Effects of pharmacological agents administered for swallowing disorders on swallowing motor activity in nerves innervating infrahyoid and laryngeal muscles

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ABSTRACT

Pharmacological agents that elevate dopamine and substance P concentrations have been suggested to prevent aspiration pneumonia and improve impaired swallowing processes. However, little is known about the effects of such agents on swallowing activities induced in motor nerves innervating the pharyngeal muscles. In the present study, we examined the effects of imidapril, cilostazol and amantadine, which are often prescribed for swallowing disorders, on swallowing motor activity. We recorded efferent activity of the cervical vagal nerve (VN), hypoglossal nerve (HN), and phrenic nerve (PN) using arterially perfused rats aged between postnatal days 21–35. VN activity was used for evaluation of swallowing motor activity. Injection of 1.25 ml of distilled water into the oral cavity or electrical stimulation of the superior laryngeal nerve (SLN) evoked synchronized swallowing bursts in the VN and HN, while inspiratory discharges were inhibited in all of those nerves. Administration of imidapril (60 ng/ml) but not cilostazol (2.5 μg/ml) and amantadine (200 ng/ml) to the perfusate increased the mean peak amplitude of orally evoked swallowing bursts in the VN. Such increase in the peak amplitude by imidapril was antagonized by administration of the NK1 receptor antagonist aprepitant (2 mg/ml) or the D1 receptor antagonist LE300 (2 mg/ml). In contrast, neither imidapril nor cilostazol caused a significant increase in swallowing bursts evoked by electrical stimulation of the SLN. These results suggest that imidapril administrations may improve impaired swallowing by enhancing pharyngeal muscle activities via an increase in substance P and dopamine.

1. Introduction

Oropharyngeal dysphagia is associated with increased risk of aspiration pneumonia, malnutrition and dehydration, and often occurs following various diseases such as acute cerebral and brainstem stroke, Parkinson's disease, schizophrenia, and sarcopenia. Although mechanisms generating swallowing disorders are not sufficiently clarified, pharyngeal neuromuscular dysfunction could contribute to swallowing disorders. The neural circuit controlling swallowing is located in the brainstem, and sensory inputs to the neural circuit from oropharyngeal mucosa and muscles regulate oropharyngeal muscle activity during swallowing (Miller, 2008). In the pharyngeal phase in swallowing, which is particularly important in relation to aspiration, sensory inputs trigger the subconscious swallowing reflex and modulate the sequential motor activity of muscles that transport the bolus through the pharynx (Steele and Miller, 2010).

A neuropeptide substance P that is stored in the peripheral ending of capsaicin-sensitive sensory nerve (Maggi and Meli, 1988) has been thought to be involved in regulation of the swallowing reflex by sensory inputs, because subcutaneous treatment of capsaicin, which could abolish substance P from the airway and upper digestive tract, attenuated the swallowing reflex in guinea pigs (Jin et al., 1994). Dopamine is a principal neurotransmitter to regulate substance P concentration. Actually, treatment of the dopamine D1 receptor antagonist decreased both the swallowing reflex and substance P-like immunoreactivities in the laryngeal and pharyngeal mucosa (Jia et al., 1998). Therefore, the following pharmacological agents, which increase substance P or dopamine, are suggested to improve impaired swallowing: angiotensin-converting enzyme (ACE) inhibitors, the phosphodiesterase type 3 inhibitor cilostazol, and the antiparkinson medication amantadine.

ACE inhibitors, which are the first-line drugs used for hypertension, increase the local substance P level by inhibiting its degradation (Sekizawa et al., 1996). Cilostazol promotes dopamine synthesis by inducing the synthesis of tyrosine hydroxylase. Amantadine is known to increase dopamine release and block dopamine re-uptake. Although the influences of these pharmacological agents on swallowing by long-term chronic administration have been investigated in both the clinical studies and animal experiments, their acute effects on the activity of the pharyngeal muscles are still unclear.

Anesthesia is known to suppress pharyngeal muscle activity during swallowing and respiration. Ketamine, for instance, depressed cough reflex (Marshall and Wollman, 1980), which is an important protective reflex in the airway to prevent aspiration pneumonia. Moreover, the hypoglossal nerve activity during swallowing reflex elicited by electrical stimulation of the superior laryngeal nerve (SLN) was progressively depressed by increasing depth of anesthesia by nitrous oxide (Nishino et al., 1985). *In situ* arterially perfused rat preparations (Paton, 1996) have been used in some experiments to study the neural circuits involved in the generation of swallowing (Bautista and Dutschmann, 2014; Bautista et al., 2014). This experimental model enabled us to study the swallowing activity without the depressant effects of anesthesia. The aim of the present study was to elucidate the effects of pharmacological agents prescribed for swallowing disorders on swallowing motor activity in the absence of anesthetics.

