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Neuropharmacology of emesis in relation to clinical response

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Summary 5-HT_3 receptor antagonists such as ondansetron, granisetron, ICS205-930 and zacopride are highly effective in the ferret, cat or dog to prevent emesis caused by cisplatin and other chemotherapeutic agents, and radiation treatment. The anti-emetic effects may be mediated centrally in the area postrema and associated structures of the emetic reflex such as the nucleus tractus solitarius, which have a very high density of 5-HT_3 receptors. Additional sites of action may be found on the 5-HT_3 receptors located on the vagus nerve or enteric neuronal elements in the gastro-intestinal tract. The precise site(s) and mechanism(s) of action of different cytotoxic treatments to induce emesis remains to be determined, but appears to involve a common action on a 5-HT_3 system. The 5-HT_3 receptor antagonists do not impair normal behaviour and, in particular, fail to affect the extrapyramidal motor system and do not cause sedation. Of potential benefit, the 5-HT_3 receptor antagonists have an anxiolytic profile of action in rodent and primate models. The 5-HT_3 receptor antagonists are revealed as an important group of drugs to prevent emesis induced by a wide range of cytotoxic treatments.

To a patient receiving chemotherapy or radiotherapy, nausea and vomiting are amongst the most feared effects of treatment. To the physician, the continuing loss of gut contents may require careful correction of electrolyte and nutritional imbalance. To the concern of both patient and physician, the lessening quality of life may finally lead to a refusal of a potential curative therapy. The traditional anti-emetics such as antihistamines, anticholinergic agents, sedatives, dopamine receptor antagonists and others have only modest efficacy, either when administered alone or frequently in elaborate combination, the latter providing eloquent testimony to the absence of any single effective treatment.

The present paper reviews the pharmacology of the 5-HT_3 receptor antagonists such as ondansetron, whose introduction has provided a novel and highly effective approach to prevent nausea and emesis caused by cytotoxic therapy (Kris *et al.*, 1989; Bonneterre *et al.*, 1990; Kaasa *et al.*, 1990; Marty *et al.*, 1990). Since nausea is a subjective experience and for which there is no reliable animal model, the present account is concerned with emesis and its antagonism.

Introduction of the 5-HT_3 receptor antagonists

5-hydroxytryptamine (5HT) is stored and released from neurones, enterochromaffin cells and blood platelets and, once released, produces its effects by binding to and stimulating receptors on the target cells (Verbeuren, 1989). A neuronally located 5-HT receptor was first identified by Gaddum and Picarelli (1957) as mediating a release of acetylcholine from cholinergic nerve terminals in the guinea pig ileum. The receptor was designated an 'M' receptor since morphine inhibited the 5-HT induced effects. Subsequently, Fozard and co-workers also identified a neuronally seated 5-HT receptor which mediated the release of noradrenaline in the rabbit heart and, in an important observation, reported that cocaine and metoclopramide were effective at inhibiting the effects of 5-HT (Fozard & Mwaluko, 1976; Fozard & Mobarok-Ali, 1978*a*,*b*). The demonstration of a 5-HT receptor which was clearly different from other 5-HT receptors (5-HT_1 and 5-HT_2), prompted the search for potent and selective antagonists of the neuronally located 5-HT receptor.

The search was centred on compounds containing an indole or tropane nucleus, or around the substituted benzamides (see reviews by Richardson & Engel, 1986; Sanger & King, 1988; Fozard, 1989) and compounds were detected by their ability to antagonise for example the actions of 5-HT to depolarise the vagus nerve and mediate the von Bezold-Jarisch reflex. The development of highly selective and specific antagonists of the neuronally located 5-HT receptor allowed a detailed characterisation of the recognition site which was classified as the 5-HT_3 receptor (Bradley *et al.*, 1986).

The possibility that 5-HT_3 receptor antagonists might prevent chemotherapy induced emesis in man began, at least with the benefit of hindsight, with the realisation that intravenous administration of high doses of metoclopramide provided the most effective anti-emetic treatment (see review by Gralla, 1983). Initially, however, the anti-emetic effects were attributed to the well known dopamine receptor blocking actions of metoclopramide. But the relative ineffectiveness of much more potent dopamine receptor antagonists as anti-emetic agents indicated that this was unlikely to be correct. Furthermore, animal studies had also established that the gastro-intestinal effects of metoclopramide to enhance gastric emptying were also dissociated from dopamine receptor blocking action. The evidence that the 5-HT_3 receptor antagonist action of metoclopramide might contribute to both the gastro-intestinal and anti-emetic effects became amenable to investigation with the introduction of the selective 5-HT_3 receptor antagonists.

