

*Critical Review***Roles of Substance P and NK₁ 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-HT₃-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 NK₁ receptor, in the brainstem nuclei in the development of emesis. Accordingly, NK₁-receptor antagonists might represent novel drugs for the management of major types of emesis.

Keywords: emesis (vomiting), neurokinin NK₁ 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 intracranial 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

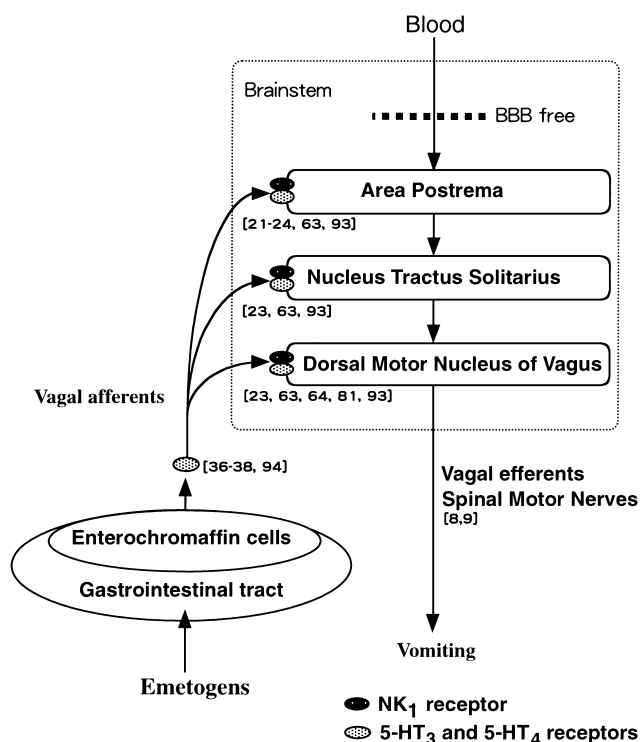
tract (7). Following stimulation of the vomiting center, vomiting is then mediated by various efferent pathways, including the vagus nerve and the phrenic nerve (8, 9). When the vomiting center is excited, the airway is closed and a marked lowering of respiration with cyanosis develops transiently. Ultimately, 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, <i>Suncus</i>	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
	<i>Suncus</i>	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	<i>Suncus</i>	resiniferatoxin	NK ₁	72
Motion	cat	CP-99,994	NK ₁	76



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 terminalis and the subcommissural organ, is a highly vascular structure

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.

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 dorso-medial 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-HT₃ and 5-HT₄, 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-HT₃-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-HT₃-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-HT₃ 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-HT₃ 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-HT₃ 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-HT₃-receptor antagonist ³H-zacopride was lower in the AP than that found in the NTS. Taking into account the limitations of the anti-emetic effect of 5-HT₃ antagonists, being restricted to

the emesis induced by anti-cancer drugs and radiotherapy, it is conceivable that the 5-HT₃ receptor relevant to emesis plays a role mainly in the peripheral system (45, 47, 58). The possibility of participation of 5-HT₃ receptor in the brainstem in emesis has not sufficiently been clarified.

Another subtype of serotonin receptor, 5-HT₄, has been added to explain the mechanism of emesis (40). It is known that serotonin receptors, 5-HT₄ as well as 5-HT₃, in the peripheral gastrointestinal tract are mostly associated with effects on emesis-related gastrointestinal motility (37). In addition, the presence of 5-HT₄ 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-HT₃ and 5-HT₄ receptors, inhibited the delayed emesis induced by intravenous injection of methotrexate, an anticancer drug, in dogs. On the basis of the neurokinin NK₁-receptor binding experiment, furthermore, they showed that 5-HT_{3/4} receptor antagonists, FK-1052 and tropisetron, had a negligible affinity for NK₁ receptor (60). They suggested that 5-HT₄ receptor in the CNS, in addition to the later-mentioned NK₁ 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 NK₁ 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 NK₁, NK₂, and NK₃, in peripheral tissues and the CNS. The NK₁ 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 NK₁ 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 NK₁-receptor antagonists in the ferret.

Several lines of evidence have indicated the existence of NK₁ 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 resiniferatoxin is within the NTS and that the mechanism relevant to anti-emetic action of NK₁-receptor antagonists in *Suncus murinus* is consistent with the broad-spectrum anti-emetic effects of NK₁-receptor antagonists in other species (63–72).

Based on experiments using paralyzed decerebrate dogs with the NK₁-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 NK₁-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 NK₁-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 NK₁-receptor antagonists are likely to have a site of action “deeper” in the brainstem than the NTS (75). On the other hand, the 5-HT₃-receptor antagonist granisetron abolished both the emetic effects of cisplatin and the c-fos expression in the NTS, because granisetron acted on 5-HT₃ 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 NK₁ and 5-HT₃ may be closely related to the difference in anti-emetic activity between these receptor antagonists. The recent observation of Andrews et al. (78) further sup-

ports that NK₁-receptor antagonists have their anti-emetic action at sites such as the central pattern generator.

NK₁-receptor antagonists show broad-spectrum anti-emetic activity in ferrets (70, 71). The most important effect of NK₁-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-HT₃-receptor antagonists effectively prevent only acute emesis (43).

Figure 1 shows a proposed scheme for the roles of NK₁ receptors in the AP, NTS, and the dorsal motor nucleus of the vagus. In these regions, the afferent substance P-ergic pathways terminate in NK₁ receptors and in the output of efferent neurons to vomiting-related muscles (81). It is interesting to note that the NK₁-receptor antagonist CP-122,721, which is more centrally active than any of the previous NK₁-receptor antagonists, blocked the NK₁ 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 NK₁-receptor antagonists suggest that substance P and NK₁ receptors play an important role in the integration of transmission induced by broad-spectrum emetic stimuli (66). Thus the potential clinical utility of NK₁-receptor antagonists for the treatment of emesis has been well recognized. Studies in animal models of emesis have shown that NK₁-receptor antagonists possess a much broader spectrum compared to known anti-emetics, including 5-HT₃-receptor antagonists, and that in the near future, the NK₁-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 emesis. Saeiki et al. (85) confirmed that the inhibitory effects of selective NK₁-receptor antagonists, HSP-117 and CP-99,994, and a 5-HT₃-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 tachy-

kinin receptor, NK₂ and NK₃, 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, NK₂ 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 NK₁-receptor agonists into the dorsal motor nucleus of vagus inhibited gastric motor activity and evoked gastric relaxation via NK₁ receptors in the dorsal motor nucleus of vagus, whereas that of the NK₃-receptor agonist senktide failed to affect these responses (64). The NK₃-receptor mRNA was detected especially in the nervous system, although the amount of NK₃ mRNA is less expressed as compared with that of NK₁ mRNA (86). Therefore, the functional significance of NK₃ 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 NK₁ 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) NK₁-receptor antagonists showed anti-emetic activity to both acute and delayed emesis in various animal models of emesis. In view of these noteworthy advantages of NK₁-receptor antagonist(s), we hope to develop a useful new class of antiemetic drugs.

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