A neurokinin-1 receptor antagonist reduced hypersalivation and gastric contractility related to emesis in dogs

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Furukawa, Naohiro, Hiroyuki Fukuda, Mizue Hatano, Tomoshige Koga, and Yasutera Shirosita. A neurokinin-1 receptor antagonist reduced hypersalivation and gastric contractility related to emesis in dogs. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G1193–G1201, 1998.—The roles of tachykinin neurokinin-1 (NK1) receptors in the induction of fictive retching, hypersalivation, and gastric responses associated with emesis induced by abdominal vagal stimulation were studied in paralyzed, decerebrated dogs. Vagal stimulation induced gradual increases in salivary secretion and activity of the parasympathetic postganglionic fibers to the submandibular gland, relaxation of the gastric corpus and antrum, and fictive retching. However, hypersalivation and increased nerve activity were suppressed and antral contractility was enhanced during fictive retching. An NK1 receptor antagonist, GR-205171, abolished the enhancement of antral contractility and fictive retching but had no effect on corpus and antral relaxation. Hypersalivation and increased nerve activity were inhibited by GR-205171 but were not completely abolished. Reflex salivation by lingual stimulation was unaffected. These results suggest that GR-205171 acts on the afferent pathway in the bulb and diminishes hypersalivation and antral contraction related to emesis as well as fictive retching but does not affect gastric relaxation or hypersalivation induced by the vagovagal, vagosalivary, and linguosalivary reflexes.

IT IS WELL KNOWN THAT abdominal vagal afferent nerves play an important role in the induction of emesis by visceral stimulation. 5-HT3 receptor antagonists have been shown to suppress emesis by blocking the stimulatory effects of the chemical and radiological treatment of cancers on peripheral vagal nerve terminals (6, 7). Recently, neurokinin-1 (NK1) receptors have attracted a great deal of attention from many investigators, because these receptors in the central nervous system are thought to be involved in the induction of emesis (1, 11–13, 25–27, 29). However, almost all of these studies have revealed the effects of NK1 antagonists only on somatomotor responses. Lang et al. (22) indicated that a series of neural mechanisms is involved in emesis: the first participates in gastrointestinal motor correlates, and the second induces somatomotor emetic responses. Furukawa and Okada (9) reported that canine salivary secretion was increased before fictive retching induced by emetic stimuli but was suppressed during fictive retching. Because the salivary center receives two opposite inputs in the preretching phase and in the retching phase, these workers suggested the existence of an afferent relay station for emesis that triggers the central pattern generator (CPG) for somatomotor emetic action (3, 4, 21) and simultaneously induces autonomic nervous responses associated with emesis. Therefore, it is necessary to investigate the effects of NK1 receptor antagonists on autonomic nervous responses associated with emesis, as well as somatomotor responses. However, there has been no previous study of the effects of NK1 receptor antagonists on autonomic nervous responses associated with emesis. This study was undertaken to investigate the effects of NK1 receptor antagonist on the activity of the afferent relay station suggested by Furukawa and Okada (9) by observing the effects on autonomic responses after emetic stimulation.

Because salivary secretion from the submaxillary gland exactly corresponds to the activity of the innervating nerves and because quantitative analysis is easy, we used salivary secretion as an index of autonomic activity. Afferent stimulation of the vagus nerves is thought to be useful for studying emesis in acute experiments in dogs, because stable emetic responses can be induced repeatedly. Therefore, afferent vagal stimulation was used to induce emesis. However, vagal stimulation also induces other reflex responses. For example, esophageal and gastric distension and afferent stimulation of the gastric branch elicits hypersalivation by the vagosalivary reflexes (9, 16, 19), and afferent stimulation of the gastric vagal branch elicits gastric relaxation by the vagovagal reflex (18). Salivary secretion is thought to be increased by vagal stimulation not only related to emesis but also via the vagosalivary reflexes. Qu et al. (23) reported that gastric antral contractility was inhibited by vagal stimulation via the vagovagal reflex but enhanced during fictive retching. Recording of gastric motility makes it possible to distinguish between the gastric response related to emesis induced by vagal stimulation and that evoked by the vagovagal reflex. For these reasons, in this study we investigated the effects of a nonpeptide NK1 receptor antagonist, GR-205171, on fictive somatomotor responses, gastric corpus and antral contractile responses, and salivary secretion associated with emesis induced by vagal stimulation. Furthermore, the effects of GR-205171 on salivary responses associated with emesis were compared with those on salivary secretion induced by the linguosalivary reflex (9, 15) to examine...
its effects on the efferent pathway to the salivary glands. Portions of the results have been reported elsewhere as an abstract (8).