5-HT_3 receptor antagonists as anti-emetic agents in animal models

The first studies were conducted in the ferret using MDL72222 and ICS205-930 (Miner & Sanger, 1986; Costall *et al.*, 1986) and both were shown to be highly effective to inhibit cisplatin-emesis. Ondansetron and granisetron were also shown to be very potent antagonists and indeed, were one hundred times more potent than metoclopramide (Costall *et al.*, 1987; Boyle *et al.*, 1987). In addition, the anti-emetic actions of an agent such as ondansetron were achieved in the complete absence of sedation or motor impairment, the latter contrasting sharply with the inhibitory actions of metoclopramide which occurred concomitant to sedation and motor impairment. Such effects almost certainly involve the dopamine antagonist actions of metoclopramide, which are also responsible for the extrapyramidal (dystonic) side effects observed following high dose treatment in man. The anti-emetic effects of the 5-HT_3 receptor antagonists established in the ferret have also been shown in the cat and dog using zacopride (Smith *et al.*, 1988; 1989).

The ability of the 5-HT_3 receptor antagonists to inhibit chemotherapeutic induced emesis has also been extended to an antgonism of radiation induced emesis in the ferret. Thus Miner and colleagues (1987) and Andrews and Hawthorn (1987) reported that renzapride antagonised radiation induced emesis; and ondansetron, granisetron, zacopride and

BMY25801 were shown to have a similar effect (Boyle *et al.*, 1987; Stables *et al.*, 1987; Bermudez *et al.*, 1988; Gylys *et al.*, 1988; King *et al.*, 1988). Granisetron has also been shown to antagonise radiation induced emesis in the dog (Harding *et al.*, 1988). Thus, the data are entirely consistent in showing that pretreatment of animals with 5-HT_3 receptor antagonists provides a highly effective regimen to protect against chemotherapy and radiation induced emesis. Furthermore, the intravenous administration of ondansetron and granisetron in ferrets or oral administration of zacopride in dogs stopped established emesis within seconds or minutes of administration (Stables *et al.*, 1987; Bermudez *et al.*, 1988; Smith *et al.*, 1989). It is clear that the 5-HT_3 receptor antagonists can prevent the development or inhibit the progression of emesis induced by cisplatin or radiation in the ferret and/or dog and cat. In these species, 5-HT_3 receptor antagonists identified above have also been shown to antagonise emesis induced by actinomycin D, adriamycin, cycloheximide, cyclophosphamide/doxorubicin, dacarbazine, mechloroethamine, mustine and trimelalol (Smith *et al.*, 1989; Davis, 1989; Hawthorn *et al.*, 1988; Gylys *et al.*, 1988; Bermudez *et al.*, 1988). Ondansetron and granisetron are particularly important tools to study the role of 5-HT_3 receptors in emesis since both compounds have a very high degree of selectivity and specificity for the 5-HT_3 receptor. In particular, neither compound has affinity for the 5-HT_4 receptor involved in intestinal motility (Craig & Clarke, 1990; 1992; Eglen *et al.*, 1990; Elswood *et al.*, 1991; Reeves *et al.*, 1991).

The ability of the 5-HT_3 receptor antagonists to inhibit chemotherapy and radiotherapy induced emesis has been shown to be specific for such stimuli. Thus granisetron fails to prevent apomorphine or morphine induced emesis in dogs or ferrets (Miner *et al.*, 1987; Bermudez *et al.*, 1988), renzapride and ICS205-930 fail to antagonise apomorphine induced emesis in ferrets (Miner *et al.*, 1987) and in this species, ICS205-930 also failed to antagonise emesis induced by lisuride and copper sulphate (Costall *et al.*, 1990). Similarly, zacopride failed to antagonise emesis evoked in dogs by copper sulphate, protoveratrine A, histamine and pilocarpine (Smith *et al.*, 1989). Also, from studies in the cat, there is no reason to believe that the 5-HT_3 receptor antagonists will be useful in motion sickness (Lucot, 1989).