2. Materials and methods

All of the experiments were performed with the approval (No. 18029) of the Institutional Animal Care and Use Committee of Showa University, which operates under Japanese Governmental Law (No. 105) for the care and use of laboratory animals. All efforts were

made to minimize the suffering and number of animals used.

2.1. Animal preparation

Seventy-nine Wistar rats of either sex aged between 21 and 35 days were used in the present study. The weights ranged from 46 to 107 g. We modified the procedures for the preparation described in detail in Tachikawa et al. (2016). The rats deeply anesthetized with isoflurane were transected caudal to the diaphragm and immersed in ice-cooled Ringer's solution (in mM: 125 NaCl, 3 KCl, 24 $NaHCO_3$, 1.25 KH_2PO_4, 1.25 $MgSO_4$, 2.5 $CaCl_2$, 10 dextrose). In the cooled Ringer's solution, the cerebral cortex was removed from the rats. The thalamus and basal ganglia remained in the preparations. Preparations were then transferred to a recording chamber, and the descending aorta was cannulated with a double lumen catheter (1317-23WG, Covidien, Dublin, Ireland). Ringer's solution containing 1.25% Ficoll (Sigma-Aldrich, St. Louis, MO) and heparin (10 unit/mL, Mochida, Tokyo, Japan) was retrogradely perfused using a roller pump (502S, Watson-Marlow, Falmouth, Cornwall, UK). The perfusate was continuously gassed with 5% CO_2/95% O_2 and warmed to 31–32 ºC using an in-line heater (TC324C; Warner Instruments, Hamden, CT). The aortic perfusion pressure was monitored via the second lumen of the catheter and maintained in the range of 30–50 mmHg by adjusting the flow between 35 and 43 mL/min.

2.2. Nerve recording

Preparations were paralyzed using vecuronium bromide (1.5–2 μg/mL; Sigma-Aldrich) prior to the isolation of the peripheral nerves. Peripheral nerves on the left side were used for nerve recording. The vagus nerve (VN) was identified between the left common carotid artery and sternothyroid muscle. The hypoglossal nerve (HN) was identified under the mylohyoid

muscle. The phrenic nerve (PN) was isolated from the pleura. Each peripheral nerve was cut distally and the proximal end was held in bipolar suction electrodes (A-M Systems, Sequim, WA). All signals were amplified using a differential amplifier (DP-304; Warner Instruments), band-pass filtered (1–3k Hz), and stored on a computer using an analog-to-digital converter (CED micro 1401; Cambridge Electronic Design, Cambridge, UK) with version 8 of the Spike2 software (Cambridge Electronic Design). Additional digital filtering was applied using DC remove with a time constant of 0.1 s (Spike2 software) when necessary to remove movement artifact. The nerve activity was rectified and integrated with a time constant of 0.1 s in the Spike2 software.

2.3. Experimental protocol

To evoke fictive swallowing, two stimulation methods were used. One is injection of distilled water into the oral cavity and the other is electrical stimulation of the SLN. For water injection, a narrow plastic tube (1 mm diameter) was inserted into the oral cavity and the caudal end was placed in the pharynx. Distilled water of 1.25 ml was injected for 10 seconds using syringe pump (CFV-3200, Nihon Kohden, Tokyo, Japan). For electrical stimulation, the SLN on the right side was identified between the trachea and the right common carotid artery and cut in the vicinity of the trachea. The proximal end was held into a bipolar suction electrode. Single pulse or repetitive pulses (5 Hz) of 100 μs duration was applied during the expiratory phase with the intensity of 10–500 μA using a Master-8 pulse generator and ISO-Flex stimulus isolator (AMPI, Jerusalem, Israel). Elicitation of fictive swallowing was performed three times at each of the following periods in each preparation: 5–30 min prior to drug administration (control) and 30–60 min after the drug administration.

