The abdominal visceral innervation and the emetic reflex: pathways, pharmacology, and plasticity

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Ces dernières années, le rôle de l’area postrema dans le réflexe émétique a été prédominant et l’implication de l’innervation viscérale abdominale plutôt négligée. Cet article tente de rétablir l’équilibre en révisant la littérature disponible sur le sujet et en présentant des études originales sur le ferret. Devant la perspective de généralisation de l’utilisation du ferret dans l’étude de l’émèse et particulièrement dans la caractérisation des actions anti-émétiques de l’antagoniste des récepteurs de 5-HT₃, on évalue l’apport de l’emploi de cette espèce pour l’étude de l’émèse. On conclut que le ferret est sensible à une vaste gamme de stimuli émétiques incluant les irritants intra-gastriques, les agonistes des récepteurs dopaminergiques et opiacés, plusieurs drogues cytotoxiques et la radiation. Le ferret est plus sensible que les autres espèces à de nombreux stimuli et, pour ce qui est de la radiation, il semble, d’après son ED₅₀₀, comme le plus sensible des animaux de laboratoire étudiés. La stimulation électrique de l’extrémité centrale de tronc pneumogastrique abdominal postérieur d’animaux anesthésiés et conscients a permis d’observer que les nerfs afférents vagaux pouvaient déclencher l’émèse. Dans des études de lésions, une implication du nerf vague dans la réponse émétique à un certain nombre de drogues cytotoxiques (par ex., cisplatine, cyclophosphamide, mustine) et à la radiation a été démontrée, bien que l’effet ait varié selon les stimuli. On tente de faire un rapprochement entre ces études et des études antérieures des effets de l’ablation de l’area postrema. Les problèmes d’interprétation des effets des lésions nerveuses sont discutés en tenant compte des faits présentés ici, indiquant qu’il pourrait y avoir un degré de plasticité dans la voie émétique suite à de telles lésions. On fait une révision des différents effets anti-émétiques des antagonistes des récepteurs de 5-HT₃ et une tentative d’identification de leur(s) site(s) d’action. Les résultats présentés suggèrent un lien entre le nerf vague et l’antagonisme des récepteurs de 5-HT₃. On discute de ces études et...
Introduction

Ingestion of food is one of the main routes by which a toxin can enter the body to cause harm to the animal, and it is therefore appropriate that the body should have protective reflexes to prevent such ingestion or to remove the agent if ingested. Using this approach, Davis et al. (1986) proposed that nausea and vomiting could be viewed as components of a hierarchical organized defence mechanism for the protection of the body against toxins. This model proposed that defence mechanisms operate at three levels and is discussed in some detail here, as it is only by viewing the vomiting reflex in a biological context that the role of the abdominal vagus and in particular the afferent nerves can be fully appreciated.

(i) Potential foods are identified as safe or hazardous before swallowing based on colour (very brightly coloured animals and fruits are often avoided), smell and taste (decaying meat and bitter foods are avoided), and individual or cultural learning. The degree of importance of each will depend upon the visual, olfactory, or gustatory discrimination in each species. For example, birds appear to place more reliance on visual cues than the rat, which is more reliant on gustatory information (Wilcoxon et al. 1971). When a toxin is identified at this level it may lead to rejection of the food (e.g., spitting out, salivation, and facial changes seen in babies when given sour or bitter substances), the induction of a learned aversion to that substance, or even the immediate induction of nausea to prevent further ingestion and facilitate the development of the aversion.

Unfortunately, many toxins are not brightly coloured nor have a bitter taste, and even these cues can be masked by other foods ingested simultaneously. In addition, the feeding habits of some animals, particularly the predatory carnivores which tend to bite and swallow their food rapidly with little chewing, minimize the time for detection of potential toxins. From these considerations it is clear that some form of postingestion toxin detection and elimination system would be an advantage.

(ii) Once the toxin contained in the food enters the stomach it can be dealt with in two main ways: it may be either rapidly ejected from the body by vomiting or possibly diarrhea in some cases or it may be neutralized by gastric acid and enzymes and diluted by gastrointestinal secretions. Afferent systems are available for the detection of toxins in the upper digestive tract and their activation can evoke the visceral and somatic components of the vomiting reflex and contribute to the genesis of nausea. In addition, conditioned taste aversions can be induced by activation of abdominal vagal afferents.

It is important that the toxin is recognized before appreciable gastric emptying has occurred, as this is likely not only to minimize absorption but also as it is the stomach that is most subject to compression during the expulsive phase of emesis the toxin can be most effectively ejected if it is still in the stomach.

Indeed the gastrointestinal motility changes occurring prior to emesis play an important role in confining gastric contents, with the two most important motility changes being relaxation of the proximal stomach and retroperistalsis of the small intestine (see Lang 1990). The former will delay gastric emptying and confine gastric contents to the proximal stomach. The function of the large retroperistaltic contraction passing from the jejunoileal region to the stomach prior to vomiting is presumably to sweep the bowel of any contaminated gastric contents that have already entered the intestine.

(iii) Considerable experimental evidence has been presented to show that a toxin detection system is present at a postabsorptive site, namely the area postrema, also often described as the chemoreceptor trigger zone for vomiting. This circumventricular organ is located at the caudal extremity of the fourth ventricle and is located outside the conventionally defined blood—brain and cerebrospinal fluid (CSF) — brain barriers, structural features that must enhance its chemoreceptor function (Leslie 1986). There is little doubt that activation of this region by agents in the circulation (dopamine D2 receptor agonists) or CSF (adrenaline) can induce emesis and probably nausea, but it is puzzling why a toxin detection system should be located at a postabsorptive site within the CNS, which can only serve to indicate that absorption has occurred and at a time when the toxin is probably having toxic effects throughout the body.

This systemic detector can be viewed in several ways; first as a “back-up” or reserve system, second as an additional system with a greater sensitivity to emetic toxins than the gut detectors; thirdly the response to toxins including emesis may be a reflection of a general chemoreceptor role for the area postrema rather than as a specific detector for emetic agents. We cannot yet distinguish between these possibilities, but there is now a considerable body of evidence showing that the area postrema has a more general chemosensitive role, being implicated in the regulation of blood pressure, fluid intake, sodium balance, food intake, respiration, and arousal (see Leslie (1986) for references). A full discussion of the role of the area postrema in vomiting is outside the scope of this review but the topic has been dealt with recently by several authors (Borison 1989; Leslie 1986; Andrews and Hawthorn 1988).

While the area postrema is the only postabsorptive site that has been implicated in emesis, an additional possible site might be the visceral afferents with receptive fields in the distribution of the hepatic portal vein.

From the above discussion it is clear that the prime functions of nausea and vomiting are to prevent further ingestion of food perceived as harmful (both in the short term and in the long term by the development of a conditioned aversion) and to eject any toxin already ingested. But how does this relate to nausea and vomiting induced by clinical conditions? In essence, all that is required is for the stimulus to be able to activate one of the above systems; in some cases the emetic response is relevant (i.e., it leads to the removal of the stimulus) but in other cases it is not. This difference can best be
Nausea occurs when there is gastric stasis (e.g., diabetic gastroparesis), and vomiting is induced probably by gastric distension, leading to removal of the stimulus and alleviation of the nausea. However, in the case of anticancer chemotherapy where cytotoxic drugs (e.g., cisplatinum) are given intravenously and emesis is evoked by the above mechanisms, the vomiting, which may last for days, serves no purpose as the stimulus remains in the system. But of course the emetic system evolved to cope with toxins entering the body via the mouth not the cardiovascular system!

From the above discussion it can be seen that the vomiting reflex is essentially centered around the gastrointestinal tract and is organized to detect, confine, and expel toxins before substantial absorption has occurred. The gastrointestinal tract receives an extensive innervation from the vagus and splanchnic nerves and a number of studies have implicated these nerves in emesis induced by a variety of agents including gastrointestinal irritants, bacterial toxins, radiation, and cytotoxic drugs. This paper reassesses the role of the abdominal innervation in emesis by using results from our own studies on the ferret reported here in conjunction with a review of the literature. After discussing the function of the visceral innervation, we review preliminary studies implicating vagal afferents as a major target site for the action of the novel 5-HT3 (5-HT, 5-hydroxytryptamine) receptor antagonist class of antiemetic agents. Finally evidence is presented to suggest that there is plasticity in the organization of the emetic system.

The use of the ferret as an animal model for the study of emesis

The ferret (Mustela putorius furo L.) is a small (<2.5 kg) carnivorous mammal, closely related to the mink, polecat, weasel, and pine marten, and has been used for the past 90 years in a variety of research areas (see Andrews (1988) for review). In the past 10 years it has been used for the study of the emetic reflex, with the first publication of which we are aware being on the effects of cisplatinum (Florczyk et al. 1981). From our own studies and those reported in the literature we can now produce a relatively comprehensive list of the emetic agents to which the ferret is sensitive. These are shown in Tables 1 and 2.