Nausea is a subjective human response and beyond a satisfactory animal model. However, prior to the development of retching and vomiting the ferrets invariably display a hunched back posture, increased activity, stretching, licking, salivation, irregular respiration and backward movements. In that such behaviour may reflect discomfort or the portent of emesis, it may be analogous to nausea. In any event, the antagonism of emesis by 5-HT_3 receptor antagonists occurs concommitant to the antagonism of such behaviour.

Sites of action of cytotoxic treatments to induce emesis

Evidence for a central action

One of the important consequences of the successful introduction of ondansetron to control emesis in animals and man has been to increase interest in the site(s) of action of emetic agents and anti-emetic treatments. Little research into the mechanisms moderating emesis had been performed since the pioneer studies of Borison and Wang (1953) whose studies provided an anatomical basis for the vomiting reflex. The area postrema acting as a 'chemoreceptor trigger zone' was thought to relay information to a 'vomiting centre' in the reticular formulation of the medulla. The area postrema was thought to be responsive to blood borne substances and a vagal afferent input. The importance of the area postrema and its input was established using ablation techniques; lesion of the area postrema abolished apomorphine induced emesis whereas lesion of the vagus nerve antagonised emesis caused by gastro-intestinal irritation. The results were interpreted to indicate a central action for emetogens such as apomorphine and a peripheral site of action for agents irritating the gastro-intestinal tract. Yet, there are obvious limitations to the interpretations made solely on the basis of lesion studies since lesion of the area postrema to abolish emesis could reflect an interruption of a drug induced effect mediated via any afferent input. For example, emesis induced by peripheral injection of tetrodotoxin in the cat is abolished by CTZ lesions, but tetrodotoxin is not emetic when given intraventricularly (Borison *et al.*, 1963; Hayama & Ogura, 1963). In contrast, the emetic response to the intraventricular injection of met-enkephalin in the dog is abolished by CT lesions but met-enkephalin is not emetic when given intravenously (Bhargava *et al.*, 1961). Also, there remains the possibility that the site of emetic action may be species dependent: in the dog radiation induced vomiting is abolished by area postrema lesions whereas vagotomy is effective in the cat (Chinn & Wang, 1954; Borison *et al.*, 1987).

With these reservations in mind, there is evidence that the area postrema/dorsal vagal complex is involved in emesis induced by chemotherapeutic agents. Thus lesions of the area postrema are reported to abolish or reduce emesis induced by cisplatin in the ferret, cyclophosphamide in the ferret and cat and mechlorethamine in the dog and cat (Borison *et al.*, 1958; Fetting *et al.*, 1982). The ability of an intraventricular injection of cisplatin in the cat to induce emesis could also be indicative of a central site of action (Smith *et al.*, 1988). However, in the latter study, the onset of action was within four minutes and contrasted with a one hour delay in onset following peripheral treatment. Higgins *et al.* (1989) have interpreted this finding as indicating that cisplatin is unlikely to mediate its effects via the area postrema. Also, there is no certainty that all chemotherapeutic agents induce emesis via the area postrema or that such agents have an identical site of action in different species.

There remains the possibility that a component of the emetic effect of cancer chemotherapeutic agents may be mediated or influenced by higher centres. The powerful control exerted by higher centres on nausea and vomiting is illustrated by psychogenic vomiting, occasioned by anticipatory factors and visual or olfactory disgust. The mechanisms mediating such response are not clear, but it is an interesting observation that after removal of forebrain influence using midbrain sections in the ferret, cisplatin (but not apomorphine) emesis was reduced (Naylor & Rudd, 1990). However, it is not possible to conclude whether cisplatin actually exerts a direct action in the forebrain or whether the forebrain facilitates an action of cisplatin at the midbrain or lower sites.

To discuss the ability of 5-HT_3 receptor antagonists to inhibit a raised mesolimbic dopamine function and reduce aversive responding in animals is beyond the scope of the present review (see Costall *et al.*, 1990). However, it remains possible that a forebrain action to reduce anxiety and stress may be beneficial to the control of emesis.

