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The effect of CP-99994 on the responses to provocative motion in the cat

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1 The NK_1 receptor antagonist CP-99994 has been shown to prevent vomiting elicited by both peripherally and centrally acting emetogens in ferrets and dogs. These results have now been extended to another stimulus, provocative motion, and another species, the cat.

2 CP-99994 displaced [^{3}H]-substance P from cat cortex with IC_{50} of 0.52 ± 0.08 nM. Following s.c. administration, peak plasma drug levels were achieved at 30 min. The plasma drug half life was 1.4 h.

3 Subcutaneous administration of CP-99994 inhibited motion-induced vomiting in the cat with an ED_{50} of 144 $\mu g\ kg^{-1}$ but did not change the epiphenomena associated with provocative motion in the cat over the dose range of 30 to 300 $\mu g\ kg^{-1}$. The antiemetic effect of CP-99994 can be attributed to antagonism of the NK_1 receptor because its enantiomer, CP-100,263, which is 900 fold weaker as an NK_1 antagonist, had no effects on any response to provocative motion.

4 The inhibitory effect of CP-99994 on motion-induced retching and vomiting is consistent with a central site of antiemetic action, potentially at the level of the motor nuclei responsible for these behaviours.

5 An investigation into whether the failure of CP-99994 to alter the epiphenomena will also predict a lack of anti-nausea effects in man will provide critical information on the neural organization of the emetic reflex.

Keywords: Emesis; motion sickness; nausea; NK_1 receptors; vomiting

Introduction

The antiemetic drugs in current use are individually effective against only one or a few emetic stimuli (e.g. Leslie *et al.*, 1990). Even the excitement generated by the 5-hydroxytryptamine $(5\text{-HT})_3$ antagonists must be tempered by the fact that they are only effective against vomiting elicited by ipecac, radiation, acute cancer chemotherapy and, to a lesser extent, recovery from anaesthesia (see Andrews, 1994 for a review). The early drugs known to have a broad spectrum of antiemetic effects were not useful clinically because they produced unacceptable depression of the central nervous system (Borison & McCarthy, 1983). The broad spectrum antiemetic effects of the 5-HT_{1A} agonists (Lucot & Crampton, 1989; Rudd *et al.*, 1992; Okada *et al.*, 1994; Wolff & Leander, 1994) have yet to be tested in human subjects at adequate doses (Lucot, 1992).

More recently, a new therapeutic approach was suggested by substance P-like immunoreactivity in areas of the brainstem intimately involved with emesis (reviewed by Watson *et al.*, 1995). Interference with substance P release by resinferatoxin in ferrets had broad spectrum antiemetic effects (Bhandari & Andrews, 1992; Andrews & Bhandari, 1993) as did potent and selective neurokinin $(NK)_1$ antagonists (McLean *et al.*, 1993). In the ferret, i.v. administration produced a dose-dependent decrease in vomiting elicited by cisplatin, with 3 mg kg^{-1} producing nearly complete suppression (Tattersall *et al.*, 1993). Intraperitoneal administration also produced dose-dependent suppression of vomiting elicited by apomorphine, with complete suppression occurring at 3 mg kg^{-1} (Tattersall *et al.*, 1994). The dose of 3 mg kg^{-1} of a racemic mixture containing CP 99994 given intraperitoneally produced from 80 to 95% inhibition of vomiting elicited by cisplatin, oral $CuSO_4$, cylcophosphamide, ipecac, morphine or radiation (Bountra *et al.*, 1993). Subcutaneous administration of CP-99994 was shown to produce dose-dependent suppression of $CuSO_4$, loperamide, apomorphine, cisplatin and ipecac emesis with complete or near suppression at 1 mg kg^{-1} (Watson *et al.*, 1995). In dogs, i.v. administration of CP-99994 significantly reduced apomorphine- and $CuSO_4$-induced retching and vomiting (Watson *et al.*, 1995).