The results with two of these agents, apomorphine and intragastric irritants, will be discussed in detail, as historically they have been used as diagnostic tests for the integrity of the central (area postrema) and peripheral (abdominal visceral nerves) components of the emetic pathway. In addition, we include results on the pattern of emesis induced by a number of cytotoxic drugs in view of the renewed interest in the design of antiemetic agents for clinical use.

Intragastric irritants

Copper sulphate

Copper sulphate is probably the stimulus most commonly used experimentally to induce emesis by activation of predominantly peripheral emetic pathways. The dose-related nature of the emetic response to copper sulphate is shown in Fig. 1 and illustrates that while the number of retches and vomits are affected by dose, the latency of onset of the response is relatively independent of dose (see NaCl below). Examination of the profile of the emetic response reveals that while this response lasts about 15 min, the majority of retches and vomits occur in the first 6 min, a pattern consistent with the anticipated rapid expulsion of an irritant from the stomach.

Hypertonic stimuli

Hypertonic stimuli, particularly in the form of sea water, have long been used as emetic agents in a clinical context on an empirical basis, but surprisingly little is known of the concentration required or even whether it is actually the osmolarity of the solution that is important.

It is necessary to understand which components of the stimulus are actually responsible so that an accurate assessment can be made of the nature of the gastrointestinal receptors responsible.

We addressed this question by constructing dose–response curves for sodium chloride (154 mM – 1.0 M) and glucose (308 mM – 2.0 M). The threshold for NaCl was 500 mM and the ED100 was 750 mM whereas for glucose the values were 1.0 and 2.0 M, respectively, suggesting that it is the osmolarity of the solution that is the emetic trigger. Emetic responses were also induced by intragastric administration of 1 M KCl, 1 M choline chloride, and 2 M mannitol at latencies similar to sodium chloride and copper sulphate.

The emetic responses to all the intragastric stimuli at the ED100 dose are summarized in Fig. 2. The latencies to vomiting range between 4 and 12 min. The actual retching and vomiting show considerable variation, with the standard intragastric agent, copper sulphate, in fact being the least emetic.

These studies show that a range of agents placed in the stomach can rapidly activate the emetic reflex leading to their ejection from the body. The site within the gut at which they act is uncertain but experiments in pyloric ligated ferrets give some insight into the site of action of 1 M NaCl. In ferrets starved overnight and in which the pylorus was occluded with a ligature 24 h earlier, 1 M NaCl induced a full emetic response with a normal latency when introduced into the stomach. Vomiting was not induced by 154 mM NaCl in these animals, and they were able to drink 50 mL of milk without vomiting. Studies in the urethane-anaesthetized ferret demonstrated that application of 1 M NaCl to the gastric antrum evoked retching (Andrews and Wood 1988). These studies only indicate that the stomach is a site at which hypertonic solutions can act to induce emesis, not that it is the only site. This view is supported by the studies of Blackshaw et al. (1987) which show the induction of retching in the urethane-anaesthetized ferret by duodenal perfusion with hypertonic solutions. Little is known of the site of action of the other osmotically active agents, but it seems reasonable to assume that they act in the same region as sodium chloride (see below). Studies in the dog have shown that while the stomach and upper duodenum are sites from which emesis can be induced by copper sulphate, the lower duodenum is the most sensitive site (Kayashima et al. 1978).

While there is little doubt that hypertonic solutions and copper sulphate in the stomach can induce nausea and vomiting in animals and humans (Barker et al. 1974; Meester 1980), it is highly unlikely that appreciable quantities of any of the agents used here would be ingested as they would probably be rejected on grounds of taste. Luminal contents may become quite hypertonic following a meal as the food is digested and this may contribute to the nausea and vomiting occasionally experienced following a large meal. While the commonly used intragastric stimuli do not represent stimuli encountered in the...
Apomorphine investigated the dose–response relationship of apomorphine given by subcutaneous and intravenous routes. A preliminary account of this work has already been presented (Andrews et al. 1986). Animals first started responding (retching with and without vomit) at a dose of 25 μg/kg given subcutaneously, with the ED₁₀₀ being 100 μg/kg. The characteristics of the response are summarized in Fig. 3. The latency of the response was typically a few minutes and it subsided in the absence, there was a tendency for the total number of retches and vomits to decrease and for the latency of the response to increase. In addition, the incidence of retching and vomiting indicates that retching is a more reliable indicator of an emetic response to apomorphine than is vomiting. These challenges were interspersed with tests using a subemetic dose of apomorphine (30 μg/kg) and injections of 154 mM NaCl. No animals had an emetic response to these stimuli.

Apomorphine was also administered intravenously over the dose range of 10–500 μg/kg. Surprisingly we were unable to find an ED₁₀₀: the best response being in three out of eight animals tested with a dose of 100 μg/kg when the response had a latency of 5 ± 2 min.

The emetic effects of apomorphine were abolished by domperidone (1 mg/kg s.c.) and metoclopramide (5 mg/kg s.c.; note this high dose was used to make this study comparable with our studies on the antiemetic effects of high dose metoclopramide against radiation and cytotoxic vomiting reported below). In a group of ferrets previously shown to respond to apomorphine, the emetic response was abolished by area postrema ablation (1 month prior to challenge) when the animals were tested with 100 and 200 μg/kg s.c. Bilateral abdominal vagotomy was without significant effect on the response to apomorphine (100 μg/kg s.c.; control retches, 30 ± 5; vagotomy, 21 ± 3).

The results from the lesion and drug studies are consistent with apomorphine having its emetic action via the area postrema and this is supported by the studies of Higgins et al. (1989) showing the induction of emesis by injection of 10 μg apomorphine into the fourth ventricle of the ferret. The reason for the differences in the emetic responses between subcutaneous and intravenous administration of apomorphine is unclear, but similar route-dependent differences have been reported for apomorphine stereotypy in the rat (Melzacka et al. 1979). The poor emetic response of the ferret to apomorphine when given intravenously has led some authors to the erroneous conclusion that the ferret is relatively insensitive particularly in comparison to the dog (e.g., Gyllys et al. 1988). The problem of apomorphine sensitivity in the ferret is complicated by studies using excessive doses (5 mg/kg; Schurig et al. 1984), which in our hands produce serious behavioural disturbances. There

<table>
<thead>
<tr>
<th>Subcutaneous</th>
<th>Intravenous or intraperitoneal</th>
<th>Per oral</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apomorphine</td>
<td>Adriamycin</td>
<td>Calcium glucuronide&lt;sup&gt;5&lt;/sup&gt;</td>
<td>X-radiation</td>
</tr>
<tr>
<td>Lisuride&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Apomorphine</td>
<td>Choline chloride</td>
<td>Vagal stimulation</td>
</tr>
<tr>
<td>Loperamide</td>
<td>Cisplatinum</td>
<td>Copper sulphate</td>
<td></td>
</tr>
<tr>
<td>Morphine</td>
<td>Cisplatin analogues</td>
<td>Emetine</td>
<td></td>
</tr>
<tr>
<td>Morphine-6-glucuronide</td>
<td>Cyclophosphamide</td>
<td>Glucose</td>
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<td></td>
<td>Cycloheximide</td>
<td>Ipecacuana</td>
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<tr>
<td></td>
<td>Diacetoxyscirpinol</td>
<td>Mannitol</td>
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<td></td>
<td>Emetine</td>
<td>Potassium chloride</td>
<td></td>
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<tr>
<td></td>
<td>Enkephalin&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Sodium chloride</td>
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<td></td>
<td>Hyperammonemia&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Syrup B.P.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mustine</td>
<td>Veratridine</td>
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<tr>
<td></td>
<td>para-chlorophenylalanine</td>
<td></td>
<td></td>
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<td></td>
<td>Trimelanol&lt;sup&gt;4&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Urethane</td>
<td></td>
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</tbody>
</table>

**Table 1. Effective emetic agents in the ferret**

Note: References for the above are found in the text or are the unpublished observations by the authors of this paper. In addition: 1. Costall et al. 1989; 2. G. L. King, personal communication; 3. Desmukh and Slope 1983; 4. Bermudez et al. 1988; 5. Basu and Passaro 1972; B.P., British Pharmacopoeia.