Evidence for a peripheral action

An early report by Walton *et al.* (1931) indicated that vagus and splanchnic nerve lesions could antagonise the emesis induced by peritonitis, and the combination lesion was shown to be necessary to abolish the emetic response to staphylococcal infection (Sugiyama *et al.*, 1966). The involvement of the abdominal vagi in radiation induced emesis has been demonstrated in a number of species by Brizzee (1956) and Borison *et al.* (1987). The most important study to indicate that cytotoxic agents may induce emesis via a peripheral site of action comes from a series of experiments using the ferret. Andrews and colleagues (1990) ligated and sectioned the dorsal and ventral abdominal vagal trunks and (or) the right and left greater splanchnic nerves. After a 7 to 10 day recovery period animals were challenged with a variety of emetic stimuli; emesis due to apomorphine was not affected by vagotomy or vagotomy plus splanchnic nerve section, whereas the vomiting caused by cycloheximide, emetine, radiation, mustine, diacetoxyscirpinol and cisplatin

was markedly reduced or abolished. The retching movements induced by the latter treatments were less affected However, it was also observed that the detailed effects of the lesion could be influenced by the route of drug administration; the effects of an intravenous drug treatment could be more effectively antagonised or abolished than for the same dose administered intraperitoneally. Further, it was noted that whilst splanchnic nerve section alone failed to affect cisplatin induced emesis, when it was combined with vagotomy it enhanced the effect of vagotomy on retching but not vomiting.

It was concluded that in the ferret the abdominal vagus either alone or in combination with the greater splanchnic nerves is involved in vomiting and retching caused by cytotoxic drugs and whole body irradiation. Radiation and cytotoxic drugs were hypothesised to cause activation of abdominal vagal afferents by the release of neuroactive agents. A cautious interpretation of peripheral nerve lesion experiments is that they may be involved in the mediation of emetic stimuli, but a limitation to such interpretations is the difficulty of assessing an efferent role for the vagus and splanchnic nerves.

The mechanism of action of cytotoxic treatments to induce emesis

The ability of 5-HT_3 receptor antagonists to inhibit cytotoxic induced emesis would indicate a role for 5-HT as a mediator of emesis. To test this hypothesis, 5-HT synthesis, storage and release processes were disrupted using reserpine, fenfluoramine and parachlorophenylalanine. All such treatments were found to antagonise cisplatin-emesis (Barnes *et al.*, 1988), and radiation induced emesis is also reported to be blocked by parachlorophenylalanine treatment (see Andrews *et al.*, 1990). This could be evidenced to support a role for 5-HT in emetic responses, subject to the limitation that none of the three agents used to disrupt 5-HT function is entirely selective for the 5-HT system, having an additional influence on the catecholamine systems. Such effects could occur at central and/or peripheral sites of action and it could be hypothesised that cytotoxic treatments should increase 5-HT release. This has been shown to occur in the ileum (Gunning *et al.*, 1987) at least as assessed by measurements of 5-HT and 5-HIAA levels, and could activate vagal afferents to induce emesis. It also remains possible (but questionable) that the 5-HT which is released in the ileum may exert a humoral influence at distant sites, such as the brain. There is a preliminary report in man that chemotherapy may increase plasma 5-HT levels (Barnes *et al.*, 1991), and also increase urinary 5-HIAA levels (Cubeddu *et al.*, 1990). But cisplatin treatment fails to change plasma 5-HT levels in the ferret (Rudd *et al.*, 1992) and, in this respect, it is interesting that cisplatin also failed to modify 5-HT/5-HIAA levels in the area postrema and other brain nuclei (Barnes *et al.*, 1988). Further, it is apparent that the carcinoid syndrome can occur in the absence of emesis.

It is clear that evidence is beginning to appear that cisplatin/cytotoxic treatments may influence 5-HT function, but further evidence is needed to link unequivocally the emetic effects of cisplatin with 5-HT release. Furthermore, it would require some evidence that 5-HT is actually emetic, and this is lacking. Thus the injection of 5-HT or 2-methyl-5-HT into the ventricles of the cat or ferret failed to induce emesis (Feldberg & Sherwood, 1954; Naylor & Gunning, unpublished data; Smith, unpublished data) or had only partial effectiveness (Higgins *et al.*, 1989). However, and more positively, the peripheral administration of 5-hydroxytryptophan in the dog or cat is reported to induce emesis (Bogdanski *et al.*, 1958; Cahen, 1964). But it is not clear whether the effects of 5-hydroxytryptophan are mediated entirely via the formation and release of 5-HT, or partly through the release of catecholamines (Cahen, 1964).

