ELSEVIER

European Journal of Pharmacology 307 (1996) 305–313

ejp

The pharmacology of the emetic response to upper gastrointestinal tract stimulation in *Suncus murinus*

Paul Andrews [a,*], Yoshifumi Torii [b], Hiroshi Saito [b], Norio Matsuki [b]

[a] *Department of Physiology, St. George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, UK*
[b] *Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113, Japan*

Received 2 November 1995; revised 19 March 1996; accepted 22 March 1996

Abstract

This paper is the first to describe aspects of the mechanics of retching in the insectivore *Suncus murinus* (house musk shrew) and in an animal of such a small size (~ 50 g). In anaesthetised animals using the novel stimulus of mechanical stimulation of the upper gastrointestinal tract as the provocative stimulus the frequency of retching was found to be about 4 retches/s, a much higher frequency than in other species (dog, cat, ferret). These studies show that quantification of retching in *Suncus* cannot be undertaken using direct observation. The temporal pattern of the emetic response was characterised in conscious *Suncus* using motion (1 Hz, 5 min) and nicotine (20 mg/kg s.c.). The ultrapotent capsaicin analogue resiniferatoxin (100 μg/kg s.c.) was discovered to be highly emetic and comparative studies showed that nicotine and resiniferatoxin induced the most intense responses with episodes (retches and a vomit) occurring every 10–15 s. The retching response to mechanical stimulation in the anaesthetised *Suncus* was not blocked by a 5-HT_3 receptor antagonist (granisetron, 1–5 mg/kg s.c.), a tachykinin NK_1 receptor antagonist (CP-99,994 20 mg/kg s.c. dihydrochloride salt (9 +)-(2*S*,3*S*)-3-(2-methoxybenzylamino)-2-phenylpiperidine) or morphine (2 mg/kg s.c.) but was blocked by the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT 100 μg/kg s.c.). *Suncus* appears to be a suitable animal in which to study the pharmacology of the emetic response to mechanical stimulation of the gut. The results are discussed in the light of studies of the pharmacology of emesis in other species.

Keywords: Emesis; Morphine; Nicotine; Tachykinin NK_1 receptor; 5-HT_3 receptor; 5-HT_{1A} receptor; Resiniferatoxin; Vomiting; Retching; (*Suncus murinus*)

1. Introduction

The majority of studies of the mechanism of emesis, particularly in relation to the emesis induced by anti-cancer chemotherapy, have been undertaken in carnivores particularly the cat and dog and more recently the ferret (Andrews and Davis, 1993 for review). Studies aimed at understanding the mechanism by which alcohol induced hepatic damage using the house musk shrew (*Suncus murinus*, an insectivore) as a model observed that the animals vomited when alcohol was administered into the stomach (H. Saito, unpublished observations). This observation prompted a more extensive investigation of the emetic reflex in this species and these studies revealed that this animal responds to a wide range of emetic stimuli including intragastric copper sulphate, veratrine, motion, cytotoxic anti-cancer drugs (e.g. cisplatin, cyclophosphamide) and abdominal radiation (Ueno et al., 1987, 1988; Kaji et al., 1991; Torii et al., 1991, 1993; Mutoh et al., 1992). In addition the emetic response to anti-cancer treatments is blocked by the clinically used 5-HT_3 receptor antagonists (e.g. granisetron, ondansetron, tropisetron, Torii et al., 1991). *Suncus* has therefore become an established model for the study of many types of emesis. *Suncus* is a member of the phylogenetically ancient order of Insectivora considered to be the most primitive and earliest eutherians and the direct ancestors of other placental mammals. Therefore studies in this species may provide important insights into the evolution of the emetic reflex and the neurotransmitters employed.

Studies of emesis in *Suncus* have typically reported only 'vomiting episodes' characterised by the oral expulsion of material from the upper gastrointestinal tract (e.g. Torii et al., 1991). Direct observation and physiological

* Corresponding author. Tel.: +44 (0)81-725-5369; fax: +44 (0)81-725-2993; e-mail: pandrews@sghms.ac.uk.

PII S0014-2999(96)00275-0

recording studies of intra-thoracic and intra-abdominal pressure mainly in the cat (McCarthy and Borison, 1974) and the ferret (Andrews et al., 1985) reveal that the somatic motor component of the emetic response usually consists of both retching and vomiting. Retching is characterised by rhythmic decreases in intra-thoracic pressure coincident with increases in intra-abdominal pressure. There is no oral expulsion of material although gastric contents may oscillate between the stomach and the oesophagus (Lang, 1990, 1992). The major features which distinguish vomiting from retching are that both intra-abdominal and intra-thoracic pressure become positive and this is associated with expulsion of material from the gut. The reason for the lack of ejection during retching is unclear but some authors have reported that the peri-oesophageal fibres of the diaphragm are active during retching but become silent during the vomit (Miller, 1990). The function of retching is unclear especially as retching and vomiting can occur in isolation. It is suggested that it may promote overcoming the restraining effect of the multi-component anti-reflux barrier between the stomach and the oesophagus (Lang, 1990, 1992; Andrews et al., 1990a).

Visual observation of emesis in *Suncus* by the authors revealed that whilst vomiting can be accurately quantified by direct observation as in other species, abdominal and thoracic movements characteristic of retching occurred prior to vomiting but at a frequency too high for individual retches to be counted. As the mechanics of emesis have never been studied in *Suncus* or indeed in an animal of such a small size it was decided to investigate whether it was possible to characterise retching in the anaesthetised *Suncus* by monitoring intra-thoracic and intra-abdominal pressure as has been done in the cat and ferret. Preliminary experiments demonstrated that it was possible to elicit retching by pharyngeal probing and this was extended to include oesophageal stimulation in order to provide a model for the induction of emesis by mechanical stimulation of the gut. The possible involvement of 5-HT_{1A}, 5-HT_3, tachykinin NK_1 receptor and opiate receptors in the response was investigated as all have been implicated in the emetic response to other types of stimuli (e.g. Lucot, 1990; Miner and Sanger, 1986; Rudd et al., 1993; Selve et al., 1994). Studies were also undertaken in conscious *Suncus* to characterise the temporal pattern of emetic episodes in response to three stimuli (motion, nicotine and resiniferatoxin) causing sustained emesis.