**Table 2. The rank order of emetic sensitivity of various species to different emetic stimuli**

<table>
<thead>
<tr>
<th>Radiation</th>
<th>Cisplatin</th>
<th>Mustine</th>
<th>Copper sulphate</th>
<th>Apomorphine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>Ferret</td>
<td>Human</td>
<td>Human</td>
<td>Human</td>
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<tr>
<td>Human</td>
<td>Dog</td>
<td>Human</td>
<td>Dog</td>
<td>Human</td>
</tr>
<tr>
<td>Dog</td>
<td>Monkey</td>
<td>Cat</td>
<td>Cat</td>
<td>Cat</td>
</tr>
<tr>
<td>Cat</td>
<td>Ferret</td>
<td>—</td>
<td>—</td>
<td>Monkey (unresponsive)</td>
</tr>
</tbody>
</table>

In the natural world, they clearly demonstrate the capacity that stimulation of the gut has for activating emesis. The relevance of these experiments to understanding emesis induced by ingested plant and animal toxins is discussed later.

**Apomorphine**

The dopamine D<sub>2</sub> receptor agonist, apomorphine, is probably the most commonly used experimental emetic agent and has been used as a standard against which the efficacy of various antiemetic agents has been tested. In the ferret we investigated the dose–response relationship of apomorphine given by subcutaneous and intravenous routes. A preliminary account of this work has already been presented (Andrews et al. 1986). Animals first started responding (retching with and without vomit) at a dose of 25 μg/kg given subcutaneously, with the ED₁₀₀ being 100 μg/kg. The characteristics of the response are summarized in Fig. 3. The latency of the response was typically a few minutes and it subsided in about 15 min. In view of the requirement to give animals multiple challenges with apomorphine over several weeks, we tested the reproducibility of the emetic response to apomorphine at a dose of 100 μg/kg in a group of five animals tested weekly over 10 weeks. The results from this study are summarized in Fig. 4 and show that while the response was reproducible in terms of presence or absence, there was a tendency for the total number of retches and vomits to decrease and for the latency of the response to increase. In addition, the incidence of retching and vomiting indicates that retching is a more reliable indicator of an emetic response to apomorphine than is vomiting. These challenges were interspersed with tests using a subemetic dose of apomorphine (30 μg/kg) and injections of 154 mM NaCl. No animals had an emetic response to these stimuli.

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does appear to be a genuine discrepancy in the literature regarding the ferret’s responsiveness to apomorphine, with animals in North America being less responsive than those in England. For example, King (1988) in the USA reported response rates of 47 and 31% with doses of 100 and 300 μg/kg s.c., respectively, whereas in England three separate groups have reported reliable short latency responses to apomorphine at doses of 100, 200, and 250 μg/kg (Andrews et al. 1986; Miner et al. 1987; Costall et al. 1989). At present it is impossible to explain this discrepancy. The only common feature of which we are aware is that the N. American studies were performed on castrated males, but this appears to be an unlikely explanation as our own studies demonstrate that female ferrets respond in the same dose range as male animals and they continue to respond throughout their reproductive cycle. In Canada the present supply of experimental ferrets all derive from a small number imported some years ago and therefore genetic factors may be involved in the response sensitivity. The recent studies of Carpenter et al. (1988) have shown that cAMP levels (in the area postrema) can influence the sensitivity of the emetic response. In dogs treated with the phosphodiesterase inhibitor, IBMX (3-isobutyl-1-methylxanthine), the dose of apomorphine required to induce emesis in 100% of animals tested was reduced. Hence subtle differences in the cellular chemistry of the area postrema could account for differences in dose sensitivity between groups of animals. It would be interesting to know whether N. American animals treated with IBMX correspond more closely to their English counterparts.

**Cytotoxic drugs**

Emesis induced by the chemotherapeutic cytotoxic drugs is one of the major side effects of this type of anticancer therapy. In the search for antiemetic drugs to combat emesis induced by this class of drugs, studies were usually performed on dogs; but with the demonstration in 1982 by Florczyk et al. that ferrets had an emetic response to cisplatin, the ferret has become the animal of choice for identifying drugs with anti-

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**Fig. 1.** The relationship between the concentration of copper sulphate instilled into the stomach (fixed volume of 30 mL) and the emetic response. Total retches and vomits from 30 min observation period. Each point is the mean ± SE from at least five ferrets.

**Fig. 2.** Summary of the emetic responses to a variety of agents instilled into the stomach in a volume of 30 mL at their ED100 concentration. K, 1 M potassium chloride; CH, 1 M choline chloride; Na, 1 M sodium chloride; Cu, 40 mg% copper sulphate; GL, 2 M glucose; MAN, 2 M mannitol. Total retches and vomits from 30 min observation period. Each value is mean ± SE from at least five ferrets and for each parameter values are shown in rank order.
emetic properties against cytotoxic drugs. In this paper we report results showing that the ferret responds to a wide range of cytotoxic agents.

**Cisplatinum**

The original studies with this compound by Schurig et al. (1984) reported that the lowest dose to reliably induce emesis was 6 mg/kg i.v., with the number of emetic episodes increasing up to a dose of 10 mg/kg i.v. and the latency decreasing from 99 to 60 min. Our own dose-response study found the most reliable dose to use was 18 mg/kg, producing emesis with a latency of 75 ± 14.5 min (n = 4). At lower doses (2–8 mg/kg i.v.) emesis was not observed, although animals showed clear behavioural changes, in particular, a "cataplectic-like" state and diarrhea. With doses up to 20 mg/kg emesis was still evoked, although no dose-response relationships were apparent. Cisplatin is also emetic in the ferret when given by the intraperitoneal route at a dose of 146 mg/kg with a latency similar to that seen with the same dose given intravenously (Hawthorn et al. 1988b).

There is some indication that different groups of ferrets have different sensitivities to cisplatin; for example, Miner et al. (1987) reported ED100 values of 7.1 and 10 mg/kg for two groups of animals. In our experience we have had animals that were refractory to cisplatin at doses up to 20 mg/kg. The lack of response was specific to cisplatin, as animals responded to apomorphine, radiation, or cyclophosphamide.

**Cycloheximide**

This potent protein synthesis inhibitor induced an emetic response after a latency of 17.2 ± 1.8 min (n = 9) when given intraperitoneally at a dose of 20 mg/kg. Retching and vomiting continued in excess of 2 h.

**Cyclophosphamide**

A dose-response study established that the ED100 was 200 mg/kg when given intraperitoneally and this dose was also effective when given intravenously. At a dose of 200 mg/kg the emetic responses were broadly similar for the intravenous route and the intraperitoneal route, with the latency to retching being 21.6 ± 8.0 min (i.v.) and 19.1 ± 3.4 min (i.p.). With the intraperitoneal route retching and vomiting were confined to the first 90 min, whereas with the intravenous dose emesis continued for at least 3 h. Cyclophosphamide (100 mg/kg i.v.) has also been given in combination with doxorubicin (4 mg/kg i.v.) in some studies (Schurig et al. 1984).

**Diaceotoxyscirpinol**

The tricothecene mycotoxin, diaceotoxyscirpinol (Angui-
dine), induced retching and vomiting after a latency of 22.0 ± 3.8 min (n = 5) at a dose of 1.5 mg/kg when given intraperitoneally. The response continued throughout the 2-h observation period.

**Emetine**

Emetine is the rigid structural analog of cycloheximide and is one of the active ingredients in the clinically used emetic ipecacuanha. Following a dose of 20 mg/kg i.p. retching began after 26.2 ± 3.9 min and vomiting after 32.8 ± 3.0 min (n = 6), and continued for about 3 h of the 4-h observation period. Profound mucoid diarrhea accompanied the emetic response. Emetine was also active as an emetic when given by the oral route in water at a concentration of 150 μg/mL in a volume of 30 mL, producing retching and vomiting with a latency of 40.7 ± 5.8 min (n = 4).

**Mustine**

A dose of 400 μg/kg i.v. failed to induce emesis although prodomata were present. Emesis was induced after a latency of 28.1 ± 3.6 min (n = 4) with a dose of 1200 μg/kg i.v. Emesis continued throughout the 2-h observation period and was accompanied by marked diarrhea.

Previous studies have emphasized the differences between compounds in terms of latency, but there are equally striking differences in the pattern of the retching and vomiting. A knowledge of the different patterns may give clues to the processes underlying the emetic response, and more significantly is critical for obtaining a true assessment of antiemetic activity as some agents may only affect particular components of the response (e.g., early or late), suggesting that different processes or pathways are implicated to different degrees in the emetic response.

