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Critical Review

Roles of Substance P and NK_1 Receptor in the Brainstem in the Development of Emesis

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Abstract. The emetic response is primarily a protective reflex occurring in a wide variety of vertebrates in response to the ingestion of toxic compounds. The role of the nuclei in the brainstem, including the area postrema, nucleus tractus solitarius, the dorsal motor nucleus of the vagus, and the central pattern generator for vomiting, as well as the involvement of the abdominal visceral innervation relevant to the emetic reflex, have all been discussed by many researchers. The introduction of serotonin 5-HT_3-receptor antagonists into clinical practice allowed for a dramatic improvement in the management of vomiting. However, vomiting still remains a significant problem. The mechanism of the emetic response is even more complicated than was first thought. This review attempts to bring together some of the evidence suggesting the roles of substance P and its receptor, neurokinin NK_1 receptor, in the brainstem nuclei in the development of emesis. Accordingly, NK_1-receptor antagonists might represent novel drugs for the management of major types of emesis.

Keywords: emesis (vomiting), neurokinin NK_1 receptor, area postrema, nucleus tractus solitarius, substance P

Participation of the nuclei of brainstem in emesis

The emetic response is a defense mechanism that is extremely complicated. Nausea and vomiting frequently occur after surgical procedures requiring general anesthesia (1, 2) and in a wide variety of disorders including peptic ulcers, peritonitis, myocardial infarction, acute systemic infections associated with fever, elevated intracranical pressure, motion sickness, and morning sickness in early pregnancy (3 – 5). In addition, apomorphine, copper sulphate, morphine, and radiation cause emesis in experimental animals, predominantly ferrets, dogs and cats (Table 1). Vomiting was thought to be a reflex integrating the visceral afferent and efferent pathways at the "vomiting center", mentioned in the next paragraph, which is considered to be situated within the medulla oblongata of the brainstem (6). This center receives input from visceral afferents that originate in such peripheral structures as the gastrointestinal tract (7). Following stimulation of the vomiting center, vomiting is then mediated by various efferent pathways, including the vagus nerve and the phrenic nerve (8, 9). When the vomiting center is excited, the airway is closed and a marked lowering of respiration with cyanosis develops transiently. Ultimately, the upper portion of the esophagus relaxes while an increase in internal abdominal pressure leads to an expulsion of the gastric contents (10, 11).

However, the exact location of the vomiting center in the brainstem is still not clear. Based on several lines of evidence, the route for the development of vomiting is shown in Fig. 1. Serotonin and Substance P are released from the enterochromaffin cells of the stomach and intestine (gastrointestinal tract) and from sensory neurons, respectively, by radiation and emetic agents such as copper sulfate or anticancer drugs (12, 13). Some abdominal neurons from both the stomach and intestines and vagal afferents may input to the area postrema (AP), and the AP has AP neurons projecting to the gelatinous areas of the nucleus tractus solitarius (NTS) (the medial part of the NTS). Furthermore, some

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Table 1. Summary of emetogens, species of tested animals, emesis inhibiting drugs, and their related receptors in AP or NTS

Emetogens	Species	Drugs	Receptors	References
Dopamine	cat	idazoxan, yohimbine	α_2	87
R(+)-7-OH-DPAT	ferret	eticlopride	D_3	88
Copper sulfate	ferret	CP-99,994	NK_1	63, 89
	ferret	HSP-117	NK_1	66, 71
	ferret, *Suncus*	resiniferatoxin	NK_1	69, 72
Cisplatin	ferret	CP-99,994	NK_1	63, 89
	ferret	tropisetron	5-HT_3	63
	ferret	ondansetron	5-HT_3	90
	ferret	dextromethorphan	NMDA	32
	Suncus	resiniferatoxin	NK_1	72
Methotrexate	dog	FK1052	5-HT_3, 5-HT_4	60
Apomorphine	ferret	CP-99,994	NK_1	63
Morphine	ferret	HSP-117	NK_1	66
	ferret	CP-99,994	NK_1	71, 89
Loperamide	ferret	naloxone	μ	91
	ferret	resiniferatoxin	NK_1	69
Ipecac	ferret	CP-99,994	NK_1	63, 71, 89
	ferret	tropisetron	5-HT_3	63
Phenylbiguanide	cat, dog, ferret	MDL72222	5-HT_3	92
Radiation	ferret	CP-99,994	NK_1	89
	ferret	resiniferatoxin	NK_1	69
Electrical stimulation of vagus	ferret	CP-99,994	NK_1	63
NMDA, Glutamate	ferret	D-AP5	NMDA	31
Nicotine	*Suncus*	resiniferatoxin	NK_1	72
Motion	cat	CP-99,994	NK_1	76