2.4. Drug application

All drugs were stored as stock solutions in distilled water. Stock solutions were diluted at least 1:1000 to the following concentration with perfusate before their application: imidapril hydrochloride (60 ng/ml, Sigma-Aldrich), cilostazol (2.5 μg/ml, Sigma-Aldrich), amantadine hydrochloride (200 ng/ml, Sigma-Aldrich), aprepitant (2 mg/ml, Sigma-Aldrich), LE300 (2 mg/ml, TOCRIS Bioscience, Bristol, UK).

2.5. Data analysis

The integrated nerve activity in the VN was used to obtain the peak amplitude and duration of the swallowing bursts. All evoked swallowing bursts were measured and averaged. Values are presented as the mean ± standard error of the mean (SEM). Data obtained before and during drug application were subjected to the Wilcoxon signed-rank test. Differences in data between groups were analyzed using a Kruskal-Wallis one-way ANOVA. The ANOVA was followed by a Bonferroni post-hoc multiple comparison test when appropriate. Probability values of less than 0.05 were considered significant. Statistical analyses were conducted using SPSS 17.0J (SPSS Japan Inc., Tokyo, Japan) and Microsoft Excel 2011.

3. Results

3.1. Effects of pharmacological agents for dysphagia on swallowing motor activity evoked by water injection into the oral cavity

We investigated the effects of pharmacological agents that often prescribed for swallowing disorders on the swallowing bursts evoked by injection of distilled water in the oral cavity. We recorded efferent nerve activity in the VN, HN and PN using suction electrodes. Prior to water injection, inspiratory motor discharges were observed in all of those

nerves in a synchronous manner (Fig. 1A). Injection of 1.25 ml distilled water in the oral cavity during the expiratory phase temporarily inhibited inspiratory discharge in all of those nerves, and evoked synchronized spindle-shaped swallowing bursts in the VN and HN (Fig. 1B). The number of swallowing bursts varied in each preparation (5–26, 14.4 ± 3.4, n = 5). The swallowing bursts were more clearly observed in the VN than HN, and hence the swallowing bursts in the VN were used to evaluate the effects of the pharmacological agents.

We first investigated the effects of the ACE inhibitor imidapril on the swallowing bursts. Administration of imidapril (60 ng/ml) into the perfusate increased the peak amplitude of the swallowing bursts (Fig. 2A). In pooled data from 9 preparations, the peak amplitude of the swallowing bursts was significantly increased by imidapril (4.1 ± 0.7 μV·s before imidapril vs. 4.6 ± 0.6 μV·s during imidapril, the Wilcoxon signed-rank test, n = 9, P = 0.002, Fig. 2B), although the burst duration was not significantly changed by imidapril (0.49 ± 0.03 s before imidapril vs. 0.52 ± 0.03 s during imidapril, the Wilcoxon signed-rank test, n = 9, P = 0.507, Fig. 2B).

Subsequently, we examined the effects of the phosphodiesterase type 3 inhibitor cilostazol. Administration of cilostazol (2.5 μg/ml) into the perfusate tended to increase the peak amplitude of the swallowing bursts in parts of preparations (Fig. 3A). However, in pooled data from 8 preparations, the difference of peak amplitude between before and during administration of cilostazol was not statistically significant (4.8 ± 0.6 μV·s before cilostazol vs. 5.5 ± 0.8 μV·s during cilostazol, the Wilcoxon signed-rank test, n = 8, P = 0.059, Fig. 3B). The burst duration was not changed by administration of cilostazol (0.45 ± 0.02 s before cilostazol vs. 0.47 ± 0.02 s during cilostazol, the Wilcoxon signed-rank test, n = 8, P = 0.167, Fig. 3B).

Finally, we examined the effects of the dopamine re-uptake inhibitor amantadine.

However, in contrast to imidapril and cilostazol, amantadine (200 ng/ml) did not increase the peak amplitude of the swallowing bursts (Fig. 4A). In pooled data from 5 preparations, both the peak amplitude and the burst duration of the swallowing bursts were not significantly changed between before and during administration of amantadine (amplitude: 5.2 ± 1.2 μV·s before amantadine vs. 4.7 ± 0.7 μV·s during amantadine, the Wilcoxon signed-rank test, $n = 5$, $P = 0.484$, duration: 0.58 ± 0.04 s before amantadine vs. 0.62 ± 0.05 s during amantadine, the Wilcoxon signed-rank test, $n = 5$, $P = 0.359$, Fig. 4B). These results indicate that imidapril rather than cilostazol and amantadine acutely enhances the swallowing motor activity in the cervical VN.