**Overall assessment of the ferret as an animal model for the study of emetic agents**

The studies described above together with those already reported in the literature show that the ferret responds to a wide range of emetic agents as the animals already established for the study of mechanisms of emesis. However, the presence of a response is not the only criterion under consideration. Ideally the animal should have a sensitivity similar to that of humans. Table 2 ranks the emetic sensitivity of six species to five emetic agents. It was not possible to include other emetic agents because of a lack of published data with which to compare the ferret. Unfortunately studies on the sensitivity of the ferret to motion stimuli have not yet been undertaken. This table illustrates that the emetic stimulus does not predict the sensitivity of the same species to another stimulus; for example, the monkey is the most sensitive to cisplatin but is unresponsive to apomorphine. In general, the cat is the least sensitive to each of the stimuli studied, although the insectivore may displace the cat as more data become available, as it is unresponsive to apomorphine and the dose of cisplatin required to induce emesis is high at 40 mg/kg i.v. (Ueno et al. 1987; Matsuki et al. 1988). With the exception of its response to cisplatin, the ferret occupies a position closer to that of the human and the dog than any other animal model. The ferret is therefore clearly an eminently suitable animal for the study of human emetic mechanisms and this has been borne out by antiemetic studies with the novel 5-HT_3 receptor antagonists in which the original ferret studies predicted efficacy in humans against a range of emetic stimuli.

Using the ferret we have undertaken a series of experiments with a variety of techniques to define the role of the abdominal visceral innervation in the mechanism of vomiting induced by a variety of stimuli.

**Evidence for the involvement of the abdominal vagi and splanchnic nerves in emesis**

From the introduction to this paper it is clear that in a biological context the gut is the second line of defence against the effect of accidentally ingested toxins and as such it would be expected to have appropriate detectors which when activated could trigger emesis. In contrast to the area postrema, relatively little attention has focused on the role of the gut in triggering emesis other than by stimuli presumed to “irritate” the gut lining such as copper sulphate. Here we present evidence from three studies in the ferret demonstrating a substantial role for the vagus in particular in the emetic response to a wide variety of emetic agents. Each set of results will be discussed separately and then the overall results will be reviewed in conjunction with those from other species in the literature and an attempt made to assess the role of the visceral innervation in emesis.

**Vagal and splanchnic nerve stimulation**

The most direct way of assessing whether activation of afferent axons in the abdominal vagus or greater splanchnic nerve can induce emesis is to stimulate them electrically in either anaesthetized or conscious animals.

**Anaesthetized animals**

Ferrers were anaesthetized with urethane (ethyl carbamate 1.5 g/kg i.p., 50% w/v in 154 mM NaCl) and prepared with cannulae for measurement of carotid arterial blood pressure, intrathoracic pressure (recorded from either a jugular venous or esophageal cannula), and gastric pressure in various combinations. Electromyographic (EMG) activity was recorded from the rectus abdominus muscles. The dorsal or ventral abdominal vagus was dissected free from the intra-abdominal esophagus over a distance of 1 cm, and ligated and cut. The left greater splanchnic nerve was identified in the region of the crus of the diaphragm, and ligated and sectioned on the spinal side of the coeliac ganglion. The central end of the vagus and splanchnic nerve were stimulated via plastic-coated silver wires wrapped around the nerves and embedded in low melting point wax. Stimuli were delivered to the electrodes from a DS2 isolated stimulator triggered by a Digitimer (D4030). Stimulus strengths were used that were supramaximal for unmyelinated axons (20 V, 0.5 ms) and the frequency (1 – 40 Hz) and stimulus duration (10 s – 10 min) varied.

Stimulation of the central end of either the dorsal or ventral abdominal vagus (30 Hz, 20 s, the minimal effective stimulus) induced an emetic response with the following components (Fig. 5). (a) Within 2 s after the start of the stimulus the blood pressure increased by 62.5 ± 5 mmHg (1 mmHg = 133.3 Pa) reaching a plateau after about 5 s. Licking and opening of the mouth began within 10 s of the start of stimulation. (b) Retching was characterized by a series of large negative-going oscillations in the intrathoracic pressure coincident with bursts of activity in the abdominal muscles and increases in the intragastric pressure. Retching began towards the end of the 20-s stimulus period or within 5 s of its end giving a mean latency of 19.4 ± 1.23 (n = 15). During each retch there was strong...
Fig. 5. (A) The retching response induced by stimulation of the central cut end of the ventral abdominal in the urethane-anaesthetized ferret. Note brief period of apnea following retching and that the abdominal EMG discharge remains elevated after the end of retching. BP, blood pressure; ITP, intrathoracic pressure. (B) The interval between the first 16 retches induced by vagal stimulation (parameters as in A) in seven animals. Note the precision of timing and the progressive increase in interval with increasing retch number. The last retch in a burst always occurs after an extended period. (C) The abolition of the retching and cardiovascular response induced by stimulation of the central end of the dorsal abdominal vagus by cervical vagotomy.
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Flexion of the spine. (c) Retching lasted for 18.5 ± 2.1 s
(18.8 ± 2.2 retches in this time) but abdominal EMG activity
remained elevated for 32.5 ± 4.1 s, although phasic activity
only occurred in association with retching. (d) In a series
of retches the interval between retches increased with time
(Fig. 5) from the onset of the burst, and following the final
retch a short (<5 s) period of apnea ensued (Fig. 5).

Retching was not induced by stimulation of the abdominal
gus in animals in which the cervical vagi had been sectioned
(Fig. 5). Stimulation of the central end of the greater splanch-
nic nerve (30 Hz, 10–60 s, 20 V, 0.5 ms) failed to evoke
retching, licking, or mouth opening, although an increase in
blood pressure comparable to that produced by vagal stimula-
tion was observed. Vagal efferent and splanchnic efferent
stimulation with the above parameters did not induce emesis.

Using prolonged periods of vagal afferent stimulation,
retching occurred in discrete bursts, each preceded by licking
and followed by a period of apnea. While the number of
retches in each burst declined, the strength of the retches as
assessed by the oscillations in intragastric pressure did not
(Fig. 5).

Conscious animals

Ferrets were sedated with ketamine (10 mg/kg s.c.) and
anaesthetized with halothane (1–4%) in N₂O and O₂. Fol-
lowing skin preparation the abdominal viscera were exposed
via a midline incision and the left and right greater splanchic
nerves were ligated and sectioned. The dorsal abdominal vagal
trunk was mobilized from the esophagus and ligated with two
ligatures about 2 mm apart at a point about 1.5 cm below the
diaphragm. The plastic insulation was removed over a 5-mm
length of plastic-coated silver wire (AG-8T, Clarke Electro-
medical) and the bare wires wrapped around the vagus sepa-
rated by 5 mm. The electrodes were secured in place by
suturing to the dorsal abdominal wall and a 1-cm length of
Portex tubing was slid over the nerve and electrodes. The elec-
trode wires were tunnelled under the skin and connected to a
socket on the dorsal surface of the neck. Wounds were closed
in layers with sutures and treated with antibiotic (Polybactrin,
Cicatrin).

Five days after surgery, animals were deprived of food
overnight. The next day they were given 50 mL of milk to
drink. The electrodes were connected to a stimulator (see
above) via a long flexible cable and the animals were placed
in an observation tank for videotape recording of activity.

Preliminary studies using a stimulus frequency of 30–
40 Hz (20 V, 0.5 ms, 60 s) are reported here. As soon as the
stimulus was turned on, the animal stopped its ongoing activity
and if it was laying on its back immediately got up. This was
followed by the animal rapidly walking backwards around the
observation cage usually accompanied by licking. After 5–
10 s of stimulation the animal stopped moving and adopted a
slightly hunched posture identical to that seen in animals
with emetic agents prior to vomiting. Immediately
before retching started the animal extended by moving
the hindlimbs. Bursts of retching culminated in vomiting and both
retching and vomiting continued after the stimulus stopped. In
one animal, following a 1-min period of stimulation (40 Hz),
retching and vomiting continued for 15 min in discrete bursts.
Throughout this time the animal was quiescent and exhibited
behaviour such as burrowing and chin rubbing, which are indi-
cators of “nausea” in this species (J. Hawthorn and P. L. R.
Andrews, unpublished observations). The emetic response to
vagal stimulation in one animal is shown in Fig. 6. It is im-
portant to point out that at no time did the animals show any
indication that they were in pain (e.g., vocalization, severe
hunching).

A full frequency–response curve has not yet been con-
structed, but we have observed emesis in animals with a frequency
of 5 Hz.

Discussion

The technique of electrical stimulation of the central end of
the abdominal vagus and splanchnic nerves in conscious and
anaesthetized animals is not new, and the results reported by
earlier authors are broadly similar to those described here. In
the decerebrate and anaesthetized dog and cat, retching and
vomiting were elicited by stimulation of the dorsal or ventral
abdominal vagus (Miller 1910; Derbyshire and Ferguson
1938). Emesis was induced after 12–40 s with a stimulus fre-
quency of 10–20 Hz. Electrical stimulation of the central end
of the abdominal vagus in man induces nausea (see Lewis
1942). One of the main findings from the present study is that
while the vagus is clearly capable of eliciting emesis, the
splanchnic nerves do not have this capacity. The reason for
this is not clear, as both nerves contain considerable numbers
of afferents involved in signalling information from the
abdominal viscera (Andrews 1986; Mei 1983; Janig and
Morrison 1986) and therefore the reason must lie in their
central connections and access to the integrative mechanisms.