2. Material and methods

2.1. Animals

Experiments were carried out on adult male (~ 5 months old) *Suncus murinus* (house musk shrew) with a mean body weight of 65 ± 2 g (n = 53) bred at The University of Tokyo. They were housed in groups of 3–5 in a temperature (25°C)- and humidity-controlled room on a 12 : 12 h light : dark cycle. Free access was allowed to water and pelleted food (The Central Institute for Experimental Animals, Kanagawa, Japan). Animals were not deprived of food prior to experiment and post-mortem measurements of gastric contents in animals which had not vomited revealed that the stomach of fed *Suncus* contained 0.58 ± 0.14 g (n = 12) of semi-solid food.

Animals were anaesthetised with a single intraperitoneal injection of sodium pentobarbital (30 mg/kg i.p.) and a depth of anaesthesia reached that was just sufficient to abolish the response to a pinch of the hind limb. The corneal blink response was also absent, although because of the small size of the eyes this response is not as obvious as in other species. Core temperature was monitored in some studies by a probe (Natume-1A-3624) inserted about 1.5 cm into the rectum and a mean temperature of 33.9 ± 0.4°C (n = 13) was measured. Animals were maintained at this temperature by an overhead lamp and a high (> 20°C) ambient temperature. The core temperature was measured in 10 conscious *Suncus* (body weight 64.0 ± 1.6 g and was found to be 37.1 ± 0.2°C. This is higher than in the anaesthetised animal and this lower temperature is probably due to immobility as the animal is constantly active and feeding. The value obtained in the conscious animal is consistent with that reported in another species of shrew (*Sorex fumeus*, 10 g body weight) of 37.8°C (Morrison and Ryser, 1952).

The animal was placed on its back and one needle (0.08 mm o.d., 0.06 mm i.d.) was inserted into the infra-cardiac region of the of the right thorax and another into the abdominal cavity in the midline. Pressure was measured in these regions by connecting the needles to pressure transducers (TMI MPU-0.5-290-0-III, Gould Inc, Valley View, OH, USA) via polythene cannulae filled with 154 mM NaCl. The pressures recorded were displayed on a chart recorder (WI-621G, Nihon Kohden, Tokyo).

After a basal period of about 15 min the emetic response was tested initially by gently repetitively probing the pharynx with a thin plastic cannula (Portex 4FG). Following this, the cannula was inserted into the body of the upper oesophagus and then slowly advanced down the oesophagus into the stomach with the cannula being advanced and withdrawn approximately 1 cm in each location.

At the end of the experiment the animals were killed by anaesthetic overdose (Euthatal, i.p.).

2.2. Analysis of records

Measurements of the frequency and timing of retches were made from the recordings of intra-thoracic pressure obtained at a high paper speed of 5 mm/s. All 70 bursts of retching occurring in 8 animals were used for the frequency analysis. Intra-thoracic pressure was used as in

general it gave a more reliable recording of events than intra-abdominal pressure.

Pressure changes (intra-thoracic for all episodes and intra-abdominal where possible) were analysed from episodes consisting of 5 retches (the most frequent number – see below) and values are given for the amplitude of first, third and fifth retch in the burst.

Episodes of vomiting were rare occurring on only four occasions and therefore it was not possible to undertake quantitative analysis so we have confined ourselves to a qualitative description.

2.3. Characterisation of emesis in conscious Suncus

In addition to studying the emetic response in anaesthetised animals, a number of studies were also undertaken in conscious animals to characterise the pattern of the emetic response induced by three different emetic stimuli. The stimuli were given at an ED_{100} dose and each was selected to produce an emetic response lasting several minutes. The stimuli used were motion (horizontal motion 1 Hz, 40 mm displacement, 5 min, Ueno et al., 1988, nicotine (10 mg/kg s.c., Ueno et al., 1987) and the ultrapotent capsaicin analog resiniferatoxin (Andrews and Bhandari, 1993) which in the course of related studies we noted caused an intense emetic response in *Suncus* at a dose of 100 μg/kg s.c.

Animals were observed in small Perspex cages (approx. 20 cm × 10 cm × 10 cm) and the time of each emetic episode noted by a trained observer using a digital stopwatch. Emetic episodes were characterised by a burst of retching (rhythmic abdominal movements) culminating in a single vomit (forceful oral expulsion of material). For each emetic stimulus the mean time of occurrence of the start of each episode after the first episode was calculated for groups of animals and the interval between the start of episodes was also calculated as indices of the intensity of the emetic episodes.

2.4. Drugs

Granisetron hydrochloride (a gift from SB Pharmaceuticals), 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT Sigma), morphine (Sigma) and CP-99,994 dihydrochloride salt (9 +)-(2*S*,3*S*)-3-(2-methoxybenzylamino)-2-phenylpiperidine, a gift from Pfizer Inc, USA) were dis-