**MATERIALS AND METHODS**

General methods of animal preparation. All of the experiments were approved by the Animal Research Committee of the Kawasaki Medical School and conducted according to the Guide for the Care and Use of Laboratory Animals. Six mongrel dogs (6–11 kg) were fasted for 16 h and used for the present study. All of the dogs were anesthetized with ketamine (25 mg/kg im). Almost all of the animals became quiet and flaccid within 5 min. If the animal was not flaccid enough ~5 min after administration, a further 10 mg/kg of ketamine was added. Midcricoid decerberation was performed during the subsequent 10 min. The dogs were paralyzed with gallamine triethiodide (2 mg/kg iv) and artificially ventilated with a respirator. Body temperature was maintained near 37.5°C by a feedback system, using an infrared heating lamp. A microtip pressure transducer was inserted into the right femoral artery, and mean systemic arterial pressure was monitored using a digital pressure monitor (Camino, M 420). To record gastric circular contractilities, two force transducers were sewn onto the wall of the gastric corpus and antrum ~3 cm orad to the pyloric sphincter in the direction of the circular muscle. A polyethylene catheter (2 mm ID, 20 cm long) was inserted into the left Wharton's duct, and drops of saliva (each drop was ~0.02 ml) from the catheter were counted using a photoelectric drop counter.

Recording of nerve discharges. The left phrenic nerve and the nerve innervating the left rectus abdominis were isolated through incisions along the cervical midline and the dorsal edge of the obliquus externus abdominis, respectively. The postganglionic branch from the right submandibular ganglion was isolated after partial removal of the masseter muscle through a midline maxillary incision. These nerves were carefully separated from the surrounding connective tissues under a stereoscopic dissecting microscope and covered with liquid paraffin. Centrifugal discharge from these nerves was monitored via bipolar platinum wire electrodes. Neural discharge was converted into frequency histograms of 200-ms, 500-ms, or 1-s bins using spike counters (Dia Medical, DSE-325 A), and the histograms were recorded (NEC San-ei, OMINACE RT2116A-08). Activities of these nerves were monitored with an oscilloscope (Nihon Kohden, VC 11). The details of these methods have been reported previously (9).

Stimulation of the lingual and vagal nerves and experimental design. The lingual nerve on the left side was isolated, and electrical afferent stimulation (1 ms, 10 V, 10 Hz) was applied via a bipolar silver wire electrode to induce reflex salivation from the left submaxillary gland. The dorsal vagal trunk was sectioned just above the diaphragm, and fictive retching was induced by electrical stimulation (1 ms, 20–25 V, 3–10 Hz) of the central part of the severed vagal trunk. Coactivating rhythm valleys of the phrenic nerve and the nerve to the rectus abdominis were used as an index of fictive retching. For the control experiments, vagal stimulation of at least 90 s was applied as emetic stimulation. When fictive retching persisted 90 s after the initiation of vagal stimulation, the stimulation was discontinued after the cessation of fictive retching. In the control experiments, two to four applications of vagal stimulation and two or three applications of lingual nerve stimulation of a 15-s period were applied. The NK<sub>1</sub> receptor antagonist GR-205171 [2-methoxy-5-(5-trifluoromethyl-tetrazol-1-yl)-benzyl]-(25-phenyl-piperidin-35-yl)-amino dihydrochloride (Glaxo Wellcome) was then adminis-
period before stimulation and within a 30-s period at the corresponding time as in the control. The statistically significant differences between the basal values before and ~10 min after the administration of NK₁ antagonist and the values before and after stimulation in the corpus and antral contractilities were analyzed. Probability values of \( P < 0.05 \) were considered significant.