Fig. 5D. The retching response to sustained dorsal vagal afferent
stimulation (7 min 30 s, 30 Hz, 20 V, 0.5 ms) in an individual ferret.
IGP, intragastric pressure; mean value from excursions during each
retching episode. Note the relationship between respiration, retching,
and licking.

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The short latency of the emetic response and the manner with which the emetic reflex can be elicits is particularly impressive when it is remembered that prior to emesis the animal goes through a compressed version of the behavioural repertoire that it would if treated with a potent emetic stimulus such as radiation or cisplatinum. This observation suggests that the behaviour may be as much a part of a motor programme as retching and vomiting. The latency of the emetic response to vagal stimulation probably represents the fastest possible activation of the emetic reflex from the viscera and is comparable to the 20 ± 6 s reported for apomorphine applied to the region of the area postrema to induce emesis in the ferret (Higgins et al. 1989). It must be pointed out that the stimulus clearly represents grossly abnormal activation of the vagus, comprising synchronous activation of all afferents at a sustained relatively high frequency for unmyelinated fibres. Studies of the frequency—response, stimulus duration—response, and current—response relationships may help reveal the main features constituting the emetic signal. For example, long periods of low frequency synchronous stimulation may be as effective as short periods of high frequency stimulation. Duration of stimulation may have an important role in preventing accidental activation of emesis by, for example, a single large amplitude gastric contraction; while it may evoke a high frequency discharge in the vagal afferents at the peak of the contraction, this will persist for perhaps only a few seconds.

The temporal patterning of the emetic response has two features worthy of comment. First, continuous vagal stimulation gives rise to discrete bursts of retching and vomiting. This suggests that although excitatory processes predominate in emesis, inhibition must also be involved to produce breaks between each burst of retching and vomiting, or that the excitatory drive builds up to a threshold, emesis ensues, and following this the excitatory mechanism becomes refractory for a short while (cf. nerve). A detailed examination of the relationships between the number of retches in a burst, whether a vomit ensues, and the interval between bursts of retching in response to a variety of stimuli may shed some light on this problem, but ultimately it can only be resolved with neurophysiological studies of the central emetic circuitry. Second, retching and vomiting continued for some time after stimulation had stopped. This observation suggests that either some type of oscillating circuit is set up which gradually decays or that vagal afferent stimulation induces the release of an agent which sustains the emetic drive after the stimulus has stopped. Studies in the anaesthetized ferret showed that abdominal vagal afferent stimulation with the parameters used in the present study induced a large increase in plasma vasopressin (but not oxytocin) from 61 ± 9 to 468 ± 63 pg/mL at 5 min (Hawthorn et al. 1988a). The vasopressin levels were elevated for at least 60 min. Nausea and retching in the human can be induced by infusions of vasopressin (Thomford and Sirinek 1975), and emesis induced by apomorphine and chemotherapy is associated with elevated vasopressin levels (Nussey et al. 1988; Fisher et al. 1982). Vasopressin may therefore be a candidate for sustaining or facilitating the emetic response but other substances should not be excluded.

Nerve lesions

The traditional method for assessing the role of the abdominal innervation in mediating the emetic response has been to study the effect of vagal and splanchnic nerve section alone or in combination on the response to a variety of emetic stimuli. Despite the simplicity and certainty of this procedure in contrast to area postrema ablation, the effects of this lesion have been less studied than area postrema ablation, leading we believe to an exaggerated role of the significance of the area postrema in emesis. Studies that have investigated the effects of visceral nerve lesions have tended to investigate agents assumed to cause emesis by gastrointestinal irritation and have infrequently studied cytotoxic drugs although radiation has been investigated. In addition, previous studies have tended to investigate only the response incidence following a lesion rather than quantifying all aspects of the residual response. In this paper we quantify the effects of abdominal vagotomy alone or in combination with greater splanchnic nerve stimulation on the response to 10 different agents in the ferret.

Methods

The methods were essentially the same as for implantation of the stimulating electrodes described above except that either the dorsal and ventral abdominal vagal trunks and (or) the left and right greater splanchnic nerves were ligated and sectioned. In these experiments animals were allowed to recover for at least 7—10 days prior to testing unless otherwise stated. Testing was carried out as described by Hawthorn et al. (1988b).

Results

**Apomorphine, loperamide, and para-chlorophenylalanine**

The responses to apomorphine (100 µg/kg s.c.), loperamide...
(an opiate receptor agonist 0.5 mg/kg s.c. tested 21 days post-vagotomy), and p-chlorophenylalanine (p-CPA, 180 mg/kg i.p.) were not significantly affected by vagotomy in terms of latency, number of retches, or number of vomits. In addition the response to apomorphine was unaffected by vagotomy and splanchnic nerve section in combination. The observation that p-CPA induced emesis was made fortuitously during a study on the effect of agents depleting neurotransmitters on the emetic response to radiation. Another fortuitous observation was that urethane (1.5 g/kg i.p.) induced emesis in animals with the vagus and splanchnic nerves cut (see below).

Cytotoxic drugs and radiation
The results of bilateral abdominal vagotomy on the emetic response to six different cytotoxic drugs and 200 cGy whole body irradiation are summarized in Fig. 7. In each case the response was influenced by the lesion. Closer inspection reveals that while vagotomy decreases both retches and vomits, it is not equally effective against both parameters: vomiting is more often abolished than is retching and even when present it is markedly reduced (e.g., cyclohexamide).

During the course of the study it became apparent that the detailed effect of the lesion could be influenced by the route of administration of the cytotoxic drug. For example, with cisplatinum (10 mg/kg) given intravenously the emetic response was totally abolished by abdominal vagotomy, whereas the same dose given intraperitoneally still induced vomiting in three of seven animals and retching in five of seven animals albeit at much reduced levels (Fig. 8). A similar finding has been reported by Hawthorn et al. 1988b with cyclophosphamide given intravenously where retching and vomiting were abolished by vagotomy and greater splanchnic nerve section, but when the drug was given by the intraperitoneal route only vomiting was substantially affected (see discussion on plasticity below). Figure 8 also illustrates another difficulty in defining the role of the visceral nerves: that of identifying a role for the splanchnic nerves. Cisplatinum splanchnic nerve section alone was without effect on retching or vomiting, but when paired with vagotomy it enhanced the effect of vagotomy on retching but not vomiting.

Discussion
The results from the visceral lesion study demonstrate that in the ferret the abdominal vagus either alone or in conjunction with the greater splanchnic nerves is involved in the induction of vomiting and retching by cytotoxic drugs and particularly whole body irradiation. We were surprised that retching and vomiting were not equally affected by the lesion, but we do not think this is due to an interference with the mechanical apparatus required for vomiting as compared with retching, as animals with similar lesions have a normal vomiting response to other agents (e.g., apomorphine). Because of the general lack of quantification of emetic responses and the range of stimuli studied here it is difficult to compare our results directly with previously published reports, but the observations on vagal—splanchnic interactions have been made by
others. For example, the emetic response to staphylococcal enterotoxin is unaffected by vagotomy or splanchectomy, whereas when lesioned in combination the response is abolished (Sugiyama and Hayama 1965). This indicates that either nerve can support the response and therefore before dismissing a role for the visceral nerves, lesions should be studied singly and in combination. Walton et al. (1931) reported similar results with emesis induced by peritonitis.

The involvement of the abdominal vagi in the emetic response to radiation has been demonstrated in several species including the cat and monkey (Borison et al. 1987; Brizzee 1956), but their role in cytotoxic-induced emesis is new. While an efferent role for the vagus and splanchnic nerves cannot be excluded (see Andrews and Davis (1990) for discussion), an afferent involvement is more likely.

It is proposed that radiation and cytotoxic drugs cause activation of abdominal vagal afferents by the release of neuroactive agents from the mucosa of the upper gastrointestinal tract. The evidence for this hypothesis has been recently reviewed (Andrews and Hawthorn 1988; Andrews et al. 1988; Andrews and Davis 1990). The evidence to date suggests that 5-HT is involved either in the activation of the afferents or in sensitizing the afferent to other agents (e.g., substance P). It must be emphasized that the involvement of vagal or other visceral afferents in the emetic response to radiation and cytotoxic drugs is based on indirect evidence, and confirmation of their involvement must await neurophysiological studies and investigations of emesis in capsaicin-treated animals.