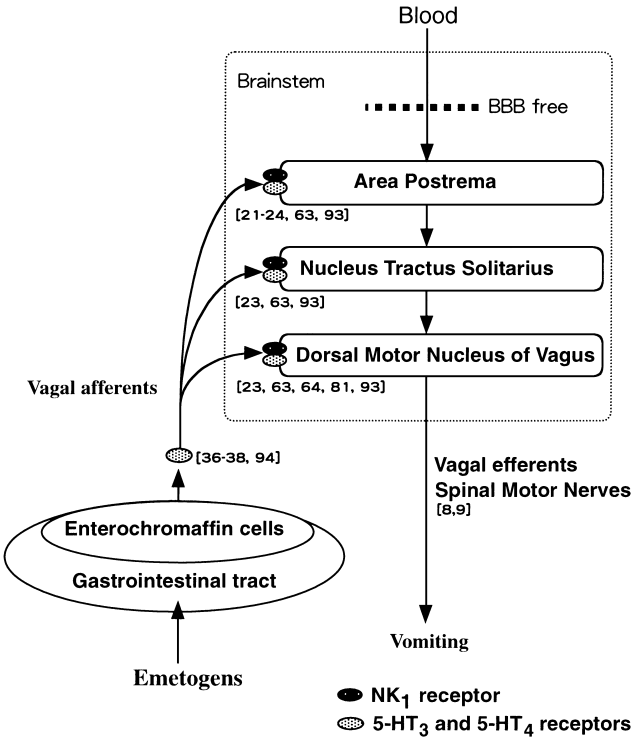

Fig. 1. The pathways involved in emesis in the ferret. Peripherally acting emetogens activate vagal afferent neurons to the group of nuclei in the brainstem enclosed by the dotted line square. The numbers in parentheses indicate the numbers of the relevant literature showing the roles of serotonin, substance P, and their receptors in each nucleus in the brainstem. BBB: blood brain barrier.

neurons from the enterochromaffin cells input directly to the NTS and the dorsal motor nucleus of the vagus in proximity to the NTS (14, 15). For convenience, these groups of nuclei, including the NTS, and neurons located in proximity to the NTS, are named the vomiting center (6).

The AP is a circumventricular organ, located in the dorsal surface of the medulla overlying the NTS (16 for review, 17). In rats and rabbits, the AP is a midline structure lying at the entrance to the central canal. In 'higher' mammals, such as humans, monkeys, cats and dogs, the AP is a bilateral structure (18). However, the fine structure of this area of the brain is conspicuously similar in all species studied (19). The AP, like other circumventricular organs such as the subfornical organum vasculosum of the lamina terminals and the subcommissural organ, is a highly vascular structure

that lacks a complete blood-brain barrier (16 – 19). In addition, the AP is a very unique site because it has various types of receptors for both neurotransmitters and hormones, and it can respond to circulating substances due to the lack of a blood-brain barrier (20 – 24). The AP, therefore, is just like a "window" mediating information from the peripheral system to the central nervous system (CNS). The AP is thought to be the site of initiation of physiological functions such as food intake, body fluid balance, the cardiovascular system, and the control of vomiting. Therefore, Wang and Borison (6) considered the AP as a chemoreceptor trigger zone (CTZ) for vomiting, and they proposed that the CTZ could detect endogenous and exogenous toxic substances.

Miller and Wilson (25), based on the results of their experiments, cast doubt on the existence of the vomiting center. Therefore, Fukuda and Koga (8) suggested the existence of "the central pattern generator for vomiting" in the dorsolateral medulla oblongata, instead of a "vomiting center", based on their experiments with dogs. They postulated that the information relevant to emesis from the gastrointestinal tract is integrated at the interface of vagal afferents and vagal efferents (afferent relay station) in or close to the central pattern generator for vomiting, which exists in the reticular area dorsomedial to retrofacial nucleus (26). Areas related to the development of emesis also exist in the brainstem; namely, the NTS, the dorsal motor nucleus of the vagus, the AP and the central pattern generator for vomiting. Therefore, the purpose of this review is to discuss the present status of the problems encountered in determining the exact site(s) of these areas involved in representative types of emesis.

Neurotransmitters in the area postrema and nucleus tractus solitarius relevant to emesis

Table 1 shows the emetic responses to various pharmacological agents, radiation, and electrical stimulation of vagus in several animal species. Several receptors in the AP and NTS involved in emesis have been reported. Glutamate is present at high concentrations, and its receptor is observed in the NTS (27). Dense glutamate immunoreactivity is also present in the nerve terminals in the AP (28). The AP neurons projecting to the NTS are excited by glutamate in dogs (29) and rats (30). Therefore, the neuronal connection of the AP to the NTS may contribute to the emetic response. Moreover, emesis induced by NMDA or glutamate can be blocked by an intravenous injection of NMDA receptor antagonists, D(−)-2-amino-5-phosphonopentanoic acid (D-AP5) and kynurenic acid (31). In addition, cisplatin-induced emesis can also be inhibited by the intravenous injection of NMDA receptor antagonists, dextromethorphan and CGS 19755 (32). However, the physiological roles of glutamate and its receptors in the AP and NTS have yet to be elucidated, and the involvement of endogenous glutamate in emesis induced by cytotoxic drugs or other emetogens is much less understood. Likewise, an inhibitory amino acid, GABA, is found in the CNS. It appears that GABA regulates the activity of serotonergic neurons of the dorsal raphe (33, 34) and of the AP (35) via $GABA_A$ receptors. There are, however, few accounts of the study of the roles of glutamate and GABA in the regulation of emesis.

Most anticancer drugs induce nausea and vomiting as distressing side effects. The common mediator for emesis is thought to be serotonin, which activates its receptors in the gastrointestinal tract (36). However, the effects of serotonin are very complex because of the presence of various subtypes of serotonin receptors. The pharmacological characteristics, distribution, and function of serotonin receptor subtypes have been reviewed (37, 38). Two serotonin receptor subtypes, 5-HT_3 and 5-HT_4, in the periphery and the CNS seem to be important in the development mechanism of emesis (39 – 41).