These studies demonstrated that CP-99994 blocks emetic responses to stimuli transduced through the vagus nerve ($CuSO_4$, ipecac, cisplatin and radiation) as well as those transduced through the area postrema (loperamide, apomorphine and morphine). Motion is distinct from these stimuli in that it relies both upon a peripheral sensory organ whose signals are not conducted by the vagus and on central connections that produce a normal emetic response in the absence of the area postrema.

We evaluated the effects of CP-99994 on emesis induced by provocative motion to extend our understanding of its mechanism and site of action. We measured the affinity of CP-99994 for the NK_1 receptor in the cat because there are species differences in affinity (McLean *et al.*, 1993). We also measured the kinetics after subcutaneous administration in cat, the preferred route of administration in this species, because this route is associated with decreased potency in the guinea-pig (McLean *et al.*, 1993).

Methods

Receptor binding

NK_1 receptor binding was assessed in cat cortical tissue obtained from Liberty Research, Waverly, NY. The tissue was homogenized in 10 vols of 0.32 M sucrose by a teflon homogenizer and the tissue centrifuged at 3200 r.p.m. for 8 min at 4°C. The supernatant was saved, the pellet rehomogenized and spun again. The combined supernatants were centrifuged at 17,000 r.p.m. for 25 min at 4°C, the pellet resuspended in 5 mM Tris HCl (pH 7.4) and spun again. This procedure was

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repeated for a total of three washes. The final pellet was resuspended in 5 mM Tris HCl at a concentration of 1 g ml^{-1} binding buffer. In 96 well polypropylene plates, incubations were initiated by adding 25 μl of tissue to each well containing 200 μl of buffer (50 mM Tris HCl, at 22°C, 50 μg ml^{-1} chymostatin, 10 μg ml^{-1} bacitracin, 40 μg ml^{-1} leupeptin, 20 μg ml^{-1} phosphoramidon, 1 mM $MnCl_2$, 1 mM $MgCl_2$ and 0.02% BSA) with 1.0 nM of [^{3}H]-substance P (final concentration; specific activity = 31 Ci $mmol^{-1}$, New England Nuclear, Boston, MA) and 25 μl of various concentrations of ligands for a final volume of 250 μl. Nonspecific binding was defined as the radioactivity remaining in the presence of 1 μM substance P. After 20 min at 22°C, the incubations were terminated by filtration onto 0.3% polyethylenimine (PEI) soaked filters (Schleicher and Schuell #30) by a Skatron cell harvester and washed with 5 ml of ice-cold 50 mM Tris at 4°C, 1 mM $MnCl_2$. The amount of radioactivity bound to the filter was determined by scintillation counting in a Betaplate counter (LKB/Wallac).

Plasma concentrations and kinetics

Two male cats previously surgically prepared with venous access ports were injected subcutaneously with 3 mg kg^{-1} CP-99994 in isotonic saline (15 mg ml^{-1}). Blood samples were obtained from the jugular vein immediately before drug administration at 0.25, 0.5, 1, 2, 3, 5 and 7 h post-dose. The blood samples were processed to obtain plasma and stored at −20°C until the day of analysis.

Plasma CP-99994 concentrations were determined by gas chromatography (g.c.) with electron capture detection after derivatization with trifluoroacetic anhydride. Briefly, an internal standard was added to plasma samples, the samples were made alkali with NaOH and extracted with methyl t-butyl ether (MTBE). The extract was back-extracted with aqueous acid, made alkali and extracted again with MTBE. The organic fraction was evaporated under nitrogen and the residue incubated with 10% trifluoroacetic anhydride in MTBE. The solvent was evaporated, the residue reconstituted in toluene and injected onto the g.c.

Motion sickness

Motion sickness was elicited by a motorized device resembling an amusement park Ferris Wheel (Crampton & Lucot, 1985). Briefly, two plexiglas boxes were suspended from the ends of a 0.89 m beam that rotated about a horizontal axle at 0.28 Hz (17 r.p.m.). The boxes were counter-rotated to maintain a horizontal floor. Each test period lasted for 30 min of rotation plus one min of observation at rest. Tests were separated by at least two weeks to prevent habituation to the motion stimulus (Crampton & Lucot, 1991).