RESULTS

Effects of vagal stimulation on the parasympathetic nerve activity to the submandibular gland, salivary secretion, and gastric motility in the control experiments. Vagal stimulation induced fictive retching, with a mean latency of 41.3 ± 7.2 s (\( n = 6 \)) in all of the dogs. Multiunit activity of the right chorda tympani nerve was slightly increased by vagal stimulation, although high-frequency activity was transiently exhibited at the onset of the vagal stimulation in some cases. This increased activity gradually increased further until fictive retching occurred. Subsequently, the excitatory effect was partially depressed in association with retching and again recovered after the cessation of retching. After the cessation of vagal stimulation, nerve activity gradually decreased and returned to the basal level ~1 min after the end of stimulation (Fig. 1A). The mean 10-s values of the activity immediately before retching (preretching phase) and immediately before (late-retching phase) and after (postretching phase) the cessation of retching and at 40–50 s after the cessation of retching were significantly larger than the basal value. There was no significant difference between the basal value and the value at 90–100 s after the cessation of retching (Fig. 2). Similar changes were observed in salivary secretion from the contralateral submandibular gland, although these changes were delayed by several seconds from the changes in nerve activity (Fig. 1A). The mean 10-s volumes at the preretching, late-retching, and postretching phases were significantly greater than the basal value. The mean volume at 120–130 s after the cessation of retching was not significantly different from the basal value before stimulation (Fig. 3).

Gastric corpus contractility was continuously decreased by vagal stimulation in five dogs. In one dog only, this contractility did not change immediately after the initiation of vagal stimulation but rather at the same time as retching occurred (Fig. 4A). Gastric

Fig. 1. Effects of a neurokinin-1 (NK₁) receptor antagonist, GR-205171, on salivary secretion and activity of parasympathetic nerve innervating the submandibular gland induced by emetic vagal stimulation. A: before administration of GR-205171 (control). B: after administration of GR-205171 (50 µg/kg iv). In B, vagal stimulation was applied 30 min after administration of GR-205171. Phrenic N., centrifugal activity of the phrenic nerve represented as frequency histograms with 200-ms bins; Abdominal M. N., centrifugal activity of an abdominal muscle branch of the L1 spinal nerve represented as frequency histograms with 500-ms bins; Saliva from L. Mand., salivary flow (1 drop was considered to be 0.02 ml) from the left submandibular gland; R. Parasympa. N., centrifugal activity of the parasympathetic nerve innervating the right submandibular gland represented as frequency histograms with 500 ms bins; imp, impulses; stim, stimulation. After GR-205171, fictive retching was not induced by vagal stimulation, and responses in salivary secretion and nerve activity were decreased but sustained. Note that salivary secretion during retching in the control experiments was equivalent to that induced by vagal stimulation after GR-205171.
Antral contractility was decreased immediately after the initiation of vagal stimulation, but was enhanced during retching in five dogs (Fig. 4A); contractility was only slightly enhanced during retching in the remaining dog. The mean value of the relative magnitude of gastric contractility was significantly decreased in the corpus and significantly increased in the antrum during fictive retching (Fig. 5).

Effects of vagal stimulation on activity in the parasympathetic nerve innervating the submandibular gland and salivary secretion after GR-205171. After the intravenous administration of 50 µg/kg of GR-205171, no fictive retching was induced in any of the dogs, although facilitatory effects on the phrenic and abdominal muscle nerves activities were sometimes observed (Fig. 4B). Vagal stimulation induced a small, but sustained, increase in parasympathetic nerve activity and salivary secretion, and the responses rapidly diminished after the cessation of stimulation (Fig. 2B). The mean values of salivary secretion and nerve activity during vagal stimulation after GR-205171 were similar to those during the retching phase in the control experiments (Figs. 2 and 3C, late retch). All of the mean 10-s values during and just after vagal stimulation (preretch, late retch, and postretch) were significantly higher than the basal value, but the facilitation in nerve activity at 40–50 s after the cessation of stimulation observed before GR-205171 was abolished. The mean 10-s value of nerve activity after GR-205171 was significantly decreased at the postretching phase and at 40–50 s after the cessation of stimulation compared with the control experiment but was not significantly changed in the preretching and late-retching phases (Fig. 2C). Similar changes were observed in salivary secretion, although these were delayed after the changes in nerve activity by several seconds (Fig. 3B). The mean 10-s value of salivary secretion after GR-205171 was significantly decreased at the postretching phase and at 40–50 s after the cessation of stimulation compared with the control experiment but was not significantly changed in the late-retching phase or at 120–130 s after the cessation of stimulation (Fig. 2C). To summarize, after GR-205171, salivary secretion was increased by vagal stimulation, but the marked increases in the preretching and postretching phases observed in the control experiments were diminished, and a small increase of the same magnitude as
that observed during fictive retching in the control experiments was sustained.

