheral and central nervous systems ¹. At present, the mechanisms underlying the emetic reflex are not fully understood, but our results suggest that HSP-117 acts within the brainstem, and probably within the nucleus tractus solitarius and area postrema.

Thus, HSP-117 is a high affinity antagonist of the ferret NK-1 receptor, and is the most potent broad-spectrum anti-emetic agent known to date. Our study suggests that NK-1 receptors are important for emesis induced by broad-spectrum emetic stimuli.

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Classification

Language: ENGLISH

Document Type: Full-length article

Publication Type: Other (Journal)

Journal Code: NSL

Subject: AMINO ACIDS, PEPTIDES & PROTEINS (90%); MAMMALS (90%); BIOCHEMISTRY (89%); NEUROSCIENCE (89%); PHARMACOLOGY (89%); BRAIN (78%); MOLECULAR BIOLOGY (78%); CANCER DRUGS (73%); REPORTS, REVIEWS & SECTIONS (73%); CATS (72%); Tachykinins; Emesis; NK-1 antagonists; HSP-117; CP-99,994

Industry: CENTRAL NERVOUS SYSTEM DRUGS (90%); HORMONES, SUBSTITUTES & ANTAGONISTS (90%); PHARMACOLOGY (89%); DOPAMINE AGENTS (78%); CANCER DRUGS (73%)

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