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European Journal of Pharmacology 339 (1997) 183–192

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Capsaicin in the 4th ventricle abolishes retching and transmission of emetic vagal afferents to solitary nucleus neurons

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Received 2 September 1997; revised 24 September 1997; accepted 26 September 1997

Abstract

Systemic tachykinin NK1 receptor antagonists and resiniferatoxin are known to abolish vomiting mediated by vagal afferents. Emetic vagal afferents have been shown to make synaptic contact with neurons in the medial solitary nucleus. These results suggest that substance P participates in the synapse as a mediator. To examine this possibility, the effects of 4th-ventricular application of capsaicin (0.033–33 mM, 20–30 μl) and resiniferatoxin (1.6–160 μM, 20–30 μl) on the activity of neurons in the medial solitary nucleus and fictive retching induced by vagal stimulation were observed in paralyzed decerebrate dogs. Capsaicin (33 mM) and resiniferatoxin (160 μM) initially increased the neuronal firing and occasionally produced retching, then abolished both neuronal and retching responses. However, stimulation of the medial solitary nucleus continued to provoke retching. Field potential changes in the medial solitary nucleus evoked by pulse-train vagal stimulation decreased in amplitude, but did not disappear. Latencies of neuronal firing and evoked potentials were about 300 ms. These results suggest that emetic vagal afferents are capsaicin-sensitive C fibers which may have substance P as an excitatory transmitter or modulator. © 1997 Elsevier Science B.V.

Keywords: Vomiting; Capsaicin; Resiniferatoxin; Solitary nucleus; Vagus nerve; (Dog)

1. Introduction

Capsaicin acts on thin sensory fibers, producing a short-lasting activation and subsequent long-term desensitization (see review by Holzer (1992)). The vagus nerve has been shown to contain capsaicin-sensitive fibers (Marsh et al., 1987; Ritter and Dinh, 1988; Holzer, 1992). The subcutaneous injection of resiniferatoxin, an ultra-potent capsaicin analogue, transiently induced emesis in *suncus murinus* in a dose-dependent manner (Matsuki et al., 1996), and blocked emesis induced by radiation and copper sulfate in the ferret (Andrews and Bhandari, 1993) and by cisplatin and copper sulfate in *suncus murinus* (Matsuki et al., 1996). Vagotomy is known to reduce the emetic effects of these stimuli (see review by Naylor and Rudd, 1992; Fukui et al., 1992, 1993). These results suggest that capsaicin-sensitive vagal afferents mediate visceral emetic stimuli to the medulla oblongata.

Capsaicin is known to release and then deplete substance P from central nerve terminals of nociceptive afferents in the spinal cord (see review of Holzer (1992)). Similarly, capsaicin has been shown to reduce substance P levels in the vagus nerve and medulla oblongata (Gamse et al., 1981, 1986; South and Ritter, 1988). Furthermore, some vagal C afferents are known to contain immunoreactive substance P (Helke and Hill, 1988; Thor et al., 1988). These results show that capsaicin-sensitive vagal afferents have substance P as a transmitter.

Neurons of the solitary nucleus have been shown to have neurokinin NK_1 receptors (Watson et al., 1995). Furthermore, new selective non-peptide NK_1 receptor antagonists, i.e., (+)-(2*S*,3*S*)-3-(2-methoxybenzylamino)-2-phenylpiperidine (CP-99,994) (Bountra et al., 1993; Tattersall et al., 1993, 1994, 1995; Gardner et al., 1995a; Watson et al., 1995), (2*S*,3*S*)-(2-methoxy-5-tetrazol-1-ylbenzyl)(2-phenylpiperidin-3-yl)amine (GR203040) (Gardner et al., 1995b) and (2-methoxy-5-(5-trifluoromethyl-tetrazol-1-yl)-benzyl)-(2*S*-phenyl-piperidin-3*S*-yl)-amine dihydrochloride (GR205171) (Gardner et al., 1996), have been shown to inhibit emesis induced by various stimuli, e.g., electrical vagal stimulation, cisplatin and