In the past decade or so, the 5-HT_3-receptor antagonists have been shown to provide relief from emesis in experimental animals as well as patients that have been treated with cytotoxic drugs such as cisplatin (42, 43). Similarly, these antagonists are effective in reducing or preventing radiation-induced emesis (44). However, 5-HT_3-receptor antagonists are not effective against motion sickness (45, 46) or against emesis induced by many drugs, such as copper sulfate, histamine, protoveratrine, pilocarpine, or apomorphine (47 – 49). In addition, 5-HT_3 antagonists such as ondansetron effectively prevent only acute emesis occurring within the first 24 h of chemotherapy (50). In contrast to acute emesis, these antagonists only partially prevent delayed emesis, which normally occurs 24 h or later after the start of chemotherapy (51, 52).

It is interesting to note that 5-HT_3 receptors are densely located in areas known to be involved in the development of the acute phase of the emetic response. For instance, there are 5-HT_3 receptors on the afferent vagus terminals in the gastrointestinal mucosa (53 – 55) and on the same afferent vagus neurons located in the brainstem, AP, NTS, and dorsal motor nucleus of vagus (43, 44, 56, 57). However, Barnes et al. (21) reported that the binding of the 5-HT_3-receptor antagonist ^{3}H-zacopride was lower in the AP than that found in the NTS. Taking into account the limitations of the antiemetic effect of 5-HT_3 antagonists, being restricted to

the emesis induced by anti-cancer drugs and radiotherapy, it is conceivable that the 5-HT_3 receptor relevant to emesis plays a role mainly in the peripheral system (45, 47, 58). The possibility of participation of 5-HT_3 receptor in the brainstem in emesis has not sufficiently been clarified.

Another subtype of serotonin receptor, 5-HT_4, has been added to explain the mechanism of emesis (40). It is known that serotonin receptors, 5-HT_4 as well as 5-HT_3, in the peripheral gastrointestinal tract are mostly associated with effects on emesis-related gastrointestinal motility (37). In addition, the presence of 5-HT_4 receptor in the brain has been demonstrated using autoradiography (22) and in situ hybridization studies (59). Yamakuni et al. (60) reported that intravenous injection of FK 1052, a potent antagonist of both 5-HT_3 and 5-HT_4 receptors, inhibited the delayed emesis induced by intravenous injection of methotrexate, an anticancer drug, in dogs. On the basis of the neurokinin NK_1-receptor binding experiment, furthermore, they showed that 5-$HT_{3/4}$ receptor antagonists, FK-1052 and tropisetron, had a negligible affinity for NK_1 receptor (60). They suggested that 5-HT_4 receptor in the CNS, in addition to the later-mentioned NK_1 receptor, may be involved in the production of delayed emesis induced by methotrexate in dogs (60).

Substance P and its receptor

As evident from Table 1, it is particularly interesting to note that the NK_1 receptor, a receptor subtype for substance P, is relevant to development of emetic responses in many species of experimental animals tested.

Substance P, a member of the tachykinin family of bioactive peptides, is believed to play an important role as a neurotransmitter in a number of sensory neurons, and it is also considered to have a similar function in the afferent pathways of the emetic reflex. The emetic action of substance P has been described by Carpenter et al. (61). Using immunohistochemical techniques, the presence of substance P in regions of the brainstem involved in emesis in humans has been demonstrated (62).

Tachykinin peptides exert a variety of pharmacological effects through the three G-protein-coupled receptor subtypes, named NK_1, NK_2, and NK_3, in peripheral tissues and the CNS. The NK_1 receptors are dense in the AP, NTS, and dorsal motor nucleus of the vagus in ferrets and rats (23, 63 – 65). Saito et al. (66), based on their results from the emetic responses in ferrets to central and peripheral emetogens and from the electrophysiological experiments using brain slices of ferret including the NTS and AP, suggested that NK_1 receptors within the NTS and AP play an important role in the emetic response in ferrets. Additionally, Ariumi et al. (24) presented evidence for an involvement of the AP in emesis using microinjection of NK_1-receptor antagonists in the ferret.

Several lines of evidence have indicated the existence of NK_1 receptors in the NTS that seem to be the site of the antiemetic action (23, 67). Previous to these studies, Andrews and Bhandari (68) reported that resiniferatoxin (100 μg/kg, s.c.), an analogue of capsaicin, which releases and depletes such sensory neuropeptides as substance P, prevented emesis in ferrets to such peripheral emetogenic stimuli like radiation and copper sulphate as well as to the central emetogen loperamide, which is thought to act in the NTS. Therefore, they proposed that the site of action of resiniferatoxin was within the NTS, where information relevant to emesis from both the AP and vagal afferents converges (68). Andrews et al. (69) proceeded to further elucidate the effects of resiniferatoxin using *Suncus murinus*, the house musk shrew, as an experimental animal. Based on their results from *Suncus murinus*, it is confirmed that the site of the anti-emetic effect of resinferatoxin is within the NTS and that the mechanism relevant to anti-emetic action of NK_1-receptor antagonists in *Suncus murinus* is consistent with the broad-spectrum anti-emetic effects of NK_1-receptor antagonists in other species (63 – 72).

Based on experiments using paralyzed decerebrate dogs with the NK_1-receptor antagonist GR205171, injected either intravenous (25 – 70 mg/kg) or microinjected into the fourth ventricle (1 μg/ml, 30 μg), Fukuda et al. (26, 73, 74) proposed that the sites of antiemetic action of the NK_1-receptor antagonist exist on the neurons in the "afferent relay station". This station is situated between the medial NTS and the central pattern generator for vomiting (26). In addition, Zaman et al. (75) reported that the NK_1-receptor antagonist CP-99,994 had no significant effect on the c-fos expression in the NTS, although it inhibited emesis induced by the opioid receptor agonist loperamide. Taking the previous references into account (26, 68, 73, 74, 76), they suggested that NK_1-receptor antagonists are likely to have a site of action "deeper" in the brainstem than the NTS (75). On the other hand, the 5-HT_3-receptor antagonist granisetron abolished both the emetic effects of cisplatin and the c-fos expression in the NTS, because granisetron acted on 5-HT_3 receptors located on the vagus projection to the NTS (77). The difference in the results of the c-fos expression between two types of receptor antagonists against NK_1 and 5-HT_3 may be closely related to the delete difference in antiemetic activity between these receptor antagonists. The recent observation of Andrews et al. (78) further sup-

ports that NK_1-receptor antagonists have their antiemetic action at sites such as the central pattern generator.

NK_1-receptor antagonists show broad-spectrum antiemetic activity in ferrets (70, 71). The most important effect of NK_1-receptor antagonists is that they are able to markedly prevent both acute and delayed emesis induced by cisplatin in ferrets (79) and humans (80). As mentioned before, 5-HT_3-receptor antagonists effectively prevent only acute emesis (43).

Figure 1 shows a proposed scheme for the roles of NK_1 receptors in the AP, NTS, and the dorsal motor nucleus of the vagus. In these regions, the afferent substance P-ergic pathways terminate in NK_1 receptors and in the output of efferent neurons to vomiting-related muscles (81). It is interesting to note that the NK_1-receptor antagonist CP-122,721, which is more centrally active than any of the previous NK_1-receptor antagonists, blocked the NK_1 receptors in these areas, and consequently it might inhibit both the acute and delayed phases of emesis in dogs induced by the anticancer drug methotrexate (60). The results of a series of experiments using NK_1-receptor antagonists suggest that substance P and NK_1 receptors play an important role in the integration of transmission induced by broad-spectrum emetic stimuli (66). Thus the potential clinical utility of NK_1-receptor antagonists for the treatment of emesis has been well recognized. Studies in animal models of emesis have shown that NK_1-receptor antagonists possess a much broader spectrum compared to known anti-emetics, including 5-HT_3-receptor antagonists, and that in the near future, the NK_1-receptor antagonists may be devoid of side effects.

As shown in Table 1, many studies on emesis have used ferrets as the experimental model. However, common experimental animals such as rats, guinea pigs and mice were not used because they do not vomit. Therefore, Mitchell et al. (82. 83) reported that poison- or motion-induced pica, eating non-nutritive substances such as kaolin in rats, is an illness-response behavior analogous to emesis in other species. Takeda et al. (84) examined the effects of anti-emetics on emetic stimuli-induced pica in rats using the methods of Mitchell et al. They reported that pica in rats is induced by various emetic stimuli such as copper sulfate, apomorphine, cisplatin, and motion. Therefore, they suggested that pica in rats may be a useful animal model for research on emeis. Saeki et al. (85) confirmed that the inhibitory effects of selective NK_1-receptor antagonists, HSP-117 and CP-99,994, and a 5-HT_3-receptor antagonist, ondansetron, on kaolin intake behavior in rats corresponded to suppression of emesis in ferrets (24, 66).

A discussion of the role of other subtypes of tachykinin receptor, NK_2 and NK_3, is outside the scope of this review. In fact, there seem to be very few reports describing the roles of these receptor subtypes in the regulation of emesis. Fundamentally, NK_2 receptor is not expressed in the nervous system (86); therefore, its functional roles in food-intake behavior and secretomotor properties of the gastrointestinal behavior have not been investigated. Krowicki et al. (64) investigated the functional effects of tachykinin peptides and their receptor subtypes on gastric motor inhibition and fundic relaxation those are prodromal events essential for emesis. They demonstrated that the microinjection of substance P and selective NK_1-receptor agonists into the dorsal motor nucleus of vagus inhibited gastric motor activity and evoked gastric relaxation via NK_1 receptors in the dorsal motor nucleus of vagus, whereas that of the NK_3-receptor agonist senktide failed to affect these responses (64). The NK_3-receptor mRNA was detected especially in the nervous system, although the amount of NK_3 mRNA is less expressed as compared with that of NK_1 mRNA (86). Therefore, the functional significance of NK_3 receptors in the dorsal motor nucleus of vagus remains to be elucidated.

Conclusions

In this review, we presented anatomical and pharmacological evidence that some presently known representative neurotransmitters may play a role in the emetic responses at the level of the brainstem, focusing on the roles of substance P and its receptor. 1) The AP is the primary area regarding biologically active chemical substances, because it lacks the blood-brain barrier. It appears to play a major role in responding to toxins in the blood or cerebrospinal fluid. 2) Substance P is one of the most likely chemical substances, in addition to serotonin, known to play a key role in emesis induced by various emetic stimuli. The NK_1 receptors are located in the AP, the central pattern generator for vomiting and/or afferent relay station, and NTS. Therefore, these areas play important roles in emesis via these receptors. 3) Many efforts to identify the site where information from the gastrointestinal tract is integrated in the CNS have not always been successful. However, more recent studies provide evidence that the central pattern generator for vomiting and neurons related to emesis in the vicinity of this generator as the most likely areas in the brainstem. 4) NK_1-receptor antagonists showed antiemetic activity to both acute and delayed emesis in various animal models of emesis. In view of these noteworthy advantages of NK_1-receptor antagonist(s), we hope to develop a useful new class of antiemetic drugs.