There remain other potential mechanisms whereby cytotoxic agents may induce emesis and peptides are an important group of emetogenic mediators (Kucharczyk & Harding, 1990). This is an area worthy of more detailed study since zacopride and granisetron are reported to antagonise peptide YY-induced emesis (Smith *et al.*, 1989; Harding *et al.*, 1989) and it is conceivable that the products of cellular breakdown caused by cancer chemo- or radiotherapy may include emetogenic proteins. It remains tobe established whether the antagonistic effects of the 5-HT_3 receptor antagonists are due to a direct antagonism of the effects of peptideYY, or reflect an inhibition of action on a 5-HT receptor which may facilitate peptide release. Such interactions reamine to be explored. In brief, it remains uncertain as to how the cellular effects of cytotoxic treatment (Vermorken & Pinedo, 1982) may influence neuronal or enterochromaffin cells to modify 5-HT or other transmitter/modulator/humoral release. Indeed, it is possible that cytotoxic agents do not directly affect the 5-HT containing cell. If 5-HT were to regulate emesis in balance with another as yet hypothetical system(s), neurotoxic disruption of the latter could permit a 5-HT dominance resulting in emesis which might then be controlled with a 5-HT_3 receptor antagonist.

The sites of action of 5-HT_3 receptor antagonists to inhibit emesis induced by cytotoxic treatments

The sites of action of the 5-HT_3 receptor antagonists are clearly those structures containing 5-HT_3 receptors, but the anti-emetic action was first established in the absence of any data as to the location of 5-HT_3 receptors either in the brain or peripheral tissues. However, the subsequent development of tritiated ligands for the 5-HT_3 receptor allowed the location and quantification of 5-HT_3 receptors both centrally and peripherally. The first ligand to be developed was the 5-HT_3 receptor antagonist ^{3}H.GR65630A (Kilpatrick *et al.*, 1987), an analogue of ondansetron, and 5-HT_3 receptors were located within structures involved in the emetic reflex. The highest density of 5-HT_3 receptors in animals brains has been located in the nucleus tractus solitarius and, to a lesser extent, the area postrema (see consensus by Pratt *et al.*, 1990). Evidence indicates that within these structures the receptors are located on vagal afferent terminals (Pratt & Bowery, 1989; Leslie *et al.*, 1990). In addition, radioligand binding studies have confirmed the presence of 5-HT_3 receptors on peripheral vagal afferent fibres and enteric neurones (Kilpatrick *et al.*, 1989; Pinkus *et al.*, 1989; Gordon *et al.*, 1989). These two major sites of action, in the brain stem nuclei or on the peripheral vagus nerve terminals/enteric neurones, provide the two potential sites of action to inhibit emesis. It should be noted that 5-HT_3 receptors have also been located in human brain stem nuclei (Barnes *et al.*, 1988*b*; Reynolds *et al.*, 1989).

In an attempt to elucidate the peripheral and/or central components of action of the 5-HT_3 receptor antagonists, the intraventricular injection of zacopride in the cat was found to antagonise emesis induced by intraventricular injection of cisplatin (Smith *et al.*, 1988). The injection of ondansetron, GR65630A and MDL72222 directly in the region of the area postrema of the ferret also inhibited the emesis induced by a peripheral injection of cisplatin (Higgins *et al.*, 1989). Such studies indicate that an antagonist action at central 5-HT_3 receptors in the area postrema and nucleus tractus solitarius may be important in the inhibition of emesis. It remains to be explained why granisetron can prevent radiation or cisplatin-induced emesis in dogs when given intravenously, but not when given into the ventricular system (Gupta *et al.*, 1988).

However, such studies do not exclude the possibility that aperipheral adminsitration of a 5-HT_3 receptor antagonist may additionally block the 5-HT_3 receptors located on the visceral vagus nerves. Studies are in progress using localised intra-arterial injections of cisplatin and ondansetron into discrete areas of the intestinal system (Rudd, unpublished data).

Using a quite different approach, quaternisation of many compounds is known to reduce their ability to penetrate the

blood brain barrier. It could be hypothesised that such compounds would exert peripheral anti-emetic effects with little or no action in the brain. Our first studies used the quaternised derivative of ICS205-930 which was found to be as potent as ICS205-930 itself to inhibit cisplatin induced emesis in the ferret (Naylor, unpublished data). However, when tested in animal models of anxiety, the quaternised ICS205-930 was found to be potent in reducing aversive responding, similar to ICS205-930 (Costall, unpublished data), indicating that the quaternary derivative had retained ability to penetrate the blood brain barrier (see Costall *et al.*, 1990 for review of the central effects of 5-HT_3 receptor antagonists). Thus reports that the ability of the quaternised ICS205–930 to antagonise cisplatin in the ferret is indicative of a peripheral site of action should be interpreted cautiously (Buchheit *et al.*, 1990). Indeed, using the potent 5-HT_3 receptor antagonist LY277359, Robertson *et al.* (1990) found that the quaternised derivative LY191617 did not antagonise cisplatin emesis in the ferret. Preliminary studies have indicated that the quaternised derivative of ondansetron is also ineffective (Rudd, unpublished data). The latter studies indicate that the anti-emetic effects of 5-HT_3 receptor antagonists may be mediated centrally. Furthermore, since the area postrema does not have an effective blood brain barrier, the central sites of action may involve the nucleus tractus solitarius and related structures. Despite the foregoing uncertainties, the quaternised derivatives may prove important tools in an elucidation of the central/peripheral sites of action of ondansetron and related agents to antagonise emesis.

Adjuvants to 5-HT_3 receptor antagonists in the control of emesis

The 5-HT_3 receptor antagonists are exceedingly effective in preventing emesis induced by cytotoxic agents. In most animals emesis is simply absent and it is difficult to envisage a more effective treatment. In the clinic, nausea and vomiting can be prevented in up to 70% of patients and be reasonably controlled in others. In those patients who do exhibit a residual emetic response it is interesting to question the aetiology. Does it, for example, reflect a cytotoxic induced effect not present in animals, or, a contributory psychogenic response to the observance by one patient of emesis in another patient. The influence of higher functions on emesis has already been considered, and is clearly a critical determinant in anticipatory emesis. The development of an anticipatory or conditioned response may be mediated via mechanism different from those affected by 5-HT_3 receptor antagonists, and may contribute to the difficulties of treating delayed emesis. But it is possible that a conditioned response may begin to develop within hours of commencing treatment in some patients, to increase the difficulties of treating even the acute phase. In brief, emesis in patients receiving cytotoxic treatments may not necessarily be the direct consequence of the emetogenic regimen.

Meaningful answers to the above speculations are not readily available, but in those patients who are not entirely controlled by ondansetron additional therapies have been considered, and dexamethosone is probably the most important example (Grunberg *et al.*, 1985). Thus Smith *et al.* (1990) report major synergism between ondansetron and dexamethasone in man in the control of emesis and in the ferret, Hawthorn & Cunningham (1990) have investigated the effects of ondansetron plus dexamethasone to inhibit cyclophosphamide induced vomiting. The latter well-designed experiments illustrated the difficulties of showing a synergistic interaction between the two drugs to inhibit emesis, when ondansetron itself was so effective. However, dexamethasone did appear to prevent the bouts of emesis occurring in the later stages of cyclophosphamide induced emesis. In studies using cisplatin induced emesis in the ferret, we have encountered similar difficulties in attempting to show a clear synergistic action between ondansetron and dexamethasone (Rudd, unpublished data). Nevertheless, such studies do not detract from the clinical findings and it will be of interest to determine more fully the profile and mechanism of action of dexamethasone as an anti-emetic agent.

Conclusion

From the initial identification of a neuronally located 5-HT receptor some 40 years ago, and its subsequent characterisation and classification as the 5-HT_3 receptor, have come compounds with a unique profile as anti-emetic agents. The 5-HT_3 receptor antagonists such as ondansetron are likely to be the future drugs of choice to prevent emesis induced by a wide range of cytotoxic treatments. The challenge remains to identify the precise sites and mechanisms of action of cytotoxic agents to induce emesis, and the precise sites of action of 5-HT_3 receptor antagonists to inhibit emesis. In turn, this will aid an understanding of the role of 5-HT in emesis and may facilitate developments for the treatment of emesis induced by many other stimuli – presently beyond a rational or effective control.

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Discussion of Professor Naylor's paper

Grunberg: Can we perhaps open the discussion by asking Professor Naylor, whether there is any response he would like to make to his respondents?

Naylor: I think Luigi was questioning the doses we used in animals. The dose chosen is one that is large enough to obtain a consistent response. This is necessary if we are screening a large number of drugs, but the doses are not lethal. It is true that we cannot use any more because the gastro-intestinal disruption is so great that the animals vomit blood, and that is not acceptable.

Tyers: If I could interject there—you haven't got a difference in doses. Toxicities are best correlated between species if you express doses on a metre squared basis. 10 mg/kg cisplatin in a ferret is around 100 mg m^{-2}—a reasonable dose—in man.

Gralla: I agree that the ferret is not a wonderful model. For example zacopride is a terrific anti-emetic in ferrets, but I have not seen any evidence that it acts as an anti-emetic in man. We should be worried about testing agents in ferrets against other causes of emesis and extrapolating to man. The ferret does not duplicate man in many areas. There are species differences and we have to be careful with our models.

Tyers: I think we mustn't forget that it was in the ferret that the $5HT_3$ receptor antagonist was identified. Zacopride is not such an anomaly – it is in fact two compounds, a mixture enantiomers – one is an agonist and one an antagonist. So the problem is not with the model but with the drug being used.

Gralla: Even given that, the fact that the same compound has different effects in different species demonstrates a species difference.

Naylor: Can I take Luigi Cubeddu's second point? We see complete protection in the ferret model yet in patients this is clearly not so. Animal studies are acute experiments lasting 2–4 h. In man observations are over a more protracted time course. Also this is a ferret—he's never experienced nor is he anticipating what is about to happen. Need I say more?

Smyth: There is one aspect of drug-induced emesis which is a constant puzzle: the variability of the emetic challenge. What is so special about anti-neoplastic agents? Most medication does not cause emesis in man. Yet cytotoxic drugs are notorious for their emetic activity. We have an extraordinary different range of chemical structures. Some of these compounds, for example antimetabolites and 5-FU, cause a lot of small bowel damage. One would think this might be the trigger for releasing serotonin, yet these are amongst the least emetogenic agents. Do we not have any clues from studying the actual chemical structures, and the pharmacology? There must be a way we can approach this experimentally.

Naylor: I think that's an extremely sound approach. I don't think any of us have looked for altered transmitter hormone or metabolite levels in response to a range of cytotoxic drugs. We have done some work with platelets but results have been negative or inconsistent. We haven't done any in the gut.

Tyers: The key experiment would be just to see whether cytotoxic drugs have a common mechanism acting directly on the enterochromaffin cells. That could be done with isolated enterochromaffin cells.

Smyth: We have done some experiments which show dexamethasone can suppress the inflammatory component of small bowel damage. This is important because if there is actual destruction of the mucosa it will produce all sorts of secondary stimuli. However we still have the anomaly that the drugs which cause the most obvious mucosal damage are those we have the least problems with.

Cubeddu: Yes, methotrexate, for example, damages the GI tract but not at the time emesis occurs, not until 242–72 h later. Also the emesis induced by methotrexate is very mild.

De Mulder: If dexamethasone is acting as an anti-inflammatory agent, which is rather a slow response, then you wouldn't expect it to work as a rescue medication. So maybe there are different mechanisms in its anti-emetic action.

Naylor: I couldn't agree more. Dexamethasone can work extraordinarily quickly to block emesis. It's unthinkable that it's due to an anti-inflammatory effect—it's too fast.

Smyth: You were trying to steer away from a central mechanism, but we should also consider that dexamethasone affects the permeability of the blood brain barrier.

Naylor: Yes it does and that is another possible mechanism we should consider.

Soukop: Are you suggesting increased permeability of the blood brain barrier increases availability of antagonist?

Tyers: It is possible.

Smyth: Have you any comments on one of the most extraordinary experiments I have seen is in the ferret model? Whole body irradiation was given to the ferret, it started vomiting, then ondansetron was given and the vomiting stopped immediately. The mechanisms of that worries me.

Naylor: We have done it with cisplatin-induced emesis. Almost before the needle is out of the vein the emesis is stopped. Very, very rapid and quite amazing. Why does it worry you?

Smyth: Well, we don't see that in man.

Selby: I would say you do see that in man but not as dramatically. In some of my early work, we were only