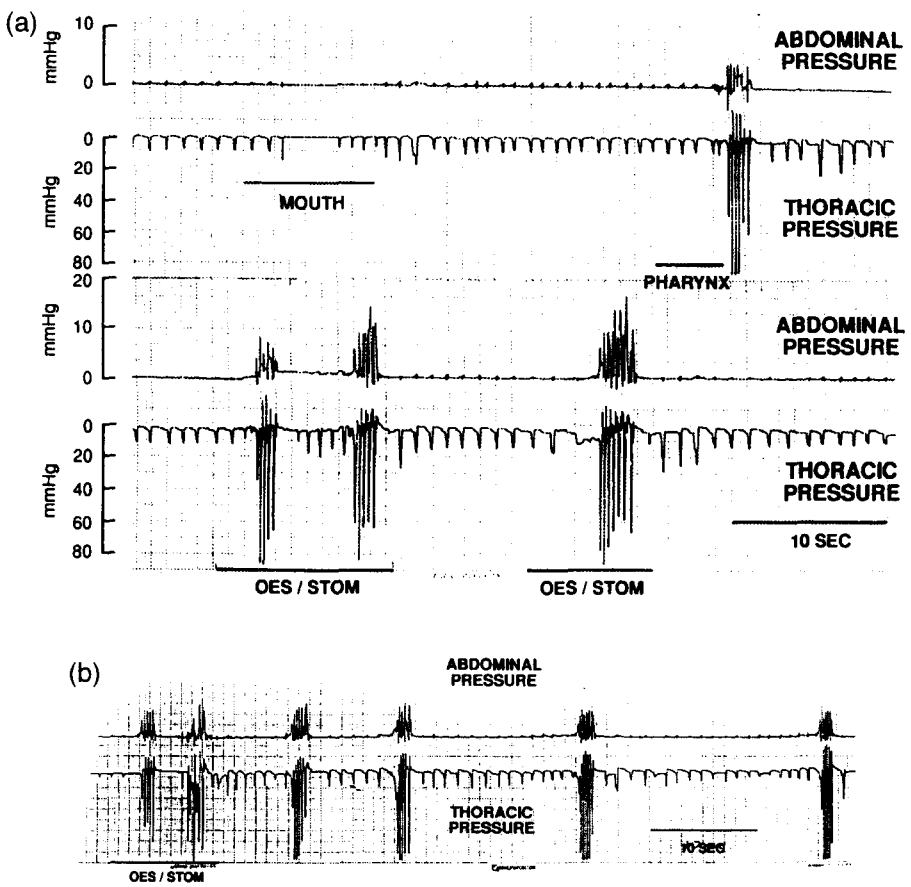

Fig. 1. Record of intra-thoracic and intra-abdominal pressure monitored in anaesthetised *Suncus*. The horizontal bars indicate mechanical stimulation of the regions indicated using a plastic rod. Note the large rhythmic oscillations in pressure indicative of retching. As Fig. 1a but note that retching continued in bursts after the initial mechanical stimulation of the distal oesophagus/stomach.

solved in 154 mM NaCl and administered subcutaneously in a volume of ~0.3 ml into the inter-scapular region. Resiniferatoxin (Sigma) was made up in Tween 80, ethanol and 154 mM NaCl. The rationale for dose selection of these drugs is reviewed in the discussion.

2.5. Statistical analysis

Results are expressed as mean ± S.E.M. (n = number of animals or observations). Significance was tested using an paired or unpaired sample Student-t test as appropriate. Correlations between the time and the occurrence of emetic episodes in the conscious animal studies were carried out using Statview 4 and Graph III software.

3. Results

3.1. Description of responses to mechanical stimulation

Placing the plastic probe in the mouth usually elicited a transient period of apnoea (Fig. 1a). Advancing the probe into the pharynx accompanied by gentle movement usually elicited repetitive swallowing unaccompanied by overt salivation. When probing was continued and made more vigorous the animal retched (Fig. 1a) and this was accompanied by movement of the tongue and ventroflexion of the head. Care was taken not to stimulate the larynx to minimise the possibility of eliciting a 'cough' response, although it is not known whether this animal is capable of coughing. Passage of the cannula through the upper oesophageal sphincter was usually associated with a brief apnoea. When respiration resumed, repetitive stimulation throughout the oesophagus and particularly when the cannula was advanced through the oesophagus into the stomach elicited multiple chains of retches sometimes followed by vomiting and oral expulsion of material. The final retch in a chain was often accompanied by wide gaping of the mouth, protrusion of the tongue and intense contraction of the anterior abdominal muscle. Although there was usually (see below) no expulsion of material, the intensity of this activity and the similarity to behaviour observed in the conscious animal is suggestive that this type of event may represent an 'unproductive vomit'. The contraction of the abdominal muscle is particularly worthy of note as during this latter event and when the animal vomited the muscle immediately below the xiphisternum contracted into a tight ball.

Retching was usually associated with profuse salivation with a pH > 9. Under these conditions retches were clearly visible with the naked eye but were at too great a frequency to count individually, although they were readily distinguishable on the pressure records (see below). Stimulation of the oesophagus or stomach evoked multiple chains of retches which often continued after cessation of oesophageal stimulation (Fig. 1b).

3.2. Quantification of pressure responses associated with retching and vomiting

3.2.1. Frequency of retching

From the records of the intra-thoracic pressure the number of retches in each burst were counted and plotted against the duration of the burst (Fig. 2). This analysis shows a linear correlation between the two parameters ($r = 0.85$) and reveals that as the number of retches in the burst increased the duration of the burst increased. From the regression equation the time/retch increases marginally. For example, in a continuous burst of 5 retches the inter-retch interval is calculated at 255 ms and for a burst of 20 retches it is 265 ms. Taking the most common number of retches/burst as 5 (see below), it can be calculated that this species retches at approximately 4 Hz or about four times the spontaneous frequency of respiration under anaesthesia (see below).

The frequency distribution of the number of retches occurring in each burst from an analysis of 70 episodes is plotted in Fig. 3 and shows that retches never occurred singly and that the most frequent number per burst was between 4 and 7 retches. Taking the number of retches in all bursts, the mean number is 7.1 ± 0.7 retches/burst with a range from 2 to 32 retches.

Although the majority of bursts of retching were continuous, about 11% showed a continual burst followed after a short delay by an additional single retch (e.g. see Fig. 1b) – a phenomenon reported in the ferret (Andrews et al., 1990a). Analysis of eight bursts showed a mean number of retches of 5.75 ± 0.41 occurring in 1.26 ± 0.86 s but inclusion of an additional retch in the burst (mean 6.75 ± 0.41) increased the time to 2.25 ± 0.21 s. Thus in these cases there is a delay of about 1 s between the main burst and the final retch.