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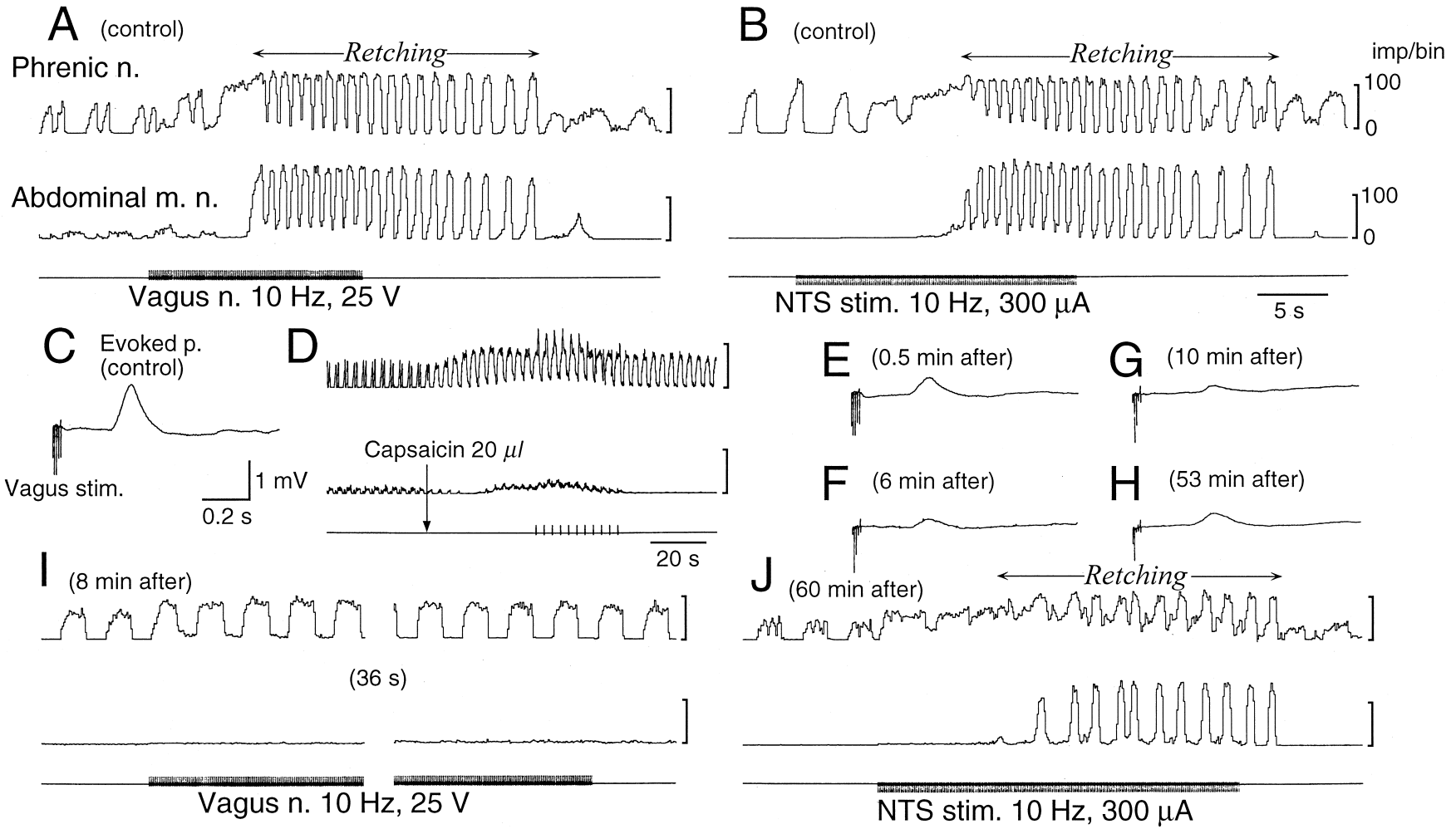

Fig. 1. Effects of capsaicin on retching and evoked potentials. From top to bottom, traces in A, B, D, I and J represent frequency histograms (100 ms bins) of efferent discharges of the phrenic and abdominal muscle nerves, and pulses representing vagal stimulation. These explanations also apply to Fig. 2 and Fig. 6. Note that the traces were recorded with the same speed in A, B, I and J, but at a lower speed in D. A: Control, retching induced by stimulation (0.5 ms duration, 10 Hz, 25 V) of abdominal vagal afferents. B: Control, retching induced by stimulation of the medial solitary nucleus (0.2 ms duration, 10 Hz, $\pm$300 μA). C: Control, field potential changes (evoked *p*) which were evoked by pulse-train stimulation (0.5 ms duration, 100 Hz, 25 V, 5 pulses, 3 s intervals) of abdominal vagal afferents and recorded from the medial solitary nucleus with a glass-coated platinum wire electrode (tip diameter of 50 μm). The evoked potentials were averaged ten times. D: Effects of capsaicin (33 mM, 20 μl) applied to the 4th ventricle. E-H; Evoked potentials of the medial solitary nucleus obtained at the indicated times after capsaicin. I: Vagal stimulation did not induce retching at 8 min after capsaicin. Traces for the times indicated were omitted at the interrupted positions. J: Retching was induced by stimulation of the medial solitary nucleus even after capsaicin treatment.

copper sulfate in the ferret, dog and *suncus murinus*. Moreover, Gardner et al. (1994) demonstrated that hindbrain administration of (D-Pro^{9},(spiro-γ-lactam)leu^{10}, Trp^{11})physalaemin-(1-11) (GR82334), a peptide NK_1 receptor antagonist, but not peripheral administration, inhibits cisplatin-induced emesis in the ferret. Therefore, the active site of NK_1 receptor antagonists has been suggested to exist in the brain stem. These results indicate that capsaicin-sensitive vagal afferents induce emesis through the release of substance P from their central terminals in the brain stem.

Koga and Fukuda (1992) demonstrated that focal cooling of the medial solitary nucleus reversibly suppresses the retching response to stimulation of abdominal vagal afferents. Furthermore, they also revealed that most neurons of the medial solitary nucleus receive abdominal vagal inputs and some project to the reticular area dorsomedial to the retrofacial nucleus and drive the central pattern generator