A total of 15 female cats previously selected for sensitivity to this motion stimulus were used. Twelve were used for the initial determination of the dose-response curve (30, 100 and 300 μg kg^{-1}). One cat was not available for the test with 300 μg kg^{-1} of the inactive isomer, CP 100263. Three cats were replaced for reasons not related to this experiment in a group of 12 that were used to test the additional dose of 170 μg kg^{-1}.

Data collected were the presence of retch/vomits, the latency to the first retch, the duration of a bout of retch/vomits from the first retch to the last vomit and the 'symptom' points from the rating scale of Suri *et al*. (1979). The research conformed with the 'Guidelines for the Use of Animals in Neuroscience Research' and the 'NIH Guide for the Care and Use of Laboratory Animals', NIH Pub. no. 85-23 (revised 1985).

Statistics

In the receptor binding assays, IC_{50} values were obtained by linear regression of individual displacement curves generated from six test concentrations run in triplicate.

The latency to the first retch and the duration of a bout of retch/vomits are in min converted to a decimal. The mean and s.e. mean reflect data from only those animals that vomited. The symptom points exclude the 16 for retch/vomits and are presented as the total for the test across animals. The ED_{50} was calculated by use of the PHARM/PCS programme for the method of Litchfield and Wilcoxon. The duration of a bout was analysed by a repeated measures ANOVA.

Drugs

CP-99994, (+)-(2**S**,3**S**)-3-(2-methoxybenzyl-amino)-2-phenylpiperidine and CP-100263, (−)-(2**R**,3**R**)-3-(2-methoxybenzyl-amino)-2-phenylpiperidine were synthesized by the Medicinal Chemistry Department at Pfizer Inc. The doses were prepared on each day of testing by dissolving in 5% mannitol to an injection of 0.1 ml kg^{-1}. Injections were s.c. 30 min before the start of motion.

Results

Binding data

CP-99994 displaced [^{3}H]-substance P from specific binding sites in cat cortex with an IC_{50} of 0.52 ± 0.08 nM. In contrast, the inactive isomer CP-100263 bound with an IC_{50} of only 4900 ± 1400 nM. By comparison, substance P bound with an IC_{50} of 12.8 ± 2.1 nM.

Kinetics

Following s.c. administration of 3 mg kg^{-1}, plasma levels peaked at 30 min (Figure 1). Levels reached a C_{max} of 815 ng ml^{-1} and exhibited an elimination of $t_{1/2}$ of 1.4 h. These data were used to select the 30 min pretreatment time and the dose range of 30 to 300 μg kg^{-1}.

Motion sickness

CP-99994 produced a dose-dependent decrease in the incidence of motion-induced vomiting with an ED_{50} of 144 μg kg^{-1} (95% C.L., 131–157 μg kg^{-1}; Figure 2). There was no change in the mean latency to the first retch, in the number of symptom points or in the duration of a bout of retch/vomits (Table 1). The apparent decrease in the duration of retch/vomits ob-

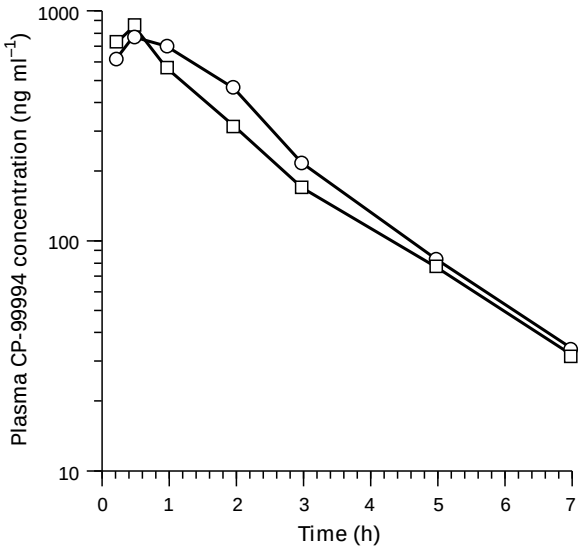

Figure 1 Plasma concentrations of CP-99994 in two individual (○, □) cats after s.c. administration of 3.0 mg kg^{-1}.