**Medullary structures influenced by abdominal vagal stimulation**

From the results presented above and those in the literature it is clear that the abdominal visceral nerves and in particular the vagi are involved in triggering the emetic response to a variety of agents. A survey of the literature reveals that the emetic effect of many of these agents is modified by area postrema ablation. Apart from invoking species differences, can these observations be reconciled with those from the present study? Anatomical studies in the cat have demonstrated abdominal vagal afferent projections to several brainstem structures including the area postrema (see Leslie (1985) for review). We can therefore speculate that if some emetic agents activated abdominal vagal afferents projecting to the area postrema, then abolition of a response by ablation of the area postrema would be incorrectly ascribed to the area postrema acting as the detector of the emetic agent. A similar conclusion may be reached if the vagal afferents instead of projecting to the area postrema merely pass close to it in their passage to the dorsomedial region of the NTS so that they are in the zone damaged by lesions directed by the area postrema. Such considerations apply not only to the mechanism of emesis but also to the effects of area postrema ablation on ingestive behaviour. For the above hypothesis to be tenable it is necessary to demonstrate a functional effect of vagal afferent stimulation on the area postrema. Here we described the results from such a study using the 2-deoxy-D-glucose (2-DG) technique.

**Methods**

The surgical procedures were the same as those used for the studies on the emetic effects of abdominal vagal afferent stimulation in the anaesthetized ferret described above. In addition, a carotid artery and external jugular vein were cannulated. The 2-DG technique employed here is in essence similar to that first described by Sokoloff et al. (1977) but modified to use [3H]-2-DG as the isotopic tracer (Herkenham and Sokoloff 1983) and to be semiquantitative in nature (Gallistler et al. 1982). In brief, after surgical preparation ferrets were left for at least 30 min, and either apomorphine (50 ng/kg i.v.) was injected or electrical stimulation (30 Hz, 20 V, 0.5 ms, 5 min) of the central cut end of the ventral abdominal vagus was started. One minute later [3H]-2-DG (1 mCi/kg; 1 Ci = 37 GBq) was injected intravenously and after a further 45 min the animal was killed by anaesthetic overdose (Euthatal). The brain was rapidly removed (<10 min) and frozen in isopentane at −45°C. The brain was coated in cryomatrix (Tissue Tec OCT compound), and sections were cut at 18–20 μm on a cryostat maintained at −18 to −20°C. Selected sections of medulla were mounted on glass coverslips and card together with appropriate [3H]Micro-scale standards (Amersham International). The sections were then applied to Ultronfilm-3H (LKB) and sealed in light-tight x-ray cassettes for 28 days, after which the exposed Ultronfilm was developed manually using standard radiographic tank-based procedures. Controls were included for positive and negative chemography.

Sections were also prepared from control animals treated in an identical way except that in these animals apomorphine was not administered and the vagus was not stimulated.

Autoradiographs were analyzed using an IBAS (Kontron, Biedanlage) computerized image analysis system, and results are expressed as optical density (OD) ratios (the OD of the structure of interest compared with that of an adjacent area of white matter; for the brain stem the pyramidal tract was used). From OD measurements the percentage change in glucose utilization for several regions was calculated.

**Results**

The medullary structures analyzed are shown in Fig. 9. It is worth noting that in the ferret the area postrema is a U-shaped structure and as such occupies a morphological position between the more bilobed structure seen in other carnivores and the midline structure in rodents and lagomorphs (see Leslie (1986) for comparative aspects of the area postrema). The results from the autoradiographs in the anaesthetized animals are summarized in Fig. 10 and show that vagal stimulation produced a significant increase in glucose utilization in the area postrema, the NTS, the dorsal vagal nucleus (DMVN), and the nucleus of the hypoglossal nerve (XII). Apomorphine increased glucose utilization in the area postrema but not in the NTS, DMVN, or hypoglossal nucleus.

**Discussion**

It is well established that apomorphine induces emesis by activation of the area postrema, and this view is supported by the results presented here showing an increase in glucose utilization by the area postrema, presumed to represent a net increase in neuronal activity (Sokoloff et al. 1977). It might have been expected that apomorphine would increase activity in the other medullary structures in view of the visceral (gastric relaxation) and somatic (licking) components of the emetic reflex. However, the dose of apomorphine used produced only sporadic retching and thus it could be argued did not evoke the full emetic response. This is an unlikely explanation, as the threshold dose of apomorphine for activating the visceral components is lower than that for the somatic components in the dog (Lang et al. 1986). A more plausible explanation is that
FIG. 8. Histology of the medulla of the ferret at the level of the area postrema: A 20-μm section of ferret brain stem at the level of the area postrema stained with 0.5% aqueous cresyl fast violet to show cyt-architectural detail. (a) Lower power (×17) view of medulla with area containing nuclei of particular interest outlined with broken black border. Scale bar = 1 mm. D, dorsal surface; V, ventral surface. (b) High power (×70) view of the area of the dorsal vagal complex. Scale bar = 200 μm. CB, cerebellum; AP, area postrema; SN, solitary nucleus; IV, fourth ventricle; X, dorsal motor nucleus of the vagus; XII, hypoglossal nucleus; ML, medial lemnisci.

The results show only a net change in activity for the whole nucleus and this does not mean that some areas of a particular nucleus have not increased their activity in response to the stimulus.

Abdominal vagal stimulation increased activity in the area postrema and this is the first demonstration of a functional connection. The vagal influence appears to be a potent one as judged by the increase in glucose utilization compared with that with apomorphine. The increase in glucose utilization by the NTS, and DMVN is consistent with current knowledge that the medullary structures are supplied directly or indirectly by abdominal vagal afferents (Leslie 1986) and of their emetic and nonemetic functions (Andrews and Hawthorn 1988). The increase in activity in the hypoglossal nucleus is presumably the neural correlate of the licking reliably induced by vagal stimulation.

Abdominal vagal afferents can clearly influence the area postrema making it a possibility that area postrema ablation may be a "superselective vagotomy." We are aware that some stimuli thought to induce emesis by vagal afferent activation are unaffected by area postrema ablation (e.g., in the cat, Beleslin and Strabac 1987), but such stimuli need not activate the same sets of vagal afferents as those projecting to the area postrema. An additional possibility is that the vagal afferents modulate the sensitivity of the area postrema, which in turn influences the sensitivity of other components of the emetic circuitry.

A number of studies have demonstrated that drugs possessing 5-HT₃ receptor antagonist activity are potent antiemetics in humans and animals. The site of action of these agents is not known, but ligand binding and functional studies have demonstrated the presence of 5-HT₃ binding sites and receptors in the dorsal medulla including the area postrema and on vagal afferent terminals in the medulla and on their cell bodies in the nodose ganglion. In this section we describe preliminary experiments that implicate the abdominal vagal afferents as the major site of action of this class of antiemetic agents.

Fig. 10. Percentage change in glucose utilization in the brain-stem nuclei of the urethane-anaesthetized ferret in response to electrical stimulation of the central end of the abdominal vagus (30 Hz, 20 V, 0.5 ms) or apomorphine (50 μg/kg i.v.). AP, area postrema; NTS, nucleus tractus solitarius; DMVN, dorsal motor vagal nucleus; XII, hypoglossal nucleus. n = 3-4 animals. *p < 0.05; **p < 0.01.
Antiemetic studies in the ferret

Our original studies were undertaken with BRL24924 (Beecham), a substituted benzamide (see Sanger and King (1988) for description of its properties). The results with this compound are summarized in Fig. 11 and show that this agent markedly reduced the emetic response to cycloheximide, emetine, and radiation (200 cGy) and abolished the emetic response to cisplatinum and that in two out of five animals treated with diacetoxyscirpinol. Irrespective of the magnitude of effect on the emetic response the latency of the response increased significantly in all cases. A similar effect has been reported for the effect of BRL24924 on cyclophosphamide-induced emesis in the ferret (Hawthorn et al. 1986).

Using the more potent (as assessed on the Bezold-Jarisch reflex; see Sanger 1990) 5-HT3 receptor antagonists BRL43694 (Granisetron, Beecham) and GR38032F (Ondansetron, Glaxo), we have demonstrated dose-related antagonism of the emetic response to whole body radiation (800 cGy), emetine, and cyclophosphamide (Andrews et al. 1987; present study). One interesting feature that emerged from the dose—-response studies was that with low doses (0.1 mg/kg s.c.) of both compounds with all stimuli so far studied, the first effect observed was an increase in the latency of the emetic response. As dose increased (0.5 mg/kg s.c.) emesis was either abolished or in animals that continued to respond the number of retches and vomits decreased but the latency of the response was not increased further.

Using doses (1 mg/kg s.c.) of the above antagonists that completely abolish the emetic response to radiation and cytotoxic drugs (such as cisplatinum and cyclophosphamide), we have been unable to demonstrate significant antagonism of the responses to apomorphine, loperamide, urethane, or p-CPA. The latency of the response to CuSO4 was increased (control 258 ± 33 vs. 422 ± 76 s, n = 5, NS) and retching and vomiting were reduced (control retches, 44 ± 8 vs. 27 ± 4 NS; control vomits, 74 ± 0.6 vs. 4.8 ± 0.4, p = 0.01) by BRL43694 (1 mg/kg s.c.).