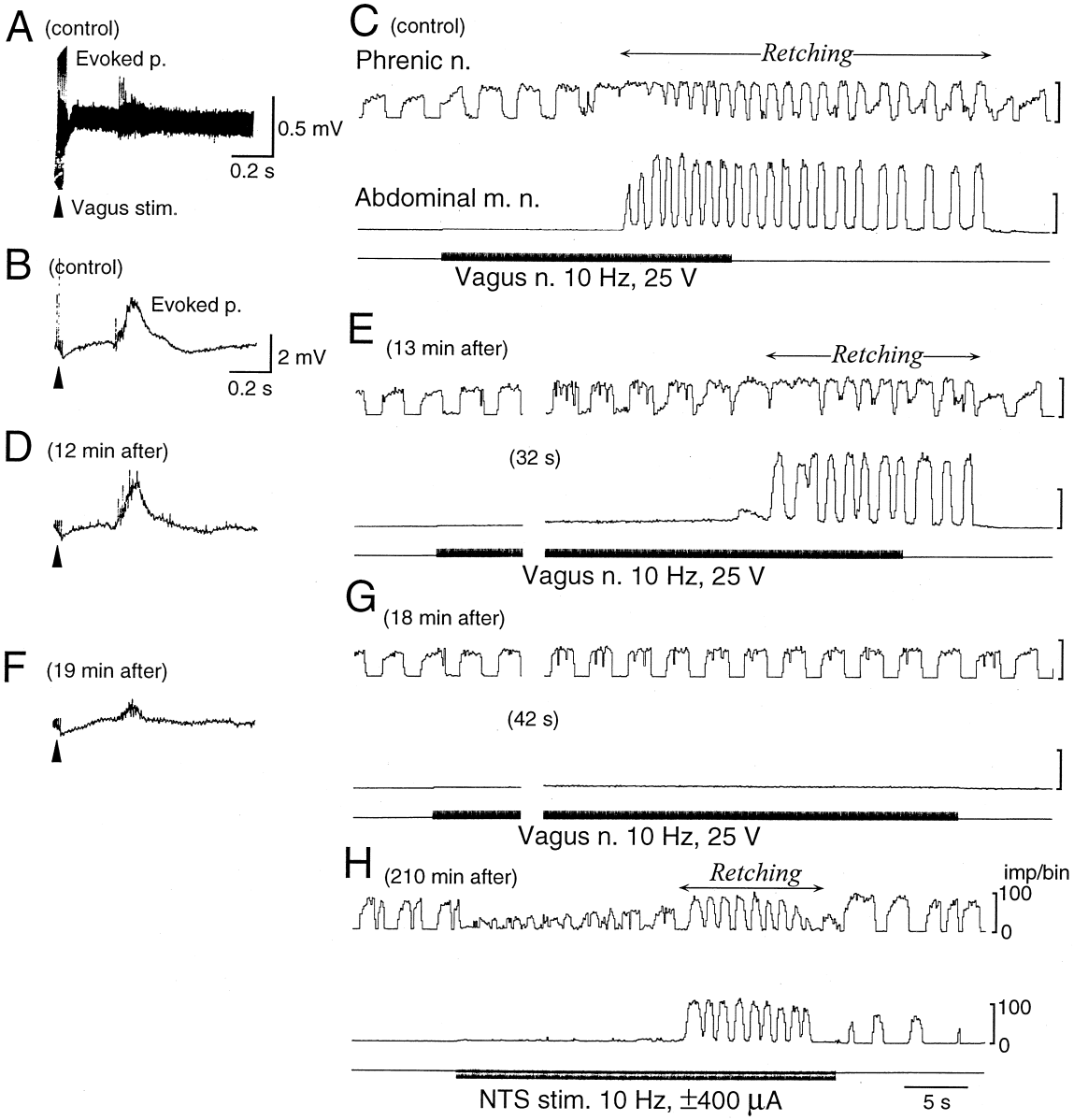

Fig. 2. Effects of resiniferatoxin on retching and evoked potentials. A: A photograph showing potential changes (evoked *p*) which were evoked by pulse-train vagal stimulation (0.5 ms, 100 Hz, 25 V, 5 pulses, 3 s intervals) and recorded from the medial solitary nucleus with a glass-coated silver wire electrode (tip diameter of about 5 μm). B: Control, the average of 10 evoked potentials. C: Control retching induced by vagal stimulation. D: Evoked potential at 12 min after resiniferatoxin (160 μM, 30 μl) applied to the 4th ventricle. E: Retching was still induced by vagal stimulation at 13 min after resiniferatoxin. Note the prolonged latency of retching. F: Evoked potential at 19 min after resiniferatoxin. G: Retching was not induced approximately 18 min after resiniferatoxin, even though vagal stimulation was continued for a longer period than that in the control (C). H: Retching was induced by stimulation (0.2 ms duration, 10 Hz, $\pm$400 μA) of the medial solitary nucleus even after resiniferatoxin.

for vomiting. The existence of a central pattern generator was postulated from the following findings: (1) vomiting was induced by stimulation of the reticular area, (2) vomiting was abolished by cutting, electrical and chemical lesions of this area (Fukuda and Koga, 1991; Koga et al., 1997); and (3) neurons in the area exhibited firing patterns appropriate to produce vomiting, when the neurons were activated by abdominal vagal afferents via neurons in the medial solitary nucleus (Fukuda and Koga, 1992, 1995, 1997).

These findings suggest the possibility that substance P works as an excitatory neuro-transmitter or modulator in the synapse between capsaicin-sensitive emetic vagal afferents and the neurons of the medial solitary nucleus which drive the central pattern generator for vomiting. To evaluate this possibility, we observed the effects of the 4th-ventricular application of capsaicin and resiniferatoxin on neuronal activity in the medial solitary nucleus and on retching in response to the stimulation of abdominal vagal afferents in paralyzed decerebrate dogs.

2. Materials and methods

The present study was performed with 22 adult dogs of either sex, each weighing 6–13 kg. Experimental protocols were approved by the Animal Research Committee of Kawasaki Medical School. The dogs were anesthetized with an intramuscular injection of ketamine hydrochloride (25 mg kg^{-1}). The animals became quite flaccid within 5 min of the injection. During the subsequent 10 min, a craniotomy using a motor-driven bone cutter and precollicular decerebration were performed. After decerebration, the animals were allowed to recover from the anesthesia. A cannula was inserted into the trachea for artificial ventilation (20 strokes min^{-1} with a tidal volume of 100–150 ml). The animal's head was then fixed on a stereotaxic head holder. Body temperature was maintained at 37–39°C, using a heating plate. Blood pressure was monitored through a cannula inserted into the femoral artery. CO_2 and O_2 concentrations in tracheal air were also monitored via the tracheal cannula.