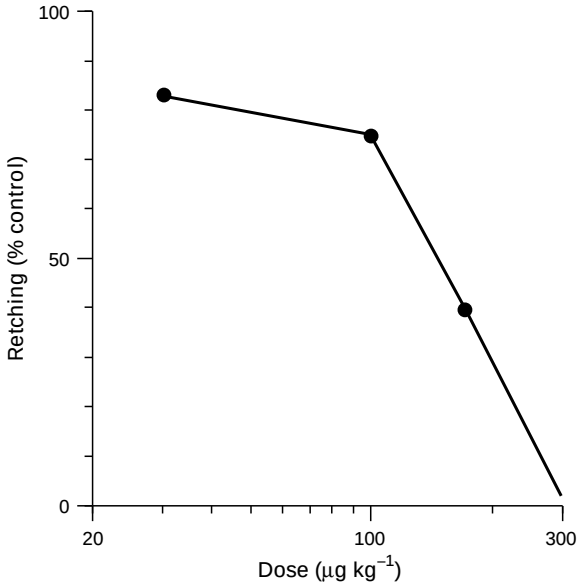

Figure 2 Effects of CP-99994 on motion-induced vomiting in cats. Doses were administered s.c. 30 min before the start of 30 min of motion followed by one min of observation at rest.

Table 1 Effects of CP-99994 on motion sickness in the cat

Treatment *Dose* (μg kg^{-1})	*Latency*	*Points*	*Duration*
CP-99994 ($n=12$)			
Control	12.43±2.87	61	0.46±0.10
30	8.38±2.74	63	0.42±0.11
100	11.28±4.58	52	0.60±0.15
300	–	56	–
Control ($n=11$)	8.57±2.16	67	0.57±0.12
Control	11.02±2.49	70	0.56±0.08
170	16.78±5.10	69	0.23±0.10
Control	12.49±3.30	63	0.48±0.09
CP-100263 ($n=11$)			
Control	11.16±2.80	45	0.49±0.10
300	11.26±2.95	46	0.57±0.08
Control	8.94±2.34	50	0.55±0.12

Injections were s.c. 30 min before motion testing. Latency to the first retch are in minutes. Points are from the Suri *et al.* (1979) scale without the points for retch/vomits. Duration of a bout of vomiting from the first retch to the last vomit are in min. Data for duration and latency are shown as means±s.e.mean.

served at the dose of 170 μg kg^{-1} failed to reach significance in those animals that vomited at this dose and on both control tests ($F_{2,4}=2.40$, $P=0.21$). Following the dose of 300 μg kg^{-1} of the isomer CP-100263, all 11 cats vomited as they did on both control tests and there was no change in the latency to the first retch, the number of symptom points or in the duration of a bout of retch/vomits. Neither CP-99994 nor CP-100263 produced behavioural changes or obvious alterations in autonomic nervous system function over the doses tested.

Discussion

CP-999994 produced a dose-dependent decrease in motion-induced vomiting with a steep dose-response curve. This study extends the emetic stimuli for which this drug is effective to include provocative motion and extends the generality of its action to another species, the cat. The lack of effect of the enantiomer CP-99994, of which is 900 times weaker as an NK_1 antagonist (McLean *et al.*, 1993), further supports NK_1 antagonism as the mechanism of the antiemetic effect of CP-99994.

The affinity of CP-99994 for the NK_1 receptor in the cat is quite high and comparable to that observed in a wide range of species, with the exception of the mouse, rat and Suncus which have a much reduced affinity (McLean *et al.*, 1993; Watson *et al.*, 1995). Therefore, the cat provides a suitable model for studying the antiemetic effects of NK_1 antagonists. The pretreatment time of 30 min was selected to correspond to the peak plasma level after s.c. administration (Figure 1) and the 31 min observation time was considerably less than the half-life of the drug. With the caveat that blood levels do not always accurately reflect levels in the central nervous system, the dose-response curve in the present study results from carefully selected testing parameters.

The ED_{50} of 144 μg kg^{-1} obtained for the motion-induced vomiting is comparable to the ED_{50} values of approximately 300 μg kg^{-1} found in the ferret for vomiting elicited by loperamide, cisplatin, ipecac, apomorphine and oral copper sulphate (Watson *et al.* 1995). The slightly low value in the cat may reflect its slightly higher affinity (IC_{50} of 0.52 vs 1.97 nM), differences in pharmacokinetics and/or differences in the relative strengths of the emetic stimuli. In dogs, i.v. infusion of 310 μg kg^{-1} during the hour before administration of emetogen, followed by continued infusion of another 150 μg kg^{-1}, significantly decreased vomiting elicited by apomorphine and abolished that induced by oral copper sulphate (Watson *et al.*, 1995). In Suncus, vomiting induced by nicotine was significantly decreased but not abolished by 3 and 10 mg kg^{-1} (Rycroft *et al.*, 1995), and motion sickness was decreased but not abolished over the dose range 3 to 30 mg kg^{-1} (Gardner *et al.*, 1995). The requirement for higher doses reflects a reduced affinity of CP-99994 for the NK_1 receptor in this species (unpublished observation, Atsushi Hagahisa). However, CP-99994 was found to be as an antiemetic in all four of the species in which it was tested.

The suppression of motion-induced vomiting occurred with no observable side effects, including sedation. A lack of side effects has been observed in the dog and also in the ferret, in which antiemetic doses of CP-99994 did not alter a variety of centrally mediated reflexes and homeostatic reflexes (Watson *et al.*, 1995). This suggests that the antiemetic effect results from blockade of a step in the emetic pathways rather than sedative or nonspecific neuromodulatory actions.

Although CP-99994 completely suppressed motion-induced vomiting, it failed to reduce the incidence of the epiphenomena that accompany motion sickness in the cat (Table 1). This is in contrast to 5-HT_{1A} agonists, which produce a dose-dependent decrease in epiphenomena that becomes significant at about 70% suppression of vomiting (Lucot & Crampton, 1989). The epiphenomena reflect altered output in the autonomic nervous system. The extent to which the neural pathways that initiate this output overlap those which subserve nausea is not known (Borison & McCarthy, 1983). In man, the variability in heart rate consistently peaks before self reports of nausea (Morrow *et al.*, 1995), suggesting temporal and neural sequencing. However, while the degree of altered gastric emptying correlates with the severity of self reported nausea, there is no temporal correlation between the two measures, suggesting independent mechanisms (Reid *et al.*, 1995). Thus, it is not clear whether the failure to block the epiphenomena at antiemetic doses in the present study will predict a failure to block nausea in man. The results from testing NK_1 antagonists as antiemetics in man will be required to settle this question.

Doses of CP-99994 which failed to abolish motion-induced vomiting also failed to alter the latency to the first retch. A similar lack of effect on latency was obtained in ferrets with vomiting elicited by cisplatin and oral copper sulphate, though there was a trend to increased latency to vomiting elicited by loperamide and ipecac (Watson *et al.*, 1995). The intermediate dose of 170 μg kg^{-1} of CP-99994 produced a non-significant

decrease in the duration of a bout of retch/vomits. Only three animals met the criteria for inclusion in the statistical analysis, rendering the lack of statistical significance questionable. Further testing of NK_1 antagonists possessing less steep dose-response curves are necessary to determine whether this mechanism decreases the duration of bouts of retch/vomits. However, the combination of suppression of vomiting with no change in the incidence of epiphenomena is a unique profile in the cat.