3.2. Effects of NK1 and D1 receptor antagonists on enhancement of swallowing motor activity by imidapril

To investigate whether substance P or dopamine is involved in enhancement of swallowing motor activity by imidapril, we next examined the effects of NK1 and D1 receptor antagonists on the swallowing bursts evoked by water injection. The concomitant administration of the selective NK1 receptor antagonist aprepitant (2 mg/ml) and imidapril (60 ng/ml) did not increase the peak amplitude of the swallowing bursts (Fig. 5A) in contrast to the administration of imidapril alone shown in Fig. 2A. The peak amplitude of the swallowing bursts normalized by the value before drug administration was significantly larger in the imidapril-administration group than in the aprepitant and imidapril-administration group and the vehicle group (imidapril: 1.19 ± 0.04, $n = 9$, aprepitant/imidapril: 0.91 ± 0.05, $n = 5$, vehicle: 0.81 ± 0.08, $n = 5$, Kruskal-Wallis one-way ANOVA and Bonferroni post-hoc multiple comparison test, imidapril vs. aprepitant/imidapril: $P = 0.048$, imidapril vs. vehicle: $P = 0.006$, Fig. 5B). The normalize peak amplitude in administration of aprepitant alone was

not different from that in the vehicle group (aprepitant: 0.89 ± 0.06, n = 5, Kruskal-Wallis one-way ANOVA and Bonferroni post-hoc multiple comparison test, P = 1.000, Fig. 5B).

The concomitant administration of the selective D1 receptor antagonist LE300 (2 mg/ml) and imidapril (60 ng/ml) also did not increase the peak amplitude of the swallowing bursts (Fig. 5C). The peak amplitude of the swallowing bursts normalized by the value before drug administration was significantly larger in the imidapril-administration group than in the LE300 and imidapril-administration group (LE300/imidapril: 0.9 ± 0.06, n = 6, Kruskal-Wallis one-way ANOVA and Bonferroni post-hoc multiple comparison test, imidapril vs. LE300/imidapril: P = 0.041, Fig. 5D), although the normalized peak amplitude in administration of LE300 alone was not different from that in the vehicle group (LE300: 0.82 ± 0.06, n = 5, Kruskal-Wallis one-way ANOVA and Bonferroni post-hoc multiple comparison test, P = 1.000, Fig. 5D). The all group was no significant difference in the normalized mean duration (Fig. 5B and 5D). These results suggest that both substance P and dopamine are involved in enhancement of swallowing motor activity by imidapril.

3.3. Swallowing motor activity evoked by electrical stimulation of the SLN

To determine whether imidapril and cilostazol also affect the swallowing bursts induced by electrical stimulation of sensory afferents as was the case for those induced by oral injection of water, we next examined the effects of imidapril and cilostazol on the swallowing motor activity evoked by electrical stimulation of the SLN. Each single pulse-stimulation with the intensity of 20–500 μA elicited a single swallowing burst in the VN and HN (Fig. 6A). In contrast, the repetitive stimulation of 5 Hz during 10 s inhibited inspiratory discharge in all recorded nerves and induced synchronized spindle-shaped swallowing bursts in the VN and HN (Fig. 6B). Both the normalized peak amplitude and duration of the swallowing bursts

evoked by single pulse-stimulation of 100 μA were not significantly different among the vehicle, the imidapril-administration group and the cilostazol-administration group (Kruskal-Wallis one-way ANOVA, peak amplitude: $P = 0.809$, duration: $P = 0.611$, Fig. 7A). Moreover, the normalized peak amplitude and duration of the swallowing bursts evoked by the repetitive stimulation of 100 μA were not also significantly different among the vehicle, the imidapril-administration group and the cilostazol-administration group (Kruskal-Wallis one-way ANOVA, peak amplitude: $P = 0.618$, duration: $P = 0.239$, Fig. 7B). As was the case with stimulation intensity of 100 μA, no significant difference was caused by administration of imidapril and cilostazol in both the peak amplitude and duration at other stimulus intensity (20–500 μA). The number of swallowing bursts evoked by the repetitive stimulation of the SLN was increased in stimulation intensity-dependent manner (Fig. 7C). However, administration of both imidapril and cilostazol did not significantly increase the number of swallowing bursts at all examined stimulation intensity (Fig. 7C). These results suggest that imidapril has different effects on swallowing induced by oral injection of water and swallowing induced by electrical stimulation of the SLN.