The effect of 5-HT3 receptor antagonists on vagal afferent activity

If we believe that the 5-HT3 receptor antagonists are acting on abdominal visceral afferents then it should be possible to demonstrate this using neurophysiological techniques. Single fibre recordings have been made in the urethane-anaesthetized ferret from vagal afferents with receptive fields in the abdominal viscera and with conduction velocities in the C-fibre range. Using functional criteria, units were identified as mechanoreceptors in the muscle of the lower esophagus, stomach, or small intestine, or chemoreceptors with receptive fields in the mucosa of the stomach or duodenum. The response to drugs was tested by injecting them into the gastrointestinal circulation via a cannula inserted into the abdominal aorta.

The majority of vagal afferents had a discharge evoked by a bolus injection of 5-HT (1–100 μg) or the 5-HT3 receptor agonist 2-methyl 5-HT (1–100 μg). The discharge was unrelated to motor changes induced by these agents (Davidson and Andrews 1988). The 5-HT3 receptor antagonist Ondansetron at an antiemetic dose (1 mg/kg i.v.) was without appreciable effect on the spontaneous activity of the afferents but abolished the response to 5-HT and 2-methyl 5-HT.

The results presented here confirm the potent antiemetic effects of the 5-HT3 receptor antagonists and extend the range of cytotoxic drugs against which they have been. Taking the results from the present study together with those reported in the literature for the ferret, dog, and human and using a variety of emetic stimuli and 5-HT3 receptor antagonists, it is possible to compile a preliminary list of the stimuli affected and unaffected by this class of antagonists (Table 3).

From our own studies in the ferret and observations from the literature, it appears that there is a relationship between the site of action of an emetic stimulus and the efficacy of a 5-HT3 receptor antagonist. This point is illustrated by com-
Table 3. A summary of the emetic agents whose effects are influenced or unaffected by 5-HT₃-receptor antagonists

<table>
<thead>
<tr>
<th>Emetic agent</th>
<th>Species</th>
<th>5-HT₃ antagonist</th>
<th>Reference</th>
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<tr>
<td></td>
<td></td>
<td>Blocked/reduced</td>
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<tr>
<td>Actinomycin D</td>
<td>Dog</td>
<td>ZAC</td>
<td>Smith et al. 1989</td>
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<tr>
<td>Adriamycin</td>
<td>Dog</td>
<td>ZAC</td>
<td>Smith et al. 1989</td>
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<td>Human</td>
<td>GRAN</td>
<td>Cassidy et al. 1988</td>
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<tr>
<td>Carboplatin</td>
<td>Human</td>
<td>GRAN</td>
<td>Cassidy et al. 1988</td>
</tr>
<tr>
<td>Cisplatinum</td>
<td>Dog</td>
<td>ZAC</td>
<td>Smith et al. 1989</td>
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<tr>
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<td>Ferret</td>
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Note: For trials with cytotoxic agents used in combination the reader is referred to Merrifield and Chaffee (1989) for references. OND, Ondansetron (GR 38032); GRAN, Granisetron (GR 43694); BAT, Batapride (BMY 25801); ZAC, Zaqride.

Comparing the reduction in emesis produced by 5-HT₃ receptor antagonists with that produced by abdominal visceral nerve lesions in the ferret (Fig. 12). This plot shows that if the vomiting response is not influenced by abdominal vagotomy either alone or in combination with greater splanchnic nerve section, then a 5-HT₃ receptor antagonist is unlikely to have an effect on such a response. This is supported by the observation that all the substances (except erythromycin) listed in...
Table 3 as not being affected by 5-HT3 receptor antagonists have either been shown to be unaffected by abdominal visceral nerve lesions or have a proposed extra-abdominal site of action (e.g., area postrema: apomorphine, opiates, histamine; nodose ganglion: prooveratrine; labyrinths: motion; fore-brain: pilocarpine; Borison 1989). In the list of emetic agents affected by 5-HT3 receptor antagonists, several of the emetic stimuli are influenced by nerve lesions and the area postrema in several cases (but see section on Nerve lesions).

The site of action of peptide YY appears to be the area postrema, but we are unaware of studies examining the effects of vagotomy.

We appreciate that the link between the abdominal visceral innervation and the effects of 5-HT3 receptor antagonists is somewhat circumstantial and although the association is compelling, it does not necessarily indicate cause and effect. Considerably more quantitative studies are required using an even wider range of emetic stimuli.

Taking the results from the lesion experiments together with those from the vagal afferent recording, an abdominal site of action for the 5-HT3 receptor antagonists appears possible (at least in the ferret) and is consistent with other studies demonstrating 5-HT3 receptors on vagal afferent neurones (Fozard 1989). The lack of effect of 5-HT3 receptor antagonists on emesis induced by agents acting primarily or exclusively at central sites (apomorphine, motion) argues in favour of a site of action outside the CNS but is not proof. The emetic response to stimulation of the central end of the abdominal vagus is not blocked by a 5-HT3 receptor antagonist at a dose that abolishes the emetic response to radiation and cytotoxic drugs. This observation again argues against a central site, but it is possible that electrical stimulation is such a potent stimulus that it cannot be surmounted by blockade of a single receptor class. Further evidence against a central site comes from the observation that the emetic response to high dose radiation (800 cGy) in a vagotomized and splanchectomized animal is unaffected by 5-HT3 receptor antagonism (see below). All these results argue against a central site and in favour of a peripheral site but we must qualify what we mean by “central.” The antiemetic agents suggested as acting at central sites have been assumed to act either on the chemoreceptor trigger zone or at the level of the “vomiting centre.” Clearly neither of these sites apply to the present class of antiemetic agents. Central sites that have received no attention are the vagal afferent terminals in the NTS and the area postrema and the neurones in the NTS and area postrema. The presence of 5-HT and its synthetic enzymes has been demonstrated in the area postrema and NTS. 5-HT has been proposed as a neurotransmitter in some vagal afferent neurones (see Leslie (1986) and Andrews and Hawthorn (1989) for references), and autoradiographic studies have demonstrated the presence of 5-HT3 receptors in the area postrema and the NTS (Higgins et al. 1989; Pratt and Bowery 1989). Studies in the rat (Pratt and Bowery 1989) and ferret (R. A. Leslie, personal communication) have demonstrated that there is a reduction in binding sites in the region of the NTS following supranodose vagotomy suggesting a location of presynaptic 5-HT3 receptors on vagal afferents. Because binding sites for 5-HT3 receptor ligands have been found in high density in the area postrema, this has been taken as evidence that the area postrema is one site at which this class of antiemetic agents acts. While this is suggestive, it must be treated with extreme caution as anyone familiar with the anatomy of the relationship between the area postrema and the NTS would agree that it is impossible to achieve separation of the area postrema for homogenate studies without including some of underlying subnucleus gelatinosus, the region where the abdominal vagal afferents terminate. The 2-DG study reported here demonstrates a clear functional influence of the abdominal vagus on the area postrema. It has been reported that injection of the 5-HT3 receptor antagonist Ondansetron into the area postrema” of the ferret blocks emesis induced by systemic cisplatinum (Higgins et al. 1989). While we do not dispute the observation, the authors describe the injection as being “made at a point approximately 1 mm below the area postrema into the hind brain”... the needle passed between the two rostral projections of the area postrema and thus did not damage the structure.” From this description we believe that this injection is into the dorsomedial NTS and not the area postrema (see Fig. 10). Irrespective of the precise site of injection the results from the study show a locus for antiemetic action for 5-HT3 receptor antagonists in the dorsomedial medulla and this is consistent with some of the autoradiographic studies. These studies only demonstrate that there is a site of action in the medulla and not that it is the site at which systemically administered 5-HT3 receptor antagonists act. At present it is difficult to see how 5-HT3 receptors present on vagal afferent terminals in the medulla could have an antiemetic effect unless 5-HT is the vagal afferent neurotransmitter and has a facilita-
tory effect on 5-HT release. In addition to these presynaptic receptors there are also 5-HT$_3$ receptors in the NTS and hence antagonists acting here could readily antagonize emesis induced by vagal or even area postrema activation.

Some evidence for an involvement of 5-HT in the central components of the emetic pathway comes from studies with p-CPA and fenfluramine showing that treatment with these 5-HT depleting agents reduced or abolished the emetic response to apomorphine, lisuride, ipecac, and cisplatinum (Barnes et al. 1988; Costall et al. 1989), and reduced that to radiation (P. L. R. Andrews and J. Hawthorn, unpublished observations). It is not possible to identify the critical site at which the p-CPA is acting, but the range of effects suggests that it may be interfering with the integrative mechanisms for emesis. Studies with this type of depleting agent clearly provide a useful insight into the nature of the neurotransmitters in the emetic pathway (Costall et al. 1989). In connection with 5-HT it is of interest that Beleslin and Nedelkovski (1988) commented that ‘‘5-HT mechanisms are not involved in the control of emesis in the area postrema, at least in the cat.’’ Studies in the cat may therefore offer an excellent opportunity to investigate whether 5-HT$_3$ receptor antagonists act in the area postrema.