The phrenic branch of the C5 spinal nerve and a branch of the L1 spinal nerve to abdominal muscles were exposed and severed at the distal end. The distal part of the proximal nerve strand was hooked on a bipolar platinum wire electrode in a liquid paraffin pool made by skin flaps. Efferent impulses were recorded by the electrode and converted into a frequency histogram of 100-ms bins, using spike counters (Dia Medical, DSE-342A) and recorded with a pen recorder.

In all of the dogs, the vagal ventral and dorsal trunks were severed at the supra-diaphragmatic region. Fictive retching was produced by continuous stimulation (10 Hz, 0.5 ms duration, 25–30 V) of the proximal nerve strand of the severed vagal ventral or dorsal trunk with a bipolar platinum wire electrode. Fictive retching was also elicited by electrical stimulation of the medial solitary nucleus (10 Hz, 0.2 ms, $\pm$200–400 μA, biphasic pulse) with a glass-coated platinum electrode. The retching responses could not be elicited by stimulation of the vagus nerve for over 1 min in 2 dogs. Apomorphine hydrochloride was injected (0.3 mg kg^{-1}, i.m.) to facilitate the retching reflex in the animals. Details of the induction of retching have been described in our previous reports (Koga and Fukuda, 1992; Fukuda and Koga, 1997). Fictive retching was defined on the basis of characteristic firing patterns of the phrenic and abdominal muscle nerves as described in several other papers (Bianchi and Grélot, 1989; Grélot et al., 1990; Koga and Fukuda, 1990; Miller and Ezure, 1992).

The field-evoked potential induced by pulse-train stimulation (100 Hz, 4–6 pulses, 0.5 ms duration, 25–30 V, 3 s intervals) of vagal afferents was recorded in the medial solitary nucleus by means of a glass coated silver wire electrode (tip diameter of about 5 μm) or a glass coated platinum wire electrode (tip diameter of 50 μm). The evoked potential was amplified and averaged 10 times. Firing was also recorded from neurons around the medial solitary nucleus with glass microelectrodes which were filled with 2% pontamine skyblue in 0.5 M sodium acetate solution. The recording sites were marked by electrophoretic injection of pontamine skyblue.

At the end of the experiments, the dogs were re-anesthetized with an overdose of pentobarbital sodium (35 mg kg^{-1}, i.v.). The brainstem was perfused with 500 ml of Tyrode's solution for about 30 min and then with 800 ml of fixative (0.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4) for about 40 min through a cannula inserted into the right vertebral artery. The common carotid arteries and left vertebral artery were

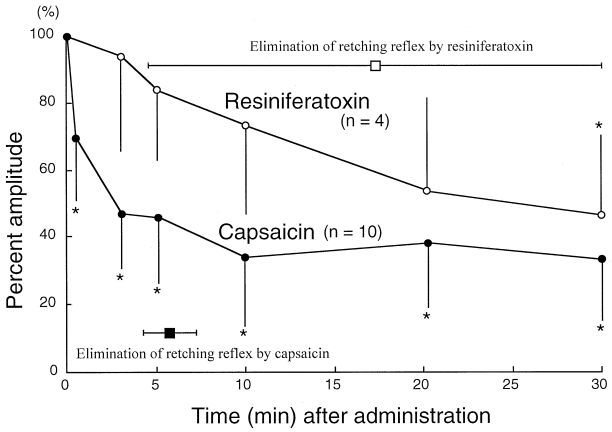

Fig. 3. Effects of capsaicin (33 mM) and resiniferatoxin (160 μM) on the amplitude of evoked potentials in response to pulse-train vagal stimulation. The average of 10 evoked-potentials recorded from the medial solitary nucleus is shown. (■) and (□) represent mean $\pm$ S.D. of the times after capsaicin and resiniferatoxin at which the disappearance of the retching response to vagal stimulation was recognized in each dog, respectively: $^{*}p < 0.05$ in Student's *t*-test.

ligated to exclude blood flow to the bulb and to prevent backflow of the perfusates. The inferior vena cava was severed in the thorax to drain the perfusates. The caudal part of the medulla oblongata was removed and additionally fixed overnight with the same fixative. The tissue was then stored in cold 0.1 M phosphate buffer for 6 h. Transverse serial sections (100 μm) were cut with a vibratome, mounted, and stained with thioneine. Recording sites were identified on the sections as spots with a diameter of about 100 μm.

Capsaicin (Sigma, USA) and resiniferatoxin (Scientific Marketing, UK) were dissolved in dimethylsulfoxide. Cap-