Effects of vagal stimulation on gastric motility after GR-205171. GR-205171 did not change basal gastric contractilities. The mean relative magnitudes of gastric contractility in the 30-s periods just before and 10 min after GR-205171 were 4.31 ± 0.39 and 4.02 ± 0.41 in the corpus and 4.33 ± 0.48 and 4.20 ± 0.55 in the antrum, respectively. There was no significant difference between the basal values before and after GR-205171. After GR-205171, antral contractility was slightly inhibited by vagal stimulation in five dogs (Fig. 4B) and was not changed in the remaining dog. The significant increase in the mean value of antral contractility during fictive retching observed in the control experiments was abolished by GR-205171 (Fig. 5). The mean value of the relative magnitude of antral contractility in the 30-s period just after vagal stimulation was significantly decreased to 3.81 ± 0.52 from the basal value of 4.05 ± 0.55.

Salivary secretion induced by lingual nerve stimulation before and after GR-205171. Salivary secretion was significantly increased by afferent stimulation of the lingual nerve (10 V, 10 Hz) in the control experiments (Fig. 3D). The salivary response to lingual nerve stimulation was not significantly changed by GR-205171. Salivary secretion was also significantly increased by lingual nerve stimulation after GR-205171. There were no significant differences in the basal values or in the stimulation response values before or after GR-205171 (Fig. 3D).

**DISCUSSION**

Salivary and gastric responses induced by vagal stimulation. Previously, Furukawa and Okada (9) reported in chloralose-anesthetized dogs that salivary

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**Fig. 3. Changes in salivary secretion associated with emetic vagal stimulation and salivary response to lingual nerve stimulation before and after GR-205171.**

A: before GR-205171. B: after GR-205171 (50 µg/kg). C: comparison of salivary responses to vagal stimulation before and after GR-205171. D: comparison of salivary responses to lingual nerve stimulation before and after GR-205171. N.: nerve. For further details, see legend to Fig. 2. *P < 0.05, **P < 0.01 vs. basal values. @P < 0.05 before vs. after GR-205171.
secretion and the activity of the parasympathetic nerve innervating the submandibular gland were facilitated before fictive retching induced by intravenous apomorphine or intragastric copper sulfate and were suppressed during retching. In the present study, similar results were obtained by vagal stimulation in decerebrated dogs, although small increases in salivary secretion and nerve activity still remained during retching. It was suggested in a previous study that an afferent relay station in the bulb that drives the CPG for the somatomotor emetic act (3, 4, 21) may simultaneously excite the salivary secretory center, and outputs from the CPG may inhibit the salivary secretory center as well as elicit somatomotor responses (Fig. 6). The difference in the degree of inhibition during retching is thought to be due to the different methods used to induce retching. Vagal stimulation elicits the vagosalivary reflex to a greater extent than intravenous apomorphine, so, in the present study, inhibitory outputs from the CPG might suppress the excitatory effects from the relay station relating to emesis, but not the vagosalivary reflex.

Gastric contractility was generally inhibited by vagal stimulation, but antral contractility was enhanced during fictive retching in the control experiments, as reported by Qu et al. (23). They suggested that this inhibitory effect in the antrum was induced by the vagovagal reflex, whereas the excitatory effect was related to emesis, because high-frequency vagal stimulation strongly inhibited antral contractility without fictive retching or antral contraction.

Effects of NK₁ antagonist on somatomotor emetic acts induced by vagal stimulation. The selective nonpeptide NK₁ receptor antagonist GR-205171 abolished fictive retching in all of the dogs in the present study. GR-205171 and other nonpeptide and peptide NK₁ receptor antagonists, e.g., CP-99,994, GR-203040, and GR-82334, have been shown to inhibit somatomotor emetic responses induced by various stimuli, i.e., afferent vagal stimulation, displatin, and copper sulfate, in the
ferret, dog, and Suncus murinus (1, 11–13, 25–27). Therefore, it is clear that NK₁ receptors play an important role in the induction of somatomotor emetic responses by various stimuli.