It could be argued that because in general 5-HT$_3$ receptor antagonists abolish the entire emetic response to a number of stimuli, whereas visceral nerve lesions tend to only reduce the response (Fig. 8), the antagonists act at sites uninfluenced by the nerve lesions, i.e., the CNS (Andrews and Hawthorn 1987). However, this may not be such a useful indicator as first thought in view of the apparent reorganization in the emetic response induced by the nerve lesion and discussed in detail below.

In conclusion we can say that a number of pieces of circumstantial evidence have identified the abdominal visceral afferents as a site at which 5-HT$_3$ receptor antagonists could act to have their antiemetic effect, while binding and microinjection studies indicate a central site (NTS, area postrema, or vagal afferent terminals). Insufficient data are available to determine which (if either) of these sites is the clinically relevant one. It is quite conceivable that there are two (or more) sites in the emetic mechanism where 5-HT$_3$ receptors play a pivotal role and the dose—response results suggest that this might be the case. At a low dose a striking feature of the 5-HT$_3$ receptor antagonists is that the latency of the emetic response is increased often before there is a substantial reduction in retches or vomits. At higher doses the retches and vomits decrease but in animals that still respond, the latency is similar to that seen with the lower dose and with still higher doses the response is abolished. It is noticeable that if the emetic response is not abolished by a visceral nerve lesion, then an increase in the latency of the response is usually observed. From these observations we propose that there are two sites of action of 5-HT$_3$ receptor antagonists: a peripheral visceral (vagal) site and a central (vagal terminals, NTS) site, the former being more ‘‘sensitive’’ to blockade than the latter. However, this hypothesis is highly speculative and awaits experimental investigation.

The idea, of course, of antiemetic agents acting to block afferents is not new, as this quote from Borison (1986) demonstrates: ‘‘Specific vomiting responses initiated from identified emetic sensory receptors are theoretically vulnerable to interruption at their afferent points of origin.’’

Is there plasticity in the organization of the emetic reflex?

During the course of our studies on the role of visceral afferents in the emetic mechanism, we noted a number of examples where there appeared to have been some reorganization of the emetic system, particularly with respect to time intervening after the lesion. We report a number of these observations together with examples of similar phenomena involving the vagus and area postrema taken from the literature.

Results

Sodium chloride and copper sulphate-induced emesis

Animals were tested prior to vagotomy, and after abdominal vagotomy (7—10 days and 21—30 days) with 1 M NaCl or 40 mg% CuSO$_4$ given into the stomach in a volume of 30 mL. The results are summarized in Fig. 13. In control animals the response latencies formed a tight group and the duration of emesis was consistent. At 7—10 days after vagotomy the latency of the response was increased and the emetic episodes became spread over 30—60 min. Animals with a greater splanchnic nerve section alone tested at this stage had responses not different from control animals. When animals were studied at 21—30 days postvagotomy, an ultra-short latency response was consistently observed although it was very rarely observed in intact animals. Animals continued to fail to respond to large volumes (>50 mL) of milk or 154 mM NaCl instilled rapidly (<5 s) into the stomach.

These changes in the response are even more striking for sodium chloride; when another group of animals was tested 3 days after vagotomy the emetic response was abolished and within 1 h the animals exhibited neurological signs indicative of sodium poisoning and were killed humanely by anaesthetic overdose.

Urethane

In animals deprived of food overnight and subsequently anaesthetized with urethane, the incidence of retching and vomiting is low (17% male, 19% female, n = 181 total), whereas in animals treated in an identical way but given an abdominal vagotomy 21 days earlier the incidence is increased dramatically (68% male, 80% female, n = 45 total). The latency of the emetic response to urethane measured in nine animals with abdominal vagotomy was 1.42 ± 0.15 min. This response is not seen in sham-operated animals or in those with section of the greater splanchnic nerves alone. In animals with vagotomy, electrical stimulation of the central cut end of the greater splanchnic nerve failed to induce emesis, indicating that there is not a gross change in the influence of the splanchnic nerves on the emetic mechanism. The emetic response was not affected by Granisetron (1 mg/kg s.c.).

Radiation

In ferrets exposed to a high dose of radiation (800 cGy) the entire emetic response is abolished by pretreatment with 1 mg/kg BRL43694 s.c. Abdominal vagotomy alone or in combination with greater splanchnic nerve section reduces and delays the response to this dose of radiation. In animals with a vagotomy and treated with an antiemetic dose of the 5-HT$_3$ receptor antagonist, the emetic response was similar to that seen in an animal with a vagotomy alone (Fig. 14). This observation suggests that in the intact animal the entire emetic response is mediated via a 5-HT$_3$ receptor dependent pathway, whereas in the vagotomized animal 5-HT$_3$ receptors are not involved in the response. The simplest explanation is that vagotomy has
induced the formation of a novel emetic pathway. As no emetic response remains in vagotomized animals exposed to 200 cGy, it appears that this novel emetic pathway is only expressed when the animal is subjected to the greater tissue damaging effects of 800 cGy. We propose that vagotomy induces the formation of an agent in the gut wall, which can be released by radiation-induced damage to enter the circulation and act on the area postrema to induce emesis. In view of the studies of Carpenter et al. (1988) a gastrointestinal peptide hormone is the most likely candidate.

**Discussion**

The results of the three studies above provide a preliminary indication that the emetic pathway exhibits a degree of plasticity. While the mechanisms involved are poorly understood (see Andrews and Davis (1990) for a full discussion), we include the observations to illustrate the difficulties in assessing mechanisms based on lesion studies alone. While we have focused on changes induced by visceral nerve lesions, there is no *a priori* reason why similar changes may not occur following area postrema ablation. The studies with copper sulphate and sodium chloride illustrate that the change induced by the lesion may alter with time after the lesion. This observation is particularly worrying as a cursory inspection of the literature reveals a wide range of post-lesion emetic test times (e.g., vagotomy, 7–10 days (Hawthorn et al. 1988b) to 60 days (Carpenter et al. 1986)), and this variation may contribute to some of the discrepancies between studies in assessing the relative roles of the area postrema and the visceral nerves.

Some indication of vagal influences on the emetic system comes from the literature. First, in contrast to other studies Rabin et al. (1985) reported that conditioned taste aversion was more reliably induced in rats with a vagotomy than in intact animals. It is possible that this represents an example of vagotomy either sensitizing the area postrema or the vagotomy provoking the formation of a systemic conditioned taste aversion inducing agent released by intragastric copper sulphate. Secondly, in the dog, Hwang et al. (1947) reported that "after vagotomy touching the balloon to the pharynx often excited nausea and sometimes vomiting, which never occurred under the same experimental conditions before the operation." They also commented that the emetic response to apomorphine was exaggerated after vagotomy.

The literature contains other examples of similar observations often reported anecdotally or dismissed as aberrant results inconsistent with other studies. The results reported here in conjunction with the other reports clearly establish that there are lesion-induced modifications in the emetic system and these cannot be ignored in attempts at understanding the mechanism by which emetic and antiemetic agents act.

**Conclusion**

**Overall assessment of the role of the abdominal visceral innervation in emesis**

The studies reported here using electrical stimulation of the vagus provide direct evidence that the abdominal vagal afferents are capable of activating the emetic reflex, and more interestingly, several components of the behaviour associated with emesis. Using nerve lesions, a previously unreported involvement of the vagus and splanchnic nerves in the emetic response induced by cytotoxic drugs has been identified. The
response to radiation in the ferret has also has been shown to involve the vagus as it does in the cat and monkey. Interpretation of the role of the visceral nerves is complicated by quantitative but not qualitative differences in the effects of the lesions depending on whether the cytotoxic drug is given intravenously or intraperitoneally. The reason for this is not clear, but it is important that this is studied because a number of cytotoxic drugs (including cisplatinum) are given clinically by intraperitoneal as well as intravenous routes.

The demonstration that abdominal vagal afferents have functional influences on the area postrema calls for some reappraisal of the area postrema in emesis; is it really the detector or is it only in the pathway for some agents?

Our studies and those of others implicate the visceral afferents (primarily the vagus) in their central and (or) peripheral course as the site of action of 5-HT$_3$ receptor antagonist antiemetics. An action on the area postrema appears unlikely but cannot yet be excluded.

The most interesting aspect of our study is the indication that there is a degree of plasticity in the emetic system, an observation which if confirmed has wide ranging implications for understanding the mechanisms by which emetic and antiemetic agents act.

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