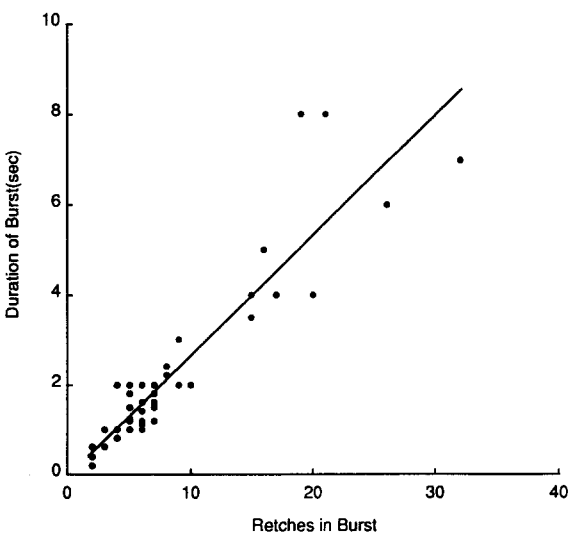

Fig. 2. The relationship between the number of retches in a burst and the duration of the burst (data points from 70 episodes of retching in 8 animals, $r = 0.85$). The emetic stimulus was mechanical stimulation of the lower oesophagus/stomach.

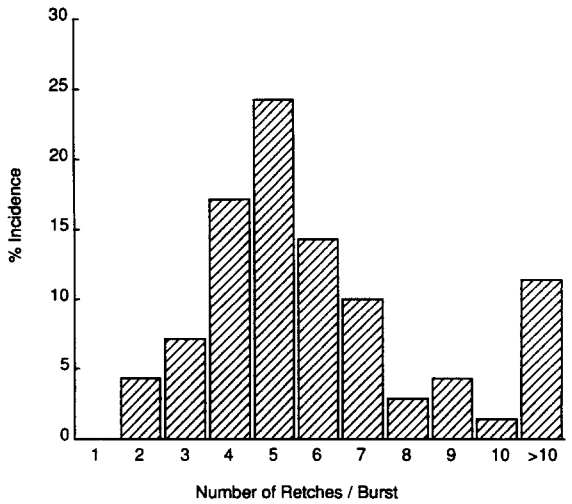

Fig. 3. The frequency distribution of the number of retches/burst from 70 episodes induced by mechanical stimulation of the lower oesophagus/stomach in 8 animals.

3.2.2. Pressure responses during retching and vomiting

Previous studies in the cat and ferret (McCarthy and Borison, 1974; Andrews et al., 1985) have monitored intra-thoracic pressure either from cannulae inserted into the superior vena cava or through the mouth into the thoracic oesophagus. The size of the animal makes the former approach difficult and the sensitivity of the animal under barbiturate anaesthesia to mechanical stimulation of the oesophagus makes the latter route impracticable. Therefore we monitored pressure via a needle inserted into the thorax via the chest wall. The major disadvantages of this technique are that the needle may modify the mechanics of the chest wall and also that the needle may become occluded by contact with the heart or lungs during the intense activity associated with retching and vomiting. Thus whilst the technique records the presence of thoracic pressure changes their magnitude and dynamics may not be identical with those recorded using less invasive methods. The results in this section should therefore be regarded more as an index of the types of change that may occur.

Retching was characterised by rapid (see above) negative going oscillations in intra-thoracic pressure. Visual inspection of the records indicated that in any burst of retches the first retch was usually the smallest – an impression that was confirmed by analysis of 10 episodes of 5 retches in six animals. The magnitude of the first retch was -40 ± 8 mm Hg, the third retch -73 ± 4 mm Hg and the fifth (last) -67 ± 4 mm Hg. For comparison, the intra-thoracic pressure excursion occurring during normal respiration in these animals was -6 ± 1 mm Hg ($n = 6$). These negative going oscillation in intra-thoracic pressure were accompanied by positive going excursions in intra-abdominal pressure: first retch 5 ± 1 mm Hg, third retch 7 ± 1 mm Hg and fifth (last) 6 ± 1 mm Hg.

Vomiting (oral expulsion of gastric contents) was only observed on four occasions in three animals and hence the description will be essentially qualitative. In each case the single vomit occurred at the end of a chain of retches ranging in number from 6 to 24 (one of the longest episodes recorded in this study) after a brief pause, the longest being 2 s. The vomit was preceded by protrusion of the tongue and licking and accompanied by wide gaping of the mouth, ventroflexion of the head and expulsion of solid material coincident with a sustained abdominal contraction. In one case the gastric contents were clearly stained yellow with bile (*Suncus* has a prominent gall-bladder embedded in the multi-lobed liver). In addition to the visual identification of vomiting it was also possible to identify vomiting from the pressure recording by the simultaneous single positive going peak in the pressure record from both the abdominal and thoracic transducers. Whilst the pressure oscillations with retching were brief (see Fig. 1a), the single peak during the vomit was of a longer duration (~ 1 s) and was accompanied by the expulsion of gastric contents (data not shown).

3.3. Effects of granisetron, morphine, CP-99,994 and 8-OH-DPAT

The 5-HT_3 receptor antagonist granisetron given at a dose of either 1 mg/kg s.c. ($n = 3$) or 5 mg/kg s.c. ($n = 3$) failed to affect overtly the swallowing or retching response to pharyngeal or oesophageal stimulation in animals tested 15 and 30 min after administration. The retching response was accompanied by overt salivation in granisetron treated as in the control animals, although it was not possible to assess whether the quantity was altered. Granisetron (1 mg/kg s.c.) was without significant effect on the respiratory rate with frequencies of 65 ± 5 breaths/min ($n = 7$ animals) being recorded in the period prior to emetic testing and 72 ± 8 breaths/min 30 min after granisetron ($n = 3$ animals). The higher dose of granisetron (5 mg/kg s.c.) was studied by direct observation and not by monitoring intra-thoracic pressure and hence respiratory frequency was not measured.

Direct observation was also used to study the effects of morphine (2 mg/kg s.c., $n = 3$ animals) and the tachykinin NK_1 receptor antagonist CP-99,994 (20 mg/kg s.c., $n = 3$ animals). Neither agent blocked the swallow, gag or retch response to pharyngeal/oesophageal probing. The retching response to pharyngeal and oesophageal probing was blocked by 8-OH-DPAT (100 μg/kg s.c.) in 3/5 animals at 15 min after treatment and in 4/5 animals 30 min after administration. In the two animals which still had a response 15 min after 8-OH-DPAT (100 μg/kg s.c.) it was noted that the intensity and duration of the stimulus required to evoke the response was greater than that required in untreated animals. In one animal the response was not blocked by 30 min after 8-OH-DPAT (100 μg/kg s.c.), although the latency of the retching response increased and

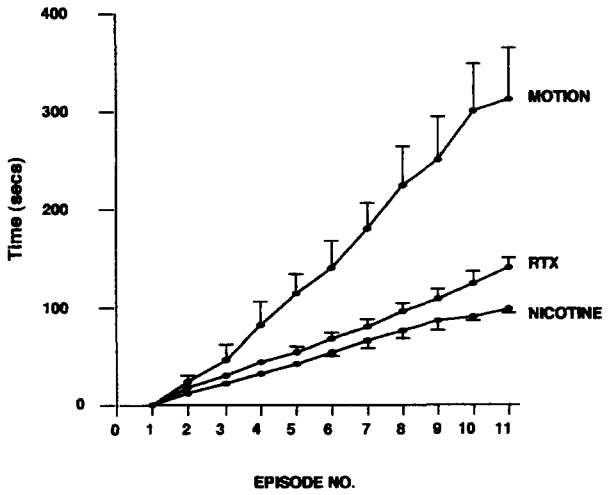

Fig. 4. The timing of the first 11 episodes of emesis in conscious *Suncus* in response to horizontal motion (1 Hz, 40 mm displacement, 5 min duration, $n = 6$ animals), nicotine (10 mg/kg s.c., $n = 11$ animals) or resiniferatoxin (100 μg/kg s.c., $n = 10$ animals). Time of occurrence is plotted as mean ± S.E.M. Where no error bar is shown it was too small to plot. (See text for details.)

more intense stimulation was required to elicit the response than in untreated animals. Both observations suggest that 8-OH-DPAT was partially effective in these animals. Animals in which the retching response was blocked appeared not to salivate in response to stimulation.

3.4. Characterisation of the emetic response in conscious Suncus to motion, nicotine and resiniferatoxin

The temporal characteristics of the emetic response were studied in conscious *Suncus* as a basis for comparison with the studies in the anaesthetised animals using mechanical stimulation. For each stimulus the time of onset of each episode of retching was noted relative to the first episode and mean times calculated for each group. Whilst in individual animals in the group episodes of retching continued beyond the time shown in Fig. 4, the analysis only continued to the point at which all animals in the group were retching. Fig. 4 shows the relationship between the retching episode number in a sequence and its time of occurrence for each stimulus. Analysis of this relationship in each animal showed that it was linear with 'r' values ranging from 0.94 to 1.0. The frequency of retching episodes was similar for nicotine and resiniferatoxin but was much lower for motion. This aspect was further analysed by comparing the intervals between the first eleven episodes of retching for each stimulus: motion, 30.4 ± 3.8 s ($n = 10$ intervals from 6 animals $P = 0.0003$ vs. resiniferatoxin and $P < 0.0001$ vs. nicotine); resiniferatoxin 14.5 ± 0.7 ($n = 10$ intervals from 10 animals, $P = 0.0001$ vs. nicotine); nicotine 10.9 ± 0.4 s ($n = 10$ intervals in 11 animals).

4. Discussion

This study shows that an emetic response can be readily induced in anaesthetised *Suncus* using mechanical stimulation of the upper gastrointestinal tract providing a novel stimulus for the investigation of the emetic pathways and their pharmacology in this species. In addition, the pattern of the emetic response in conscious *Suncus* has been characterised using three different emetic stimuli including the ultrapotent capsaicin analogue resiniferatoxin which is the most potent emetic so far investigated in *Suncus* (Andrews and Matsuki, unpublished observations). The results from this study describe experiments on both the mechanics and aspects of the pharmacology of the emetic response in *Suncus* and therefore for convenience these two aspects will be discussed separately.

4.1. The mechanics of emesis in Suncus

This study is, as far as we are aware, the first to quantify aspects of the mechanics of vomiting in an insectivore (*Suncus murinus*, the house musk shrew) and an animal of such a small size (~ 65 g), although there is a descriptive report of the occurrence of vomiting in an even smaller species of insectivore, *Sorex unguiculatus*, weighing ~ 10 g (Matsuki et al., 1993). As indicated in the results, the measurements of the magnitude of intra-thoracic and intra-abdominal pressure should be regarded with caution until more accurate techniques can be used. For this reason the discussion will be confined to measurements of the frequency of retching.

A study in the conscious ferret characterising the retching response to a wide range of emetic stimuli revealed that the median number of retches/burst was 6–9 with the maximum observed being 14 (Andrews et al., 1990a). This value is similar to that in the cat (decerebrate) with the number of retches/burst averaging 4–6 with a maximum of 14 being reported (McCarthy and Borison, 1974) and the conscious dog where a mean number of retches/vomit of 9.2 ± 1.3 was reported (Lang, 1992). It appears from the values obtained in *Suncus* of a mean number/burst of 7.1 ± 0.7 and most episodes having 4–7 retches that the average number of retches in an episode may be relatively fixed even across species. This requires substantiation by detailed measurements in a greater range of species. Such studies could provide a novel insight into the puzzling question of the function of retching (see Andrews et al., 1990b for discussion of this topic).

Few studies have directly studied the frequency at which animals retch but this aspect is commented upon sufficiently to allow some limited comparison of the results from *Suncus* with those in other species. In the dog (Monges et al., 1978; Lang, 1992) and ferret (Andrews et al., 1990a) the frequency of retching is about 1/s and in

the cat 0.8–1/s (Brizzee, 1990). In each of these species the resting respiratory frequency is similar (dog 28–30/min [MacArthur, 1987], cat 20–40/min [Hurni and Rossbach, 1987], ferret 31–36 [Pyle, 1940; Andrews et al., 1979]) at about 0.5 breaths/s whereas in *Suncus* it is about 1/s. Thus whilst the dog, cat and ferret retch at about twice their resting respiratory frequency, on average *Suncus* retches at about four times its respiratory frequency.

Studies describing the detailed pattern of emesis in conscious *Suncus* show that based on the number of emetic episodes the stimuli investigated produce responses of differing intensity. Nicotine and resiniferatoxin induced episodes every 10–15 s in contrast to the less intense response to motion where episodes occurred every 30 s. When it is considered that these times are from the onset of one episode to the onset of the next and this will include the time spent retching (~4 retches/s) as well as the vomit at the end of the retches, it can be seen that the emetic reflex can reset very rapidly when the stimulus is sufficiently intense. Further studies are required using video-tape analysis of retching and vomiting in *Suncus* to examine the precise temporal relationships between retching and vomiting so that the mechanisms responsible (eg central pattern generator) can be elucidated.

4.2. Pharmacology of the response

The 5-HT_3 receptor antagonist granisetron has been shown to reduce or block the emetic response to a range of cytotoxic drugs in a variety of species including *Suncus* (see Andrews, 1994 for review; Torii et al., 1991). In general, the anti-emetic effects of granisetron and other 5-HT_3 receptor antagonists (e.g. tropisetron and ondansetron) are only seen at higher doses (> 1 mg/kg) in *Suncus* in contrast to other species such as the ferret where doses < 1 mg/kg are required (Torii et al., 1991; Stables et al., 1987). The reason for this apparent species difference is unclear but may be another expression of the species differences already reported between 5-HT_3 receptors in the ferret, rat, rabbit and mouse (Butler et al., 1990; Newberry et al., 1991). The apparent lack of effect of granisetron on the emetic response to mechanical stimulation was not unexpected as the 5-HT_3 receptor antagonists are not considered to have a 'broad-spectrum' anti-emetic action. It was not possible to study the pathway involved in the emetic response to oesophageal and gastric distension as simultaneous bilateral cervical vagotomy caused apnoea resulting in death (unpublished observations). However, stimulation of mucosal or muscle mechanoreceptors with afferents in the vagus (Grundy and Scratcherd, 1989) projecting to the nucleus tractus solitarius in the brainstem provides the most likely mechanism. Although 5-HT_3 receptors are located both pre-synaptically in vagal afferents and post-synaptically in the nucleus tractus solitarius and area postrema (Pratt and Bowery, 1989; Leslie et al., 1990), emesis induced by electrical stimulation of abdominal vagal afferents is not blocked by 5-HT_3 receptor antagonists in the cat or ferret (Milano et al., 1990, Andrews et al., 1990b). In addition, whilst the predominant site of anti-emetic action of the 5-HT_3 receptor antagonists against both radiation and cytotoxic drugs is considered to be peripheral (Andrews, 1994), neurophysiological studies in the ferret have shown that neither granisetron nor ondansetron blocks the activation of muscle or mucosal receptors by mechanical stimulation (Andrews and Davidson, 1990; Blackshaw and Grundy, 1993).

The apparent lack of effect of the tachykinin NK_1 receptor antagonist CP-99,994, even at a high dose of 20 mg/kg, was surprising as this agent has a broad spectrum anti-emetic effect in other species and is proposed to have a site of action in the brainstem (e.g. ferret, dog, Bountra et al., 1993; Tattersall et al., 1993; Watson et al., 1995). However, the previous studies did not test the effect of CP-99,994 on the emetic response induced by mechanical stimulation of the gut, although Watson et al. (1995) noted that the gag reflex evoked by pharyngeal probing was unaffected. It is possible that in *Suncus* CP-99,994 differentially affects brainstem pathways processing visceral mechanoreceptor as opposed to chemoreceptor information. A similar explanation could apply to the lack of effect of morphine which previous studies in *Suncus* have shown can block the emetic response to systemic nicotine and serotonin (Selve et al., 1994). The 5-HT_{1A} receptor agonist 8-OH-DPAT and other agents active at this receptor (e.g. buspirone, flesinoxan) have been shown to reduce or block emesis induced by apomorphine, cisplatin, copper sulphate, morphine, motion and xylazine (Lucot, 1990, 1992, 1994; Lucot and Crampton, 1987; Rudd et al., 1992, 1993). This study extends the list by demonstrating that the emetic response to mechanical stimulation of the upper gut can also be blocked by 8-OH-DPAT, presumed to be acting on 5-HT_{1A} receptors at this dose (see Lucot, 1994 and Okada et al., 1994 for discussion). The dose of 8-OH-DPAT at which the anti-emetic effect in *Suncus* was observed is worthy of comment as it is similar to the dose that markedly reduces motion-induced emesis in the cat (Lucot, 1992). In contrast, in the ferret the doses of 8-OH-DPAT of 250–750 μg/kg/s.c. are required for the anti-emetic effects and with the exception of the response to apomorphine, full blockade of the response is still not achieved even at the dose of 750 μg/kg (Rudd et al., 1992). Although we have not studied the full dose-response curve for the anti-emetic effects of 5-HT_{1A} receptor agonists in *Suncus*, the preliminary results from this study indicate that this species may be particularly suitable for investigating the involvement of 5-HT_{1A} receptors in emesis. This is supported by the recent study of Okada et al., 1994 in *Suncus* which demonstrated a blockade of emesis induced by veratrine, motion, nicotine copper sulphate and cisplatin by a number of 5-HT_{1A} receptor agonists (e.g. 8-OH-DPAT, buspirone and SUN 8399).

The site of the anti-emetic effects of 8-OH-DPAT is argued to be in the brainstem because this is the convergence point for the pathways involved in the induction of the various types of emesis. In the context of the present study the most likely sites are either presynaptic receptors (inhibitory) located on the terminals of the abdominal vagal afferents projecting to the nucleus tractus solitarius or post-synaptic (inhibitory) located within the nucleus tractus solitarius where 5-HT_{1A} binding sites and receptor mRNA have been demonstrated (Lucot, 1992). The results from the present study are consistent with the demonstration of a reduction by 8-OH-DPAT of fictive emesis induced in the decerebrate cat by electrical stimulation of vagal afferents (Milano and Grelot, 1992).

In conclusion, this study has demonstrated that retching can be readily induced in anaesthetised *Suncus* by mechanical stimulation of the upper gastrointestinal tract providing a novel stimulus for the study of the pharmacology of the emetic pathway. The response to mechanical stimulation was blocked by a 5-HT_{1A} receptor agonist but not by morphine, a 5-HT_3 or a tachykinin NK_1 receptor antagonist. In conscious *Suncus* the ultrapotent capsaicin analogue resiniferatoxin is identified as a novel emetic agent producing an emetic response of similar intensity to nicotine but more intense than motion.

Acknowledgements

The authors would like to thank SmithKline Beecham and Merck Sharpe & Dohme for financial support.

References

Andrews, P.L.R., 1994, 5-HT_3 receptor antagonists and antiemesis, in: 5-Hydroxytryptamine$_3$ Receptor Antagonists, eds. F.D. King, B.J. Jones and G.J. Sanger (CRC Press, Boca Raton, USA) p. 255.

Andrews, P.L.R., P. Bhandari, 1993, Resiniferatoxin, an ultrapotent capsaicin analogue, has anti-emetic properties in the ferret, Neuropharmacology 32, 799.

Andrews, P.L.R. and H.I.M. Davidson, 1990, Activation of vagal afferent terminals by 5- hydroxytryptamine is mediated by the 5-HT_3 receptor in the anaesthetised ferret, J. Physiol. 422, 92P.

Andrews, P.L.R. and C.J. Davis, 1993, The mechanism of emesis induced by anti-cancer therapies, in: Emesis in Anti-cancer Therapy, Mechanisms and Treatment, eds. P.L.R. Andrews and G.J. Sanger (Chapman and Hall Medical, London, UK) p. 113.

Andrews, P.L.R., A.J. Bower and O. Illman, 1979, Some aspects of the physiology and anatomy of the cardiovascular system of the ferret, Lab. Anim. 13, 215.

Andrews, P.L.R., S. Bingham and C.J. Davis, 1985, Retching evoked by stimulation of abdominal vagal afferents in the anaesthetised ferret, J. Physiol. 358, 103P.

Andrews, P.L.R. and P.B. Bhandari, S. Garland, S. Bingham, C.J. Davis, H.I.M. Davidson, R.R. Roylance and S. Lane, 1990a, Does retching have a function?: an experimental study in the ferret, Pharmacodyn. Ther. (Life Sci. Adv.) 9, 135.

Andrews, P.L.R., C.J. Davis, S. Bingham, H.I.M. Davidson, J. Hawthorn and L. Maskell, 1990b, The abdominal visceral innervation and the emetic reflex: pathways, pharmacology and plasticity, Can. J. Physiol. Pharmacol. 68, 325.

Blackshaw, A. and D. Grundy, 1993, Effects of 5-hydroxytryptamine on discharge of vagal mucosal afferent fibres from the upper gastrointestinal tract of the ferret, J. Auton. Nerv. Syst. 45, 41.

Bountra, C., D.B. Briggs, T. Dale, C. Gradner, C. Jordan, D. Twissell and P. Ward, 1993, Anti-emetic profile of a non-peptide neurokinin NK_1 receptor antagonist, CP-99,994, in ferrets, Eur. J. Pharmacol. 249, R3.

Brizzee, K.R., 1990, Mechanics of vomiting: a minireview, Can. J. Physiol. Pharmacol. 68, 221.

Butler, A., C.J. Elswood, J. Burridge, S. Ireland, K.T. Bunce, G.J. Kilpatrick and M.B. Tyers, 1990, The pharmacological characterization of 5-HT-3 receptors in three isolated preparations derived from guinea-pig tissues, Br. J. Pharmacol. 101, 591.

Grundy, D.G. and T.S. Scratcherd, 1989, Sensory afferents from the gastrointestinal tract, in: Handbook of Physiology, Section 6, Vol. 1 (Part 1), ed. J.D. Wood (American Physiological Society, Bethesda, MD, USA) p. 593.

Hurni, H. and W. Rossbach, 1987, The laboratory cat, in: The UFAW Handbook on the Care and Management of Laboratory Animals, 6th edn., ed. T. Poole (Longman Scientific and Technical, UK) p. 476.

Kaji, T., H. Saito, S. Ueno, T. Yasuhara, T. Nakajima and N. Matsuki, 1991, Role of histamine in motion sickness in *Suncus murinus*, Aviat. Space Environ. Med. 62, 1054.

Lang, I.M., 1990, Digestive tract motor correlates of nausea and vomiting, Can. J. Physiol. Pharmacol. 68, 242.

Lang, I.M., 1992, New perspective on the mechanisms controlling vomitus expulsion, in: Mechanisms and Control of Emesis, eds. A.L. Bianchi, L. Grelot, A.D. Miller and G.L. King (Colloque INSERM, John Libbey Eurotext) Vol. 223, p. 71.

Leslie, R.A., D.J.M. Reynolds, P.L.R. Andrews, G.G. Grahame-Smith, C.J. Davis and J.M. Harvey, 1990, Evidence for presynaptic 5-hydroxytryptamine$_3$ recognition sites on vagal afferent terminals in the brainstem of the ferret, Neuroscience 38, 667.

Lucot, J.B., 1990, Effects of serotonin antagonists on motion sickness and its suppression by 8-OH-DPAT in cats, Pharmacol. Biochem. Behav. 37, 283.