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References

1 Ahmed SS, Gupta RC and Brancato RR: Significance of nausea and vomiting during acute myocardial infarction. Am Heart J **95**, 671 – 672 (1978)
2 Bailey PL, Streisand JB, Pace NL, Bubbers SJ, East KA, Mulder S and Stanley TH: Transdermal scopolamine reduces nausea and vomiting after outpatient laparoscopy. Anesthesiology **72**, 977 – 980 (1990)
3 Barnes JH: The physiology and pharmacology of emesis. Mol Asp Med **7**, 397 – 508 (1984)
4 Herlihy T, McIvor ME, Cummings CC, Siu CO, Alikahn M: Nausea and vomiting during acute myocardial infarction and its relation to infarct size and location. Am J Cardiol **60**, 20 – 22 (1987)
5 Jarnfelt-Samsioe A: Nausea and vomiting in pregnancy: a review. Obst Gynecol Surv **42**, 422 – 427 (1987)
6 Wang SC and Borison HL: A new concept of organization of the central emetic mechanism: recent studies on the sites of action of apomorphine, copper sulfate and cardiac glycosides. Gastroenterology **22**, 1 – 12 (1952)
7 Carpenter DO: Neural mechanisms of emesis. Can J Physiol Pharmacol **68**, 230 – 236 (1990)
8 Fukuda H and Koga T: The Bötzinger complex as the pattern generator for retching and vomiting in the dog. Neurosci Res **12**, 471 – 485 (1991)
9 Hyland NP, Abrahams TP, Fuchs K, Burmeister MA and Hornby PJ: Organization and neurochemistry of vagal preganglionic neurons innervating the lower esophageal sphincter in ferrets. J Comp Neurol **430**, 222 – 234 (2001)
10 Lang IM: Digestive tract motor correlates of vomiting and nausea. Can J Physiol Pharmacol **68**, 242 – 253 (1990)
11 Knox AP, Strominger NL, Battles AH and Carpenter DO: The central connections of the vagus nerve in the ferret. Brain Res Bull **33**, 49 – 63 (1994)
12 Freeman AJ, Cunningham KT and Tyers MB: Selectivity of 5-HT3 receptor antagonists and anti-emetic mechanisms of action. Anticancer Drugs **3**, 79 – 85 (1992)
13 Andrews PL: 5-HT_3 receptor antagonists and anti-emesis. *In* 5-HT3 Receptor Antagonists, Edited by King FD, Jones B and Sanger GJ, pp 255 – 317, CRC Press, Boca Raton (1994)
14 Gwyn DG, Leslie RA and Hopkins DA: Gastric afferents to the nucleus of the solitary tract in the cat. Neurosci Lett **14**, 13 – 17 (1979)
15 Ciriello J, Hrycyshyn AW and Calaresu FR: Glossopharyngeal and vagal afferent projection to the brainstem of the cat. J Auton Nerv Sys **4**, 63 – 79 (1981)
16 Johnson AK and Loewy AD: Circumventricular organs and their role in visceral functions. *In* Central Regulation of Autonomic Functions, Edited by Loewy AD and Spyer KM, pp 247 – 267, Oxford University Press, New York (1990)
17 Strominger NL, Knox AP and Carpenter DO: The connectivity of the area postrema in the ferret. Brain Res Bull **33**, 33 – 47 (1994)
18 Wislocki GB and Putnam TJ: Further observations on the anatomy and physiology of the area pastremae. Anat Rec **27**, 151 – 156 (1924)
19 Leslie RA: Comparative aspects of the area postrema: fine-structural considerations help to determine its function. Cell Mol Neurobiol **6**, 95 – 120 (1986)
20 Stefanini E and Clement-Cormier Y: Detection of dopamine receptors in the area postrema. Br J Pharmacol **74**, 257 – 260 (1981)
21 Barnes JM, Barnes NM, Costall B, Naylor IL, Naylor RJ and Rudd JA: Topographical distribution of 5-HT3 receptor recognition sites in the ferret brain stem. Naunyn Schmiedebergs Arch Pharmacol **342**, 17 – 21 (1990)
22 Jakeman LB, To ZP, Eglen RM, Wong EHF and Bonhaus DW: Quantitative autoradiography of 5-HT4 receptors in brains of three species using two structurally distinct radioligands, [^{3}H]GR113808 and [^{3}H]BIMU-1. Neuropharmacology **33**, 1027 – 1038 (1994)
23 Baude A and Shigemoto R: Cellular and subcellular distribution of substance P receptor immunoreactivity in the dorsal vagal complex of the rat and cat: a light and electron microscope study. J Comp Neurol **402**, 181 – 196 (1998)
24 Ariumi H, Saito R, Nago S, Hyakusoku M, Takano Y and Kamiya H: The role of tachykinin NK-1 receptors in the area postrema of ferrets in emesis. Neurosci Lett **286**, 123 – 126 (2000)
25 Miller AD and Wilson VJ: 'Vomiting center' reanalyzed: an electrical stimulation study. Brain Res **270**, 154 – 158 (1983)
26 Furukawa N, Fukuda H, Hatano M, Koga T and Shiroshita Y: A neurokinin-1 receptor antagonist reduced hypersalivation and gastric contractility related to emesis in dogs. Am J Physiol **275**, G1193 – G1201 (1998)
27 Bednar I, Qian M, Qureshi GA, Källström L, Johnson AE, Carrer H and Sodersten P: Glutamate inhibits ingestive behaviour. J Neuroendocrinol **6**, 403 – 408 (1994)
28 Walberg F and Ottersen OP: Neuroactive amino acids in the area postrema. An immunocytochemical investigation in rat with some observations in cat and monkey (*Macaca fascicularis*). Anat Embryol **185**, 529 – 545 (1992)
29 Carpenter DO, Briggs DB and Strominger N: Response of neurons of canine area postrema to neurotransmitters and peptides. Cell Mol Neurobiol **3**, 113 – 126 (1983)
30 Migita K, Hori N, Manako J, Saito R, Takano Y and Kamiya H: Effects of arginine-vasopressin on neuronal interaction from the area postrema to the nucleus tractus solitarii in rat brain slices. Neurosci Lett **256**, 45 – 48 (1998)
31 Price MT and Olney JW: Excitatory amino acid antagonists as anti-emetics. Society for Neuroscience **16**, Abstract, 377 (1990)
32 Lehmann A and Kärrberg L: Effects of *N*-methyl-D-aspartate receptor antagonists on cisplatin-induced emesis in the ferret. Neuropharmacology **35**, 475 – 481 (1996)

33 Kalen P, Strecker RE, Rosengren E and Bjorklund A: Regulation of striatal serotonin release by the lateral habenula-dorsal raphe pathway in the rat as demonstrated by in vivo microdialysis: role of excitatory amino acids and GABA. Brain Res **492**, 187 – 202 (1989)

34 Becquet D, Hery M, Francois-Bellan AM, Giraud P, Deprez P, Faudon M, Fache MP and Hery F: Glutamate, GABA, glycine and taurine modulate serotonin synthesis and release in rostral and caudal rhombencephalic raphe cells in primary cultures. Neurochem Int **23**, 269 – 283 (1993)

35 Cechetto DF, Diab T, Gibson CJ and Gelb AW: The effect of propofol in the area postrema of rats. Anesth Analg **92**, 934 – 942 (2001)

36 Tyers MB and Freeman AJ: Mechanism of the anti-emetic activity of 5-HT3 receptor antagonists. Oncology **49**, 263 – 268 (1992)

37 Briejer MR, Akkermans LM and Schuurkes JA: Gastrointestinal prokinetic benzamides: the pharmacology underlying stimulation of motility. Pharmacol Rev **47**, 631 – 651 (1995)

38 Barnes NM and Sharp T: A review of central 5-HT receptors and their function. Neuropharmacology **38**, 1083 – 1152 (1999)

39 Higgins GA, Kilpatrick GJ, Bunce KT, Jones BJ and Tyers MB: 5-HT_3 receptor antagonists injected into the area postrema inhibit cisplatin-induced emesis in the ferret. Br J Pharmacol **97**, 247 – 255 (1989)

40 Bhandari P and Andrews PL: Preliminary evidence for the involvement of the putative 5-HT4 receptor in zacopride- and copper sulfate-induced vomiting in the ferret. Eur J Pharmacol **204**, 273 – 280 (1991)

41 Endo T, Minami M, Monma Y, Yoshioka M, Saito H and Parvez HS: Vagotomy and ondansetron (5-HT3 antagonist) inhibited the increase of serotonin concentration induced by cytotoxic drugs in the area postrema of ferrets. Biogenic Amines **9**, 163 – 175 (1992)

42 Kris MG, Gralla RJ, Clark RA and Tyson LB: Phase II trials of the serotonin antagonist GR38032F for the control of vomiting caused by cisplatin. J National Cancer Inst **81**, 42 – 46 (1989)

43 Marty M, Pouillart P, Scholl S, Droz JP, Azab M, Brion N, Pujade-Lauraine E, Paule B, Paes D and Bons J: Comparison of the $5\text{-hydroxytryptamine}_3$ (serotonin) antagonist ondansetron (GR 38032F) with high-dose metoclopramide in the control of cisplatin-induced emesis. N Engl J Med **322**, 816 – 821 (1990)

44 King GL and Landauer MR: Effects of zacopride and BMY25801 (batanopride) on radiation-induced emesis and locomotor behavior in the ferret. J Pharmacol Exp Ther **253**, 1026 – 1033 (1990)

45 Lucot JB: Blockade of $5\text{-hydroxytryptamine}_3$ receptors prevents cisplatin-induced but not motion- or xylazine-induced emesis in the cat. Pharmacol Biochem Behav **32**, 207 – 210 (1989)

46 Stott JR, Barnes GR, Wright RJ and Ruddock CJ: The effect on motion sickness and locomotor function of GR 38032F, a 5-HT3-receptor antagonist with anti-emetic properties. Br J Clin Pharmacol **27**, 147 – 157 (1989)

47 Andrews PL, Davis CJ, Bingham S, Davidson HI, Hawthorn J and Maskell L: The abdominal visceral innervation and the emetic reflex: pathways, pharmacology, and plasticity. Can J Physiol Pharmacol **68**, 325 – 345 (1990)

48 Smith WL, Alphin RS, Jackson CB and Sancilio LF: The anti-emetic profile of zacopride. J Pharm Pharmacol **41**, 101 – 105 (1989)

49 Bermudez J, Boyle EA, Miner WD and Sanger GJ: The anti-emetic potential of the $5\text{-hydroxytryptamine}_3$ receptor antagonist BRL 43694. Br J Cancer **58**, 644 – 650 (1988)

50 Andrews PL and Hawthorn J: Evidence for an extra-abdominal site of action for the 5-HT3 receptor antagonist BRL24924 in the inhibition of radiation-evoked emesis in the ferret. Neuropharmacology **26**, 1367 – 1370 (1987)

51 Kris MG, Tyson LB, Clark RA and Gralla RJ: Oral ondansetron for the control of delayed emesis after cisplatin. Report of a phase II study and a review of completed trials to manage delayed emesis. Cancer **70**, Suppl, 1012 – 1016 (1992)

52 Goedhals L, Heron JF, Kleisbauer JP, Pagani O and Sessa C: Control of delayed nausea and vomiting with granisetron plus dexamethasone or dexamethasone alone in patients receiving highly emetogenic chemotherapy: a double-blind, placebo-controlled, comparative study. Ann Oncol **9**, 661 – 666 (1998)

53 Butler A, Hill JM, Ireland SJ, Jordan CC and Tyers MB: Pharmacological properties of GR38032F, a novel antagonist at 5-HT3 receptors. Br J Pharmacol **94**, 397 – 412 (1988)

54 Round A and Wallis DI: The depolarizing action of 5-hydroxytryptamine on rabbit vagal afferent and sympathetic neurones in vitro and its selective blockade by ICS 205-930. Br J Pharmacol **88**, 485 – 494 (1986)

55 Ireland SJ and Tyers MB: Pharmacological characterization of 5-hydroxytryptamine-induced depolarization of the rat isolated vagus nerve. Br J Pharmacol **90**, 229 – 238 (1987)

56 Kilpatrick GJ, Jones BJ and Tyers MB: Identification and distribution of 5-HT3 receptors in rat brain using radioligand binding. Nature **330**, 746 – 748 (1987)

57 Kilpatrick GJ, Jones BJ and Tyers MB: Binding of the 5-HT3 ligand, [^{3}H]GR65630, to rat area postrema, vagus nerve and the brains of several species. Eur J Pharmacol **159**, 157 – 164 (1989)

58 King GL and Landauer MR: Effects of zacopride and BMY25801 (batanopride) on radiation-induced emesis and locomotor behavior in the ferret. J Pharmacol Exp Ther **253**, 1026 – 1033 (1990)

59 Vilar'o MT, Cort'es R, Gerald C, Branchek TA, Palacios JM and Mengod G. L:calization of 5-HT4 receptor mRNA in rat brain by in situ hybridization histochemistry. Mol Brain Res **43**, 356 – 360 (1996)

60 Yamakuni H, Sawai H, Maeda Y, Imazumi K, Sakuma H, Matsuo M, Mutoh S and Seki J: Probable involvement of the $5\text{-hydroxytryptamine}_4$ receptor in methotrexate-induced delayed emesis in dogs. J Pharmacol Exp Ther **292**, 1002 – 1007 (2000)

61 Carpenter DO, Briggs DB and Strominger N: Peptide-induced emesis in dogs. Behav Brain Res **11**, 277 – 281 (1984)

62 McRitchie DA and Törk I: Distribution of substance P-like immunoreactive neurons and terminals throughout the nucleus of the solitary tract in the human brainstem. J Comp Neurol **343**, 83 – 101 (1994)

63 Watson JW, Gonsalves SF, Fossa AA, McLean S, Seeger T, Obach S and Andrews PL: The anti-emetic effects of CP-99,994 in the ferret and the dog: role of the NK1 receptor. Br J Pharmacol **115**, 84 – 94 (1995)

64 Krowicki ZK and Hornby PJ: Substance P in the dorsal motor nucleus of the vagus evokes gastric motor inhibition via neurokinin 1 receptor in rat. J Pharmacol Exp Ther **293**, 214 – 221 (2000)

65 Shiroshita Y, Koga T and Fukuda H: Capsaicin in the 4th ventricle abolishes retching and transmission of emetic vagal

afferents to solitary nucleus neurons. Eur J Pharmacol **339**, 183 – 192 (1997)
66 Saito R, Suehiro Y, Ariumi H, Migita K, Hori N, Hashiguchi T, Sakai M, Saeki M, Takano Y and Kamiya H: Anti-emetic effects of a novel NK-1 receptor antagonist HSP-117 in ferrets. Neurosci Lett **254**, 169 – 172 (1998)
67 Tattersall FD, Rycroft W, Francis B, Pearce D, Merchant K, MacLeod AM, Ladduwahetty T, Keown L, Swain C, Baker R, Cascieri M, Ber E, Metzger J, MacIntyre DE, Hill RG and Hargreaves RJ: Tachykinin NK1 receptor antagonists act centrally to inhibit emesis induced by the chemotherapeutic agent cisplatin in ferrets. Neuropharmacology **35**, 1121 – 1129 (1996)
68 Andrews PL and Bhandari P: Resinferatoxin, an ultrapotent capsaicin analogue, has anti-emetic properties in the ferret. Neuropharmacology **32**, 799 – 806 (1993)
69 Andrews PL, Okada F, Woods AJ, Hagiwara H, Kakaimoto S, Toyoda M and Matsuki N: The emetic and anti-emetic effects of the capsaicin analogue resiniferatoxin in *Suncus murinus*, the house musk shrew. Br J Pharmacol **130**, 1247 – 1254 (2000)
70 Gardner CJ, Twissell DJ, Dale TJ, Gale JD, Jordan CC, Kilpatrick GJ, Bountra C and Ward P: The broad-spectrum anti-emetic activity of the novel non-peptide tachykinin NK1 receptor antagonist GR203040. Br J Pharmacol **116**, 3158 – 3163 (1995)
71 Tattersall FD, Rycroft W, Hill RG and Hargreaves RJ: Enantio-selective inhibition of apomorphine-induced emesis in the ferret by the neurokinin1 receptor antagonist CP-99,994. Neuro-pharmacology **33**, 259 – 260 (1994)
72 Bountra C, Bunce K, Dale T, Gardner C, Jordan C, Twissell D and Ward P: Anti-emetic profile of a non-peptide neurokinin NK1 receptor antagonist, CP-99,994, in ferrets. Eur J Pharmacol **249**, R3 – R4 (1993)
73 Fukuda H, Koga T, Furukawa N, Nakamura E, Shiroshita Y: The tachykinin NK1 receptor antagonist GR205171 prevents vagal stimulation-induced retching but not neuronal transmission from emetic vagal afferents to solitary nucleus neurons in dogs. Brain Res **802**, 221 – 231 (1998)
74 Fukuda H, Koga T, Furukawa N, Nakamura E, Shiroshita Y: The tachykinin NK1 receptor antagonist GR205171 abolishes the retching activity of neurons comprising the central pattern generator for vomiting in dogs. Neurosci Res **33**, 25 – 32 (1999)
75 Zaman S, Woods AJ, Watson JW, Reynolds DJ and Andrews PL: The effect of the NK1 receptor antagonist CP-99,994 on emesis and c-fos protein induction by loperamide in the ferret. Neuropharmacology **39**, 316 – 323 (2000)
76 Lucot JB, Obach RS, McLean S and Watson JW: The effect of CP-99994 on the responses to provocative motion in the cat. Br J Pharmacol **120**, 116 – 120 (1997)
77 Reynolds DJ, Barber NA, Grahame-Smith DG and Lesli RA: Cisplatin-evoked induction of c-fos protein in the brainstem of the ferret: the effect of cervical vagotomy and the anti-emetic 5-HT3 receptor antagonist granisetron (BRL 43694). Brain Res **565**, 231 – 236 (1991)
78 Andrews PL, Kovacs M and Watson JW: The anti-emetic action of the neurokinin$_1$ receptor antagonist CP-99,994 does not require the presence of the area postrema in the dog. Neurosci Lett **314**, 102 – 104 (2001)
79 Rudd JA, Jordan CC and Naylor RJ: The action of the NK1 tachykinin receptor antagonist, CP 99,994, in antagonizing the acute and delayed emesis induced by cisplatin in the ferret. Br J Pharmacol **119**, 931 – 936 (1996)
80 Kris MG, Radford JE, Pizzo BA, Inabinet R, Hesketh A and Hesketh PJ: Use of an NK1 receptor antagonist to prevent delayed emesis after cisplatin. J Natl Cancer Inst **89**, 817 – 818 (1997)
81 Baude A, Lanoir J, Vernier P and Puizillout JJ: Substance P-immunoreactivity in the dorsal medial region of the medulla in the cat: effects of nodosectomy. J Chem Neuroanat **2**, 67 – 81 (1989)
82 Mitchell D, Wells C, Hoch N, Lind K, Woods SC and Mitchell LK: Poison induced pica in rats. Physiol Behav **17**, 691 – 697 (1976)
83 Mitchell D, Krusemark ML and Hafner D: Pica: a species relevant behavioral assay of motion sickness in the rat. Physiol Behav **18**, 125 – 130 (1977)
84 Takeda N, Hasegawa S, Morita M and Matsunaga T: Pica in rats is analogous to emesis: an animal model in emesis research. Pharmacol Biochem Behav **45**, 817 – 821 (1993)
85 Saeki M, Sakai M, Saito R, Kubota H, Ariumi H, Nago S, Takano Y and Kamiya H: Effects of a novel NK-1 receptor antagonist, HSP-117, on cisplatin-induced pica as a new assess-ment of delayed emesis in rats. Jpn J Pharmacol **86**, 359 – 362 (2001)
86 Nakanishi S: Mammalian tachykinin receptors. Ann Rev Neuro-sci **14**, 123 – 136 (1991)
87 Jovanovic-Micic D, Samardzic R and Beleslin DB: The role of alpha-adrenergic mechanisms within the area postrema in dopamine-induced emesis. Eur J Pharmacol **272**, 21 – 30 (1995)
88 Yoshikawa T, Yoshida N and Hosoki K: Involvement of dopa-mine D3 receptors in the area postrema in R(+)-7-OH-DPAT-induced emesis in the ferret. Eur J Pharmacol **301**, 143 – 149 (1996)
89 Bountra C, Gale JD, Gardner CJ, Jordan CC, Kilpatrick GJ, Twissell DJ and Ward P: Towards understanding the aetiology and pathophysiology of the emetic reflex: novel approaches to antiemetic drugs. Oncology **5**, Suppl **1**, 102 – 109 (1996)
90 d'Allens H, Aubert B, Bons J and Pappo M: Ondansetron: a specific 5-HT3 serotonin receptor inhibitor, a new antiemetic in oncology. Bull Cancer **78**, 1139 – 1146 (1991)
91 Bhandari P, Bingham S and Andrews PL: The neuropharma-cology of loperamide-induced emesis in the ferret: the role of the area postrema, vagus, opiate and 5-HT3 receptors. Neuro-pharmacology **31**, 735 – 742 (1992)
92 Miller AD and Nonaka S: Mechanisms of vomiting induced by serotonin-3 receptor agonists in the cat: effect of vagotomy, splanchnicectomy or area postrema lesion. J Pharmacol Exp Ther **260**, 509 – 517 (1992)
93 Kilpatrick GJ, Jones BJ and Tyers MB: The distribution of specific binding of the 5-HT3 receptor ligand [^{3}H]GR65630 in rat brain using quantitative autoradiography. Neurosci Lett **94**, 156 – 160 (1988)
94 Ireland SJ and Tyers MB: Pharmacological characterization of 5-hydroxytryptamine-induced depolarization of the rat isolated vagus nerve. Br J Pharmacol **90**, 229 – 238 (1987)