Effects of an NK₁ antagonist on changes in gastric contractility induced by vagal stimulation. In the present study, GR-205171 abolished the excitatory response in the antrum as well as fictive retching, whereas the inhibitory responses in the corpus and antrum remained unaffected. Because the excitatory response and inhibitory responses are thought to be related to emesis and the vagovagal reflex, respectively, as mentioned above, GR-205171 may suppress not only retching activity but also the accompanying phenomena associated with emesis, without inhibiting other physiological reflexes. Similar results obtained for salivary secretion seem to support this hypothesis, as mentioned below.

Effects of an NK₁ antagonist on changes in salivary secretion induced by vagal stimulation. In the control experiments, salivary secretion and the activity of the parasympathetic nerve innervating the submandibular gland were greatly increased before retching (preretching phase) and immediately after the cessation of retching (postretching phase). After GR-205171, salivary secretion at the corresponding times as in the control was significantly reduced, whereas the mean value during the retching phase (late-retching phase) in the control was equivalent to that at the corresponding time after the NK₁ antagonist. We previously reported that the frequency of parasympathetic nerve activity was related to the volume of salivary secretion (9). In the present study, the difference between the discharge rates before and after GR-205171 in the preretch phase was not significant, whereas the mean value during the retching phase was decreased, whereas the mean value during the retching phase was decreased. Therefore, the NK₁ receptor antagonist may exist on neurons in afferent relay station. CPG is thought to exist in the area dorsal to the retrofacial nucleus (3, 20), and the afferent relay station may be situated at the medullar area medial to the most rostral part of the nucleus ambiguus (5).
secretion more potently than substance P itself. In the present study, however, salivary secretion induced by lingual nerve stimulation was not significantly different before and after GR-205171. Therefore, GR-205171 seems to have little or no effect on the efferent pathway of the salivary response induced by emetic stimulation under our experimental conditions in dogs. Because salivary secretion and parasympathetic nerve activity were strongly suppressed during retching induced by apomorphine (9), as mentioned above, the following conclusions should be considered. 1) The increased response in salivary secretion and nerve activity in the control experiments may reflect the sum of the response to the vagosalivary reflex and that related to emesis. 2) During the retching phase in the control experiments, only the response to the vagosalivary reflex may be present. 3) GR-205171 may diminish only the response related to emesis but leave the response to the vagosalivary reflex unaltered, which appeared immediately after the initiation of vagal stimulation with constant magnitude.

Possible location of NK$_1$ receptors, and schema of the central mechanisms for induction of the salivary and gastric responses related to emesis. Gardner et al. (11) reported that a peptide NK$_1$ receptor antagonist, GR-82334, inhibited cisplatin-induced emesis in the ferret after hindbrain administration but not when given peripherally. It has been shown that some vagal C fibers contain immunoreactive substance P (28) and are sensitive to capsaicin (10, 17). Koga and Fukuda (20) recently, Shiroshita et al. (24) reported in dogs that capsaicin or reserpine toxin, an ultrapotent capsaicin analog, administered into the fourth ventricle abolished fictive retching induced by vagal afferent stimulation but did not abolish the retching induced by stimulation of the medial NTS. These results suggested that substance P from capsaicin-sensitive vagal afferent nerves mediates visceral emetic signals to the medial NTS. However, very recently, Fukuda et al. (5) suggested that microinjection of GR-205171 at the medullar area medial to the most rostral part of the nucleus ambiguous not only abolished fictive retching but also decreased hypersalivation before retching. This area is situated between the medial NTS and the CPG, which may exist in the area dorsal to the retrolental nucleus (3, 21). Therefore, it seems that vagal afferent fibers that mediate emetic signals are capsaicin sensitive, but the relevant transmitter may not be substance P, although further studies are required to clarify the transmitter from the vagal afferent fibers.

On the basis of the results of the present study and previous work, the following conclusions are postulated (Fig. 6). 1) Emetic vagal afferent nerves excite the neurons in the NTSs, but NK$_1$ receptors may not play a major role in this excitation. 2) The outputs from the neurons in the NTS excite an afferent relay station that drives the CPG and simultaneously facilitates salivary secretion and antral contractility. 3) NK$_1$ receptors may exist on the neurons in the relay station. 4) The outputs from the CPG inhibit the salivary secretory centers and elic it somatomotor responses. This inhibition may be induced via the relay station.

In conclusion, an NK$_1$ receptor antagonist may abolish salivary and gastric responses related to emesis as well as fictive retching but does not change the responses induced by the lingusalivary reflex and vago-vagal gastric reflexes.

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