Lucot, J.B., 1992, Prevention of motion sickness by 5-HT-1A agonists in cats, in: Mechanisms and Control of Emesis, eds. A. Bianchi, L. Grelot, A.D. Miller, G.L. King (Colloque INSERM, John Libbey, Eurotext) Vol. 223, p. 195.

Lucot, J.B., 1994, Antiemetic effects of flexsinoxan in cats: comparisons with 8-hydroxy-2-(di-*n*-propylamino) tetralin, Eur. J. Pharmacol. 253, 53.

Lucot, J.B. and G.H. Crampton, 1987, 8-OH-DPAT suppresses vomiting in the cat elicited by motion, cisplatin or xylaxine, Biochem. Behav. 33, 627.

MacArthur, J.A., 1987, The dog, in: the UFAW Handbook on the Care and Management of Laboratory Animals, 6th edn., ed. T. Poole (Longman Scientific and Technical, UK) p. 456.

Matsuki, N., Y. Torii, T. Kji, N. Nishiyama, J. Ishiyama, N. Chida and H. Saito, 1993, Emetic responses of *Sorex unguiculatus*, Exp. Anim. 225.

McCarthy, L.E. and H.L. Borison, 1974, The respiratory mechanics of vomiting in decerebrate cats, Am. J. Physiol. 266, 738.

Milano, S. and L. Grelot, 1992, Differential blocking effects of buspirone and 8-OH-DPAT on vagal-induced emesis in decerebrate cats, in: Mechanisms and Control of Emesis, eds. A.L. Bianchi, L. Grelot, A.D. Miller and G.L. King (Colloque INSERM, John Libbey, Eurotext) Vol. 223, p. 353.

Milano, S., L. Grelot, Z. Chen and A.L. Bianchi, 1990, Vagal-induced vomiting in decerebrate cat is not suppressed by specific 5-HT-3 receptor antagonists, J. Auton. Nerv. Syst. 31, 109.

Miller, A.D., 1990, Respiratory muscle control during vomiting, Can. J. Physiol. Pharmacol. 68, 237.

Miner, W. and G.J. Sanger, 1986, Inhibition of cisplatin vomiting by

selective 5-hydroxytryptamine-M receptor antagonism, Br. J. Pharmacol. 88, 497.

Monges, H., J. Salducci and B. Naudy, 1978, Dissociation between the electrical activity of the diaphragmatic dome and crura muscular fibres during esophageal distention, vomiting and eructation. An electromyographic study in the dog, J. Physiol. (Paris) 74, 541.

Morrison, P.R. and F.A. Ryser, 1952, Weight and body temperature in mammals, Science 116, 231.

Mutoh, M., H. Imananishi, Y. Torii, M. Tamura, H. Saito and N. Matsuki, 1992, Cisplatin-induced emesis in *Suncus murinus*, Jpn. J. Pharmacol. 58, 321.

Newberry, N.R., S.H. Cheshire and M.J. Gilbert, 1991, Evidence that the 5-HT_3 receptors of the rat, mouse and guinea-pig superior cervical ganglion may be different, Br. J. Pharmacol. 102, 615.

Okada, F., Y. Torii, H. Saito and N. Matsuki, 1994, Anti-emetic effects of serotonergic 5- HT-1A receptor agonists in *Suncus murinus*, Jpn. J. Pharmacol. 64, 109.

Pratt, G.D. and N.G. Bowery, 1989, The 5-HT-3 ligand [3H] BRL43694 binds to presynatic sites in the nucleus tractus solitarius of the rat, Neuropharmacology 28, 1367.

Pyle, N.J., 1940, The use of ferrets in laboratory and research investigations, Am. J. Pub. Health 30, 787.

Rudd, J.A., K.T. Bunce and R.J. Naylor, 1992, The effect of 8-OH-DPAT on drug-induced emesis in the ferret, Br. J. Pharmacol. 106, 101P.

Rudd, J.A., K.T. Bunce and R.J. Naylor, 1993, The effect of 5-HT-1A receptor ligands on copper sulphate-induced emesis in the ferret, Br. J. Pharmacol. 110, 99P.

Selve, N., E. Friderichs, W. Reimann, and S. Reinartz, 1994, Absence of emetic effects of morphine and loperamide in *Suncus murinus*, Eur. J. Pharmacol. 256, 287.

Stables, R., P.L.R. Andrews, H.E. Bailey, B. Costall, S.J. Gunning, J. Hawthorn, R.J. Naylor and M.B. Tyers, 1987, Antiemetic properties of the $5HT_3$ receptor antagonist, GR38032F, Cancer Treat. Rev. 14, 333.

Tattersall, F.D., W. Rycroft, J. Hargreaves and R.G. Hill, 1993, The tachykinin NK_1 receptor antagonist CP-99,994 attenuates cisplatin-induced emesis in the ferret, Eur. J. Pharmacol. 250, R5.

Torii, Y., H. Saito and N. Matsuki, 1991, 5-hydroxytryptamine is emetogenic in the house musk shrew, *Suncus murinus*, Naunyn-Schmied. Arch. Pharmacol. 344, 564.

Torii, Y., M. Mutoh, H. Saito and N. Matsuki, 1993, Involvement of free radicals in cisplatin-induced emesis in *Suncus murinus*, Eur. J. Pharmacol. Env. Toxicol. Pharmacol. 248, 131.

Ueno, S., N. Matsuki and H. Saito, 1987, *Suncus murinus*: a new experimental model in emesis research, Life Sci. 41, 513.

Ueno, S., N. Matsuki and H. Saito, 1988, *Suncus murinus*: a new experimental model for motion sickness, Life Sci. 43, 413.

Watson, J.W., S.F. Gonsalves, A.A. Fossa, S. McLean, T. Seeger, S. Obach and P.L.R. Andrews, 1995, The anti-emetic effects of CP-99,994 in the ferret and the dog: role of NK_1 receptors, Br. J. Pharmacol. 115, 84.