The site of the antiemetic action of CP-99994 is most likely to be within the central nervous system. The initial studies with CP-99994 used stimuli which act through vagal afferents and/or the area postrema (see Introduction), leaving peripheral sites as candidates for the site of antiemetic action. Provocative motion requires the vestibular apparatus, the signals from which undergo unknown processing within the central nervous system and pass immediately subadjacent to the area postrema (see Brizzee, 1990 for a review). While the vestibular end organs possess substance P-like immunoreactivity (Ylikoski *et al*., 1984), the signal to the vestibular nuclei relies on an excitatory amino acid (Raymond *et al*., 1984; Gallagher *et al*., 1987), making a peripheral site of action for CP-99994 unlikely in the present study. Injection of CP-99994 but not CP-100263 into the hindbrain of ferrets blocked cisplatin-induced vomiting (Gardner *et al*., 1994). Further, systemic administration of a quarternized NK_1 antagonist failed to block cisplatin-induced emesis in the ferret, even at doses equivalent to effective doses of CP-99994 (Tattersall *et al*., 1995). Combined, these studies provide strong evidence that the antiemetic effect of CP-99994 results from blockade of NK_1 receptors on a component of the emetic pathway within the central nervous system.

The unique profile of CP-99994 in that it is able to abolish retching and vomiting without altering epiphenomena fits into both of the major conceptual models for the neural organization of the emetic reflex. The 'vomiting centre' model holds that diffusely spread neurones (Miller & Wilson, 1983) orchestrate the activity of specific nuclei which execute the components of the emetic reflex (Borison & Wang, 1949). CP-99994 could be blocking NK_1 receptors on the emetic motor nuclei that are activated by output from the vomiting centre, whereas the nuclei involved in epiphenomena do not rely on NK_1 receptors. The other model describes nuclei arranged in series such that each step in the sequence leads to the execution of a specific component of the emetic reflex (Davis *et al*., 1986). In this model, CP-99994 could block transmission between those nuclei which mediate the epiphenomena and the subsequent nuclei which lead to the mechanical acts associated with vomiting. While the present results are compatible with both models and do not permit selection between them, they support a key role for NK_1 receptors very late in the emetic signalling pathway, perhaps at the level of the cells responsible for the motor output.

The specific neural pathways which organize the emetic reflex are poorly understood. Lesion studies in the cat suggest that the reflex is organized in the section of brainstem between the retrofacial nucleus and the obex, with the nucleus tractus solitarius serving as a beginning of the final common pathway for vomiting (Miller *et al*., 1994). Studies in the dog conclude that the respiratory muscle component of the reflex is organized in the Botzinger complex and that the nucleus tractus solitarius serves as a relay (Fukuda & Koga, 1991). Vomiting elicited by either ipecac, loperamide or cisplatin produced increased cfos-like immunoreactivity in the medial nucleus of the tractus solitarius and nucleus gelatinosus of ferrets (Leslie & Reynolds, 1992). Similarly, injection of a cocktail of emetic drugs into the cat produces cfos-like immunoreactivity in an arc radiating from the area postrema, the nucleus tractus solitarius, especially the gelatinosus and medial nuclei, and the lateral tegmental field to the ventrolateral medulla (Miller & Ruggiero, 1994).

Autoradiography of substance P in the ferret revealed dense binding in the nucleus gelatinosus and dorsal motor nucleus of the vagus, with slightly less in the rest of the nucleus tractus solitarius (Watson *et al*., 1995). The nucleus gelatinosus receives input from the vagus and area postrema (Fukuda & Koga, 1991) and is a structure necessary for motion sickness (Brizzee, 1990), but seems too early in the sequence for the site of action of CP-99994 because this antagonist did not change the epiphenomena. Similar reasoning applies to the medial nucleus where both vagal and primary vestibular inputs also converge, occasionally onto the same neurones (Grelot *et al*., 1993). Thus, the site of action of CP-99994 may be in other portions of the nucleus tractus solitarius or in emetic nuclei outside the nucleus tractus solitarius. Resolving this question will contribute much to the understanding of the neural circuitry involved in the organization of vomiting.

In conclusion, blockade of NK_1 receptors with CP-99994 produced a dose-dependent suppression of motion sickness in cats, while its inactive isomer had no effect. These data support a central site for the antiemetic effect of CP-99994, potentially at the level of the motor nuclei that drive emesis. More definitive determination of its site of action, coupled with the observation that the epiphenomena associated with motion sickness were not altered, would provide important information on the neural circuitry organizing the emetic reflex.

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