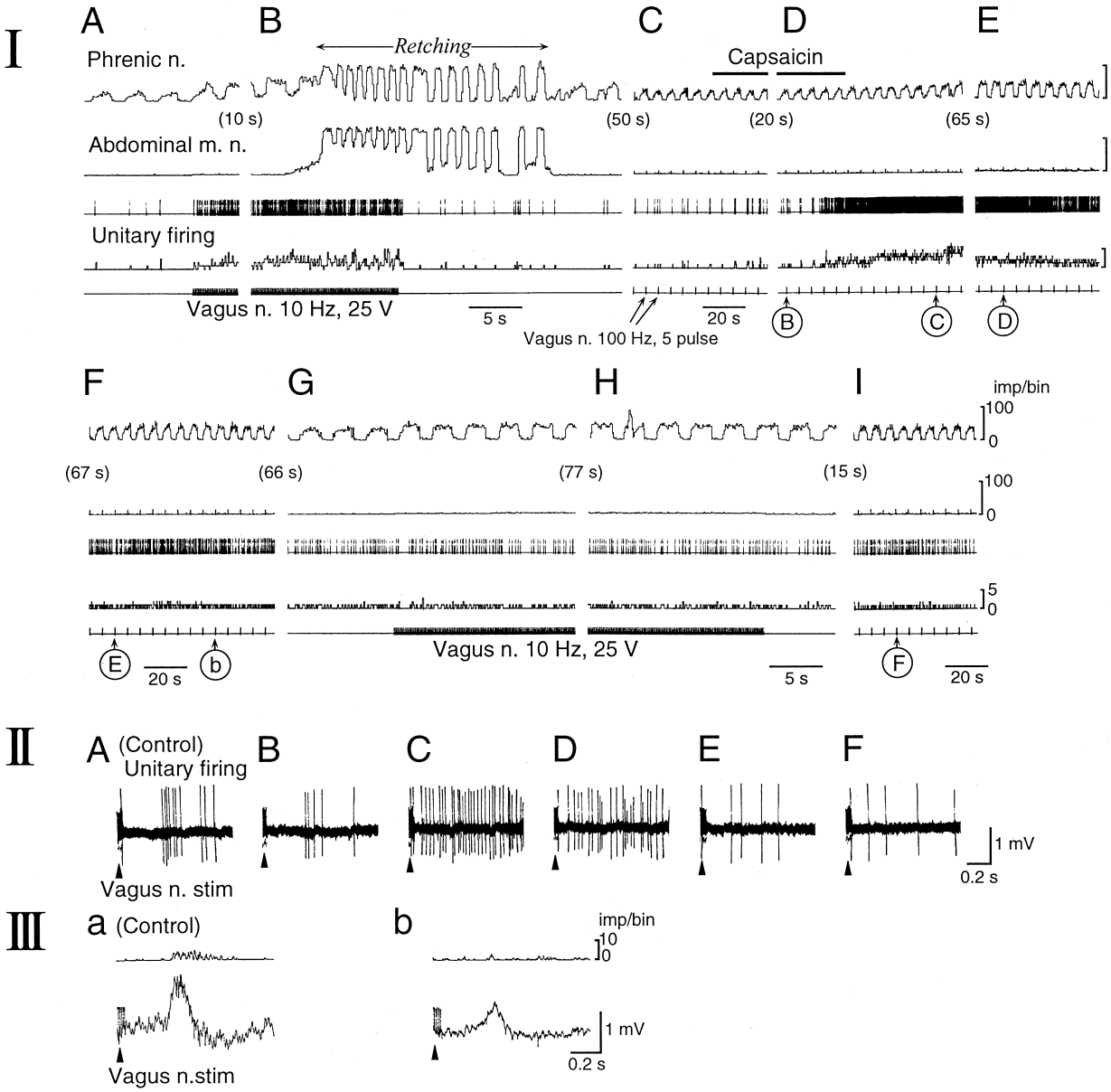

Fig. 4. Effects of capsaicin on retching and neuronal firing recorded from the medial solitary nucleus. (I)A–I: From top to bottom, traces represent frequency histograms of efferent discharges of the phrenic and abdominal muscle nerves, pulses representing firing of a neuron of the medial solitary nucleus, frequency histogram of the neuronal firing and pulses representing vagal stimulation. Traces for the times indicated were omitted at the interrupted positions. Note the higher recording speed in A, B, G and H than that in C–F and I. A–B: Firing of the neuron increased during vagal stimulation. Capsaicin (33 mM, 20 μl) was injected into the 4th ventricle during the period indicated by a horizontal bar in C and D. Neuronal firing increased after capsaicin (D–I). G, H: Vagal stimulation produced neither retching nor any changes in neuronal firing. I: Note that firing gradually subsided. (II)A–F: Neuronal responses to pulse-train vagal stimulation (0.5 ms duration, 25 V, 100 Hz, 5 pulses, 3 s intervals). Each photograph was obtained at the time indicated by the corresponding letter in (I). Note that the neuronal response disappeared in F. The recording site of this unit is shown in Fig. 5A(a). (III)a–b, The upper and lower traces represent the firing frequency of the medial solitary nucleus neuron and field potential changes in response to pulse-train vagal stimulation, respectively. Both responses were recorded with the same glass microelectrode and simultaneously averaged ten times. (a): Control. (b): Responses obtained at the period indicated by (b) in (I).

saicin (0.033–33 mM, 20–30 μl) and resiniferatoxin (1.6–160 μM, 20–30 μl) were applied to the 4th ventricle by using a micro-injector (Nihon Koden, XF-320J).

3. Results

3.1. Effects of capsaicin and resiniferatoxin applied in the 4th ventricle on fictive retching

Fictive retching was induced by continuous stimulation of abdominal vagal afferents. Retching disappeared within 10 min after the 4th-ventricular application of capsaicin (33 mM, 20–30 μl, $n = 12$) (Fig. 1). Similarly, resiniferatoxin (160 μM, 20–30 μl, $n = 7$) completely suppressed retching within 40 min after application (Fig. 2). Retching was still induced after the 4th-ventricular application of lower doses of capsaicin (33 and 330 μM) and resiniferatoxin (1.6 μM). Capsaicin (33 mM, 20–30 μl) itself induced retching without vagal stimulation in 2 dogs at 3 and 5.5 min after treatment. However, stimulation of the medial solitary nucleus induced fictive retching in all of the animals after retching in response to vagal stimulation was abolished by capsaicin or resiniferatoxin (Figs. 1 and 2). Treatment with the same amount of the vehicle, dimethylsulfoxide, had no effect on either type of retching caused by stimulation of vagal afferents or of the medial solitary nucleus.

3.2. Effects of capsaicin and resiniferatoxin on evoked potentials

A field potential change was evoked by pulse-train stimulation of abdominal vagal afferents, recorded from the medial solitary nucleus and averaged ten times. The vehicle had no effect on the amplitude and latency of the evoked potential. The mean ± S.D. latency of the onset of the evoked potential was 303.0 ± 29.9 ms in 15 dogs. The effects of capsaicin (33 mM, 20–30 μl) and resiniferatoxin (160 μM, 20–30 μl) on the evoked potential were observed in 10 and 4 dogs, respectively, as shown in Fig. 1C, E–H and Fig. 2B, D, F. The changes in amplitudes of the evoked potential after the 4th-ventricular application of capsaicin or resiniferatoxin are summarized in Fig. 3. The amplitudes were promptly reduced by capsaicin and reached 33.5 ± 17.7% of the control amplitude at 30 min after application ($n = 10$), while resiniferatoxin produced a more gradual decrease to 46.6 ± 22.5% of the control ($n = 4$) 30 min after application. Neither capsaicin nor resiniferatoxin completely inhibited the evoked potential or increased the latency (Figs. 1–3). With a decrease in the amplitude, retching in response to vagal stimulation was also abolished.

3.3. Effects of capsaicin and resiniferatoxin on firing of solitary nucleus neurons

Neuronal firing was recorded from the medial solitary nucleus and from around the hypoglossal nucleus. Responses to pulse-train stimulation of abdominal vagal afferents were observed in 16 neurons, as shown in Fig. 4(II)A. The mean latency of the responses was 332.7 ± 42.0 ms. The effects of capsaicin were observed in 4 neurons of the medial solitary nucleus, and an example is shown in Fig. 4. Firing of this neuron increased in response to pulse-train vagal stimulation as well as during continuous vagal stimulation applied to induce retching (Fig. 4(I)A, B; (II)). Neuronal firing also increased after the application of capsaicin ((I)D, E) and then gradually subsided ((I)F–I). After application, neuronal responses to pulse-train vagal stimulation gradually decreased and finally disappeared, as shown in Fig. 4(II). Evoked-potentials that were recorded simultaneously with neuronal firing from the same electrode similarly decreased in amplitude, but did not completely disappear (Fig. 4(III)a, b). Continuous vagal stimulation applied 8 min after capsaicin failed to increase neuronal firing and to induce retching (Fig. 4(I)G, H). The recording site of this neuron is shown in Fig. 5A(a). Similar effects of capsaicin were observed in 3 other neurons in the medial solitary nucleus. Fig. 5 shows the recording sites of the 4 neurons (open circle) in which effects of capsaicin were observed.

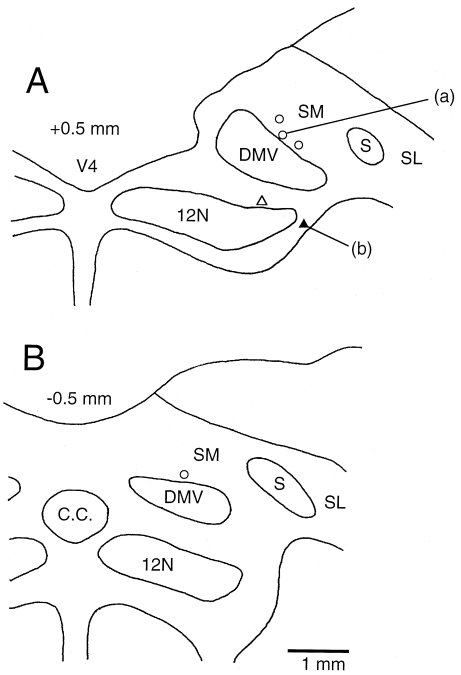

Fig. 5. Recording sites for neuronal firing. The levels of the schematically represented transverse planes of the medulla oblongata are shown as the distances from the obex. (○) represents recording sites of the units in which the response to pulse-train vagal stimulation disappeared after capsaicin together with disappearance of the retching response. (△) and (▲) represent recording sites of the units in which the response to pulse-train vagal stimulation disappeared after resiniferatoxin and continued after resiniferatoxin, respectively. (a) and (b) represent the recording sites of the unit shown in Fig. 4 and the unit shown in Fig. 6, respectively. 12N; hypoglossal nucleus. C.C.; central canal. DMV; dorsal motor nucleus of the vagus. S; solitary tract. SL; lateral solitary nucleus. SM; medial solitary nucleus. V4; 4th ventricle.

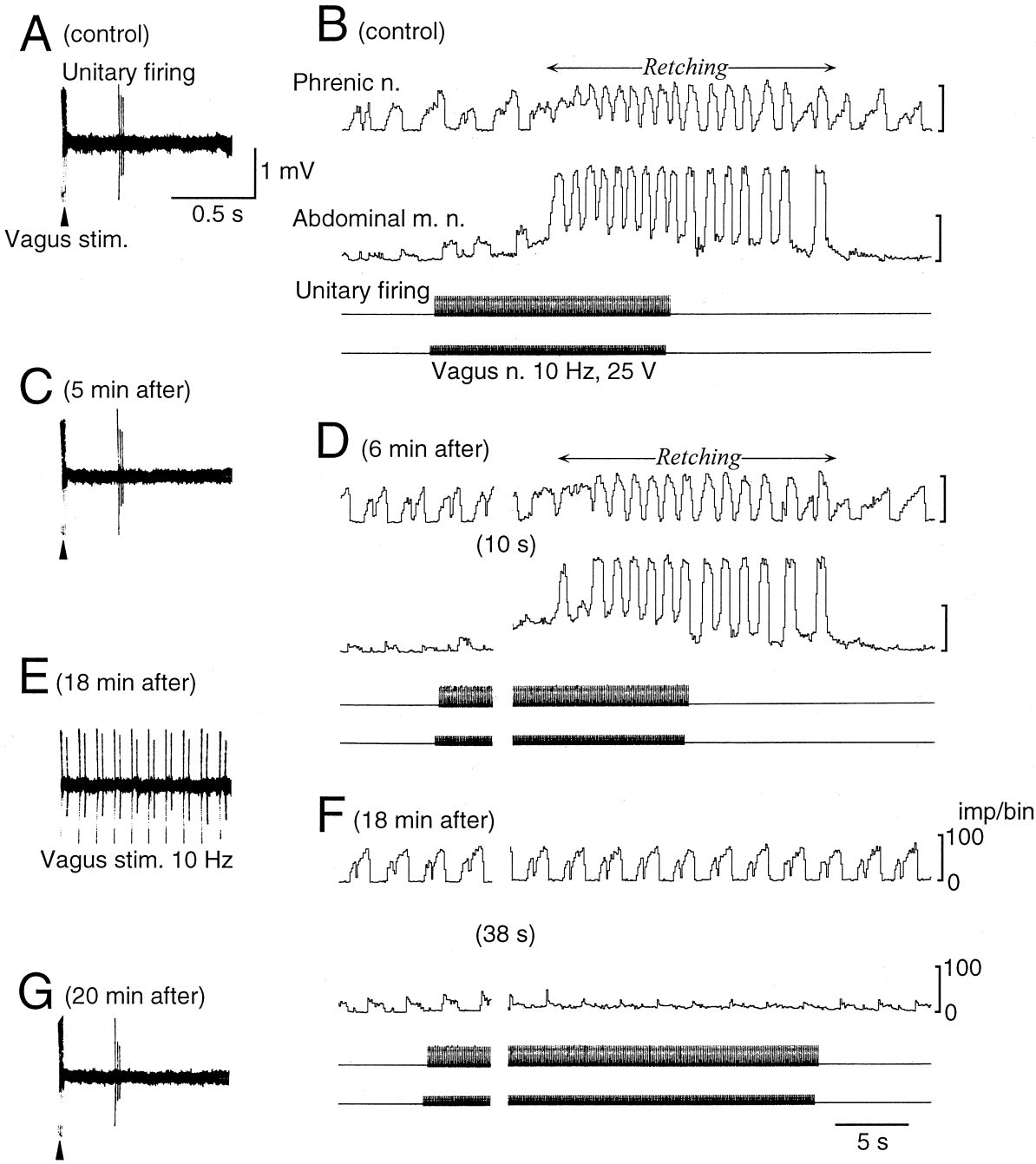

Fig. 6. Effects of resiniferatoxin on neuronal firing recorded from the reticular site just lateral to the hypoglossal nucleus. The recording site of this unit is shown in Fig. 5A(b). (A) Control response of the unit to pulse-train vagal stimulation (0.5 ms, 25 V, 100 Hz, 3 pulses, 3 s intervals). (B) Control retching induced by vagal stimulation. (C, G) Neuronal responses to pulse-train vagal stimulation obtained at the indicated times after resiniferatoxin (160 μM, 30 μl). (D) Retching induced 6 min after resiniferatoxin. Note the prolonged latency. (E): Neuronal response to vagal stimulation applied to induce retching in F. (F) Vagal stimulation did not produce retching 18 min after resiniferatoxin.

Resiniferatoxin had similar effects in a neuron from which recordings were made at a site in the ventral part of the nucleus intercalatus, just dorsal to the hypoglossal nucleus (Fig. 5A, open triangle). Responses of this neuron to pulse-train and continuous vagal stimulation disappeared just before the retching reflex was suppressed by the application of resiniferatoxin (160 μM, 20 μl). However, the other neuron, from which recordings were made at a site just lateral to the hypoglossal nucleus (Fig. 5A(b)), still responded to pulse-train as well as continuous vagal stimulation even after the retching response had disappeared after application of the same dose of resiniferatoxin (Fig. 6). Moreover, firing of this neuron was not increased by resiniferatoxin treatment.

4. Discussion

In the present study, we examined whether or not transmission in the synapse between emetic vagal afferents and neurons in the medial solitary nucleus is blocked by capsaicin or resiniferatoxin. Administration of capsaicin into the 4th ventricle initially enhanced the firing of neurons in the medial solitary nucleus and induced fictive retching in 2 dogs. This enhanced firing gradually subsided and the response of these neurons to stimulation of abdominal vagal afferents disappeared. Simultaneously, fictive retching induced by vagal stimulation disappeared and the amplitude of the field potential changes evoked in the medial solitary nucleus by pulse-train vagal stimulation decreased to about 30% of the control amplitude. Stimulation of the medial solitary nucleus produced retching in all of the animals examined even after capsaicin abolished the retching response to vagal stimulation. Similar results were also observed after resiniferatoxin.

4.1. Possible mechanisms underlying the effects of capsaicin and resiniferatoxin

We previously demonstrated that emetic vagal afferents comprise C fibers that form synapses with neurons of the medial solitary nucleus in dogs (Koga and Fukuda, 1992; see Section 1). The latencies of the evoked potentials and neuronal firing in this study are consistent with our previous results and suggest that they are mediated by vagal C afferent fibers. Marsh et al. (1987) observed the effects of capsaicin on the vagal sensory neurons of rats in vitro and revealed that capsaicin increases Na^+ and Ca^{2+} conductance of vagal C afferent fibers and cell bodies, and consequently depolarizes the neurons and blocks conduction in the fibers. This depolarization seems to cause short-lasting activation and subsequent long-term desensitization, and also the release and subsequent depletion of transmitter(s) when depolarization occurs at nerve terminals (see review by Holzer (1992)).

These previous studies suggest that the mechanisms underlying the present results may be as follows: capsaicin applied to the 4th ventricle seems to increase the Na^+ and Ca^{2+} conductance of the terminal portion of vagal C afferent fibers in the medial solitary nucleus, and consequently depolarizes the terminal portion and releases transmitter(s) from the terminal. The transmitter(s) may enhance the firing of neurons in the nucleus and induce retching. The depolarization is thought subsequently to inactivate the terminal portion (desensitization). Thus, action potentials conducted along vagal C afferents from a thoracic stimulating site seem to be blocked at the terminal portion. In this study, the firing of medial solitary nucleus neurons and retching in response to vagal stimulation disappeared after capsaicin administration, and the amplitude of the evoked potentials decreased. However, medial solitary nucleus neurons continued to fire at a frequency higher than before the application of capsaicin. This firing indicates that the persistently depolarized terminal portion still exhibits spontaneous release of transmitter(s), and that medial solitary nucleus neurons also remain excitable. Thus, stimulation of the medial solitary nucleus provoked retching even after the retching and neuronal responses to vagal stimulation were abolished. Therefore, these results indicate that capsaicin-sensitive emetic vagal afferents make synaptic contact with medial solitary nucleus neurons which drive the central pattern generator for vomiting (see Section 1).

However, about 30% of the amplitude of the evoked potentials in the medial solitary nucleus remained after the retching response to vagal stimulation was abolished by the 4th-ventricular administration of capsaicin. This result suggests that some vagal C afferents are not affected by capsaicin, and that capsaicin-insensitive vagal afferents and their target neurons in the medial solitary nucleus do not subserve the retching reflex.

As mentioned in Section 1, systemic resiniferatoxin has been shown to induce transient emesis (Matsuki et al., 1996) and then to block emesis mediated by vagal afferents (Andrews and Bhandari, 1993; Matsuki et al., 1996). These previous findings are thought to be consistent with the present results and show that emetic vagal afferents are capsaicin-sensitive. However, these previous studies did not elucidate the site of the emetic and anti-emetic actions of systemic resiniferatoxin. The present results suggest that the terminal portion of capsaicin-sensitive emetic vagal afferents in the medial solitary nucleus is the site of both actions of systemic resiniferatoxin.

4.2. Possible transmitter of emetic vagal afferent fibers

As described above, the present results suggest that capsaicin applied to the 4th ventricle initially releases transmitter(s) from the central terminals of capsaicin sensitive emetic vagal afferents, and that the transmitter(s) activates the neurons of the medial solitary nucleus which drive the central pattern generator for vomiting. Previous findings suggest that capsaicin-sensitive vagal afferents have substance P as a transmitter. Furthermore, neurons in the solitary nucleus have been shown to have binding sites for substance P (Watson et al., 1995). Therefore, it may be assumed that substance P is the transmitter in the synapse between capsaicin-sensitive emetic vagal afferents and the neurons of the medial solitary nucleus which drive the central pattern generator for vomiting.

Accordingly, the present results suggest that the synapse between emetic vagal afferents and the secondary neurons in the medial solitary nucleus is the site of the anti-emetic action of tachykinin NK_1 receptor antagonists. As mentioned in Section 1, many previous studies consistently demonstrated that NK_1 receptor antagonists act on the brain stem and abolish emesis mediated by emetic vagal afferents. However, these previous studies did not show

the precise site of the anti-emetic action of NK_1 receptor antagonists.

In summary, we postulate the following as the neuronal mechanism through which stimulation of emetic vagal afferents produces vomiting: capsaicin-sensitive emetic vagal afferents release substance P and activate neurons of the medial solitary nucleus. The neurons in turn drive the central pattern generator for vomiting (see Section 1). The central pattern generator consists of the two main groups of neurons which generate the firing patterns appropriate for producing the vomiting activity of phrenic and abdominal muscle motoneurons, respectively. Both patterns of activity of the neurons of the central pattern generator are sent to appropriate motoneurons via inspiratory and expiratory premotoneurons in the caudal part of the ventral respiratory group (Koga, 1991; Koga and Fukuda, 1994, 1997; Koga et al., 1997). As a result, vomiting motions are produced.

4.3. Other possible mechanisms

Our present results did not allow identification of the transmitter which was released from emetic vagal afferents by administration of capsaicin and resiniferatoxin in the 4th-ventricle. Vagal afferents have been shown to contain not only substance P but also glutamate, 5-hydroxytryptamine (5-HT), cholecystokinin (CCK), vasoactive intestinal peptide (VIP), calcitonin gene-related peptide (CGRP) and somatostatin (Van Giersbergen et al., 1992). Among these transmitters, capsaicin is known to release substance P, somatostatin and CGRP in the spinal cord, but not glutamate, 5-HT, VIP or CCK (Holzer, 1992). However, these differences in the effects of capsaicin on vagal afferents are not fully understood. Therefore, the present results can not exclude the possibility that one of these transmitters other than substance P mediates emetic vagal inputs to neurons of the medial solitary nucleus. Further studies are required to define the site of action of NK_1 receptor antagonists and substance P. In particular, the effects of microinjection of NK_1 receptor antagonists into the medial solitary nucleus on neuronal activity should be studied.

In conclusion, retching induced by vagal stimulation disappeared after the 4th-ventricular administration of capsaicin and resiniferatoxin, concomitantly with a decrease in the amplitude of evoked potentials of the medial solitary nucleus induced by pulse-train vagal stimulation and with the disappearance of neuronal responses to vagal stimulation in the medial solitary nucleus. The site of action of capsaicin and resiniferatoxin is considered to be in the central terminal portion of the emetic vagal afferents. These results demonstrate that capsaicin-sensitive vagal afferents mediate emetic visceral inputs to neurons of the medial solitary nucleus, which in turn drive the central pattern generator for vomiting.

Acknowledgements

This study was supported in part by a Research Project Grant (No. 8-609) from Kawasaki Medical School.

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