4. Discussion

In the present study, we demonstrated that short-term administration of the ACE inhibitor imidapril enhanced the swallowing bursts in the VN induced by injection of distilled water in the oral cavity using *in situ* arterially perfused rat preparations. Many previous studies have shown that long-term treatment with ACE inhibitors inhibited the occurrence of aspiration pneumonia (reviewed by El Solh and Saliba, 2007). For instance, patients with hypertension who had received ACE inhibitors (imidapril, enalapril or captopril) for 2 years had a 2.65-fold reduction in the risk of developing pneumonia compared with those who received other

antihypertensive drugs (calcium-channel blocker or β-blocker) (Sekizawa et al., 1998). Arai et al., (2003) reported that treatment with imidapril for 12 weeks significantly improved silent aspiration in normotensive elderly patients with stroke. In addition, the latency of the swallowing reflex was also improved by treatment with imidapril for 2 weeks in normotensive elderly patients with aspiration pneumonia (Nakayama et al., 1998). Our results suggest that the novel possibility of acute effects of imidapril to enhance activity of the pharyngeal muscle innervated by the VN in addition to its chronic effects on improving swallowing.

In addition to the action of ACE that converts angiotensin I to angiotensin II, ACE metabolizes many other bioactive peptides, including bradykinin, substance P, chemotactic peptides and opioid peptides (Igic and Behnia, 2003; Skidgel and Erdös, 2004). Among these substances, substance P is considered to play important roles in regulating swallowing. Jin and co-workers (1994) reported that when substance P but not calcitonin gene-related peptide or acetylcholine is administered into the pharynx of guinea pigs, the number of induced swallowing reflex is increased. In contrast, the concentration of substance P in sputum of elderly patients with aspiration pneumonia was reduced to one-seventh of those in healthy elderly people (Nakagawa et al., 1995). Such decrease in substance P was also observed in serum of patients with hypertension and symptomless dysphagia, while treatment with imidapril recovered serum substance P concentration to control level (Arai et al., 1998). Our data showed that enhancement of swallowing bursts evoked by oral water injection after imidapril administration was antagonized by the selective NK1 receptor antagonist aprepitant. It is therefore plausible that an increase in substance P was involved in effects of imidapril on the swallowing bursts.

Our data also showed that the selective D1 receptor antagonist LE300 antagonized

enhancement of the water injection-induced swallowing bursts by imidapril. These results suggest that imidapril does not only directly promote an increase in substance P by suppressing its degradation but may also affect dopamine system. Both ACE (Strittmatter et al., 1984) and angiotensin type 1 receptor (Rodriguez-Pallares et al., 2008) are present not only in the periphery but also in various brain regions including the substantia nigra with the highest densities. It was previously reported that chronic cerebral hypoperfusion in spontaneous hypertensive rats reduces putative dopaminergic neurons in the substantia nigra that show tyrosine hydroxylase immunoreactivity, which is improved by long-term administration of perindopril, an ACE inhibitor (Ikeda et al., 2015). Moreover, dopamine dialysate levels in striatum are elevated in rats treated for 1 week with perindopril via drinking water (Jenkins et al., 1997). Therefore, imidapril may increase extracellular dopamine concentration by inhibiting the function of ACE in the central nervous system including the basal ganglia.

In the present study, neither imidapril nor cilostazol enhanced the swallowing bursts evoked by electrical stimulation of the SLN, while imidapril increased the peak amplitude of orally evoked swallowing bursts. There are two possible explanations for this discrepancy. First, imidapril increases substance P in the posterior oral cavity and the pharynx, which is likely to activate the NK1 receptors located on the sensory endings (Carlton et al., 1996). This may facilitate induction of the swallowing reflex induced by oral injection of water but does not increase the activation of the SLN caused by the electrical stimulation. Actually, when substance P is administered into the pharynx of guinea pigs, the number of induced swallowing reflex is increased (Jin et al., 1994), as mentioned above. Imidapril might also be able to increase substance P through the increase in dopamine content because chronic subcutaneous administration of D1 receptor antagonist reduces substance P content in the

laryngeal and pharyngeal mucosa in guinea pigs (Jia et al., 1998).

Second, the sensory nerves innervating the posterior oral cavity and pharynx are involved in not only the vagus nerve but also the glossopharyngeal nerves. The most effective receptor regions for elicitation of the pharyngeal phase in swallowing are innervated both by fibers of the glossopharyngeal nerve which run through the pharyngeal plexus, and by the SLN of the vagus nerve (Miller 1982). Since the glossopharyngeal nerve afferent and the SLN afferent terminate in the separate areas in the nucleus solitary tract and almost did not overlap (Sweazey and Bradley, 1986), the neural circuit underlying swallowing reflex induced by sensory inputs via afferent fibers in the SLN may be, at least in part, different from that via afferent fibers in the glossopharyngeal nerve. Thus, it is possible that imidapril enhances the swallowing reflex induced by activation of the glossopharyngeal nerve during the oral water injection but not by activation of the SLN during the water injection and electrical stimulation. Since imidapril suppresses degradation of substance P, substance P may be increased in the central nervous system. Furthermore, administration of the non-selective dopamine receptor agonist in rats increases expression of substance P in the striatum (Li et al., 1987). Therefore, dopamine increased by imidapril may also increase substance P in the central nervous system. In this case, it is possible that the increase in substance P in the central nervous system may also contribute to enhancement of swallowing burst induced by activation of the glossopharyngeal nerve.

Improvement of swallowing by administration of cilostazol and amantadine has been reported in both clinical studies and animal experiments. In a rat chronic cerebral hypofusion model, the number of swallows was improved by administration of cilostazol for 2–6 weeks (Zhang et al., 2009) or amantadine for 6 weeks (Ikeda et al., 2015). Although the effect of cilostazol was associated with an increase in tyrosine hydroxylase in the substantia nigra and

substance P in the striatum and the effect of amantadine was accompanied by an increase in substance P in the striatum, neither cilostazol nor amantadine significantly enhanced the swallowing bursts and increased the in the present study. Thus, it is possible that long-term administration may be necessary for cilostazol and amantadine to enhance swallowing or both drugs can only be effective on the patient with swallowing disorder.

5. Conclusion

We found that short-term administration of imidapril increased the peak amplitude of orally evoked swallowing bursts in the VN. Such increase in the peak amplitude was antagonized by administration of both the NK1 receptor antagonist and the D1 receptor antagonist. Our data suggest that imidapril may improve impaired swallowing by enhancing pharyngeal muscle activities via an increase in substance P and dopamine.

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Conflict of interest statement

None.

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Figure legends

Fig. 1. Swallowing motor activity evoked by injection of distilled water in the oral cavity. A: An example of VN, HN, and PN activity before injection of distilled water. Original and integrated traces are shown for each nerve. B: Representative fictive swallowing elicited by oral injection of 1.25 ml distilled water. A black bar shows the period of oral injection of distilled water. Oral injection evoked sequential swallowing bursts (black arrows) in the VN and HN.

Fig. 2. Effects of imidapril on swallowing motor activity evoked by injection of distilled water into the oral cavity. A: Representative swallowing bursts in the VN before (Control, left traces) and after (Imidapril, right traces) administration of imidapril (60 ng/ml) in a preparation. B: The summarized peak amplitude (left) and duration (right) of the swallowing bursts before and after administration of imidapril (n = 9). Each bar graph and vertical bar indicate mean and SEM. An asterisk shows significant difference between before and after administration of imidapril ($P < 0.05$). Black dots indicate raw data in each preparation.

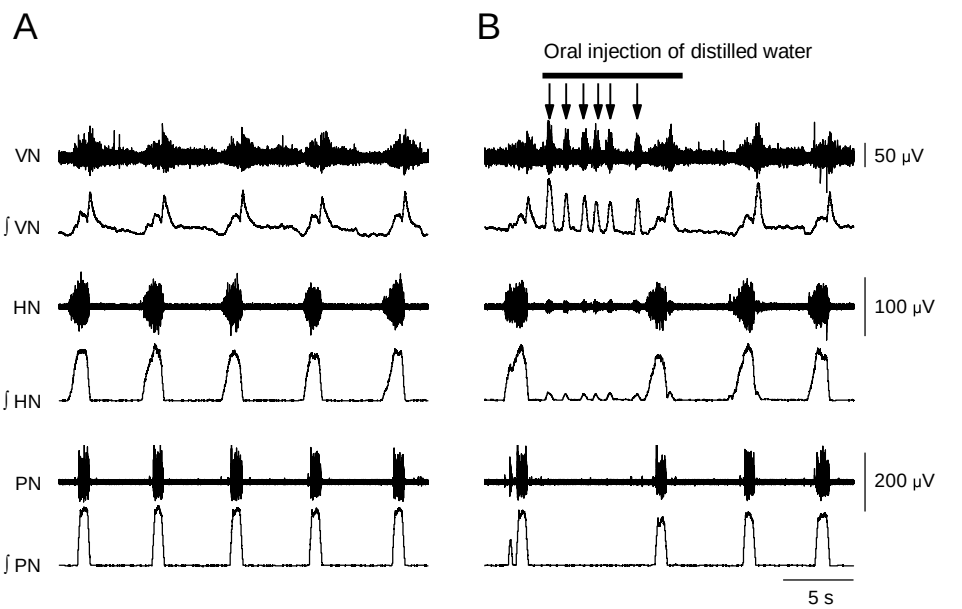
Fig. 3. Effects of cilostazol on swallowing motor activity evoked by injection of distilled water into the oral cavity. A: Representative swallowing bursts in the VN before (Control, left traces) and after (Cilostazol, right traces) administration of cilostazol (2.5 mg/ml) in a preparation. B: The summarized peak amplitude (left) and duration (right) of the swallowing bursts before and after administration of cilostazol (n = 8). Each bar graph and vertical bar indicate mean and SEM.

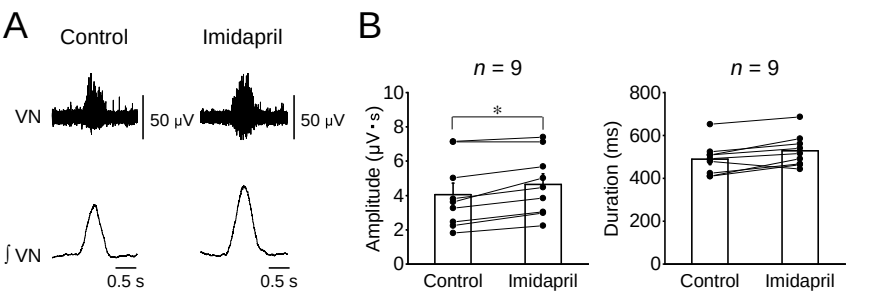
Fig. 4. Effects of amantadine on swallowing motor activity evoked by injection of distilled water into the oral cavity. A: Representative swallowing bursts in the VN before (Control, left traces) and after (Amantadine, right traces) administration of amantadine (200 ng/ml) in a preparation. B: The summarized peak amplitude (left) and duration (right) of the swallowing bursts before and after administration of amantadine (n = 5). Each bar graph and vertical bar indicate mean and SEM.

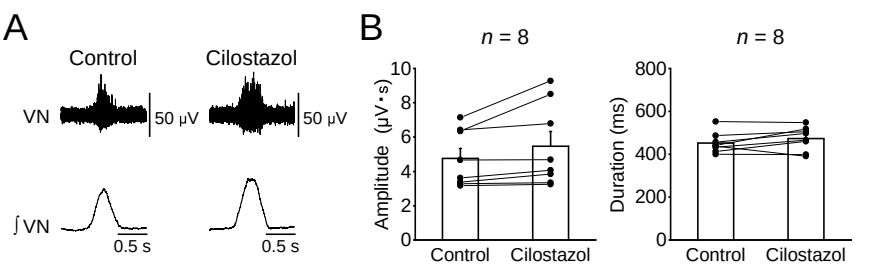
Fig. 5. Effects of NK1 and D1 receptor antagonists on imidapril-induced activation of swallowing motor activity. A: Representative swallowing bursts in the VN before (Control, left traces) and after (Aprepitant/imidapril, right traces) concomitant administration of imidapril (60 ng/ml) and an NK1 receptor antagonist, aprepitant (2 mg/ml). B: The normalized peak amplitude (left) and duration (right) of the swallowing bursts in four groups (vehicle, imidapril, aprepitant, aprepitant/imidapril). In each preparation, the data were normalized at the peak amplitude or duration before drug administration. Each Bar graph and vertical bar indicate mean and SEM. †P < 0.05, vehicle vs. imidapril, imidapril vs. aprepitant/imidapril. C: Representative swallowing bursts in the VN before (Control, left traces) and after (LE300/imidapril, right traces) concomitant administration of imidapril (60 ng/ml) and a D1 receptor antagonist, LE300 (2 mg/ml). D: The normalized peak amplitude (left) and duration (right) of the swallowing bursts in four groups (vehicle, imidapril, LE300, aprepitant/LE300). In each preparation, the data were normalized at the peak amplitude or duration before drug administration. Each bar graph and vertical bar indicate mean and SEM. †P < 0.05, vehicle vs. imidapril, imidapril vs. LE300/imidapril.

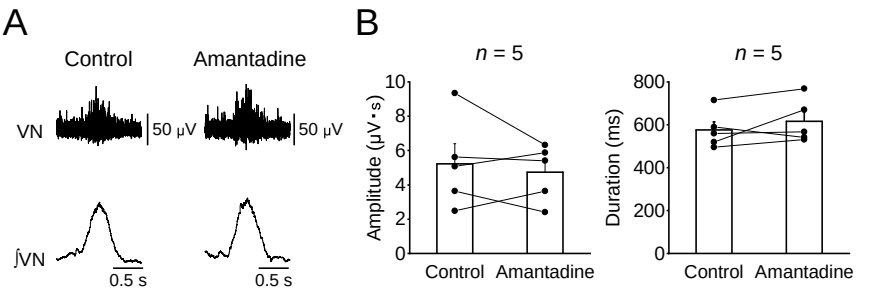
Fig. 6. Swallowing motor activity elicited by electrical stimulation of the SLN. A: An example of the swallowing burst evoked by SLN stimulation with single electric pulse (100 μA, 100 μs). An open arrowhead and a black arrow indicate an electrical stimulation artifact and the swallowing burst, respectively. B: An example of the swallowing bursts evoked by SLN stimulation with repetitive electric pulses (100 μA, 100 μs, 5 Hz). A white bar and black arrows indicate the period of electrical stimulation and evoked swallowing bursts, respectively.

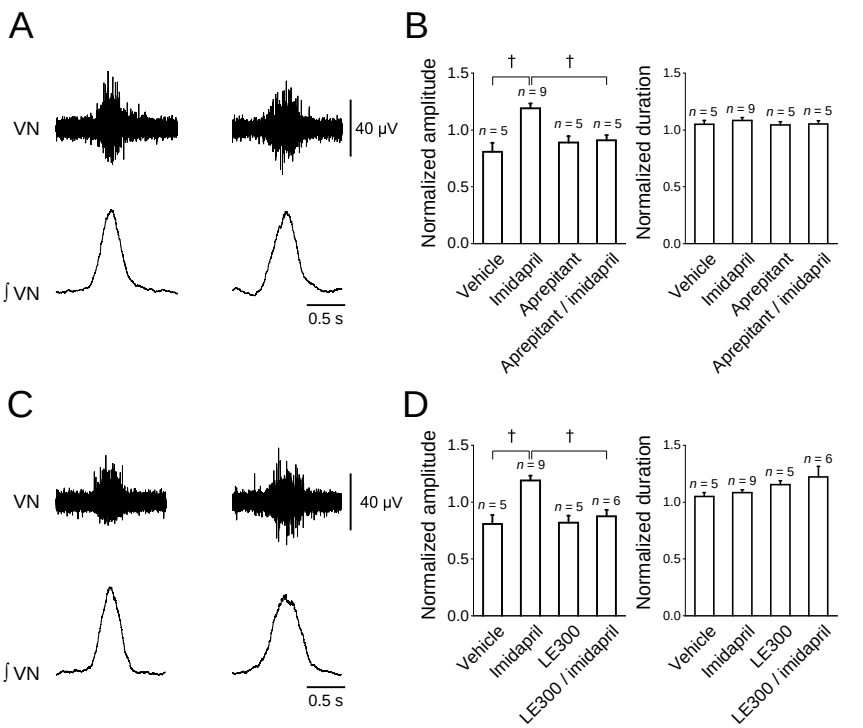
Fig. 7. Effects of imidapril and cilostazol on swallowing motor activity elicited by electrical stimulation of the SLN. A: The normalized peak amplitude (left) and duration (right) of the swallowing bursts evoked by SLN stimulation with single electric pulse (100 μA, 100 μs) in three groups (vehicle, imidapril, cilostazol). In each preparation, the data were normalized at the peak amplitude or duration before drug administration. Each bar graph and vertical bar indicate mean and SEM. B: The normalized peak amplitude (left) and duration (right) of the swallowing bursts evoked by SLN stimulation with repetitive electric pulses (100 μA, 100 μs, 5 Hz) in three groups (vehicle, imidapril, cilostazol). In each preparation, the data were normalized at the peak amplitude or duration before drug administration. Each bar graph and vertical bar indicate mean and SEM. C: The number of swallowing bursts evoked by the repetitive stimulation of the SLN on each stimulation intensity before and during administration of imidapril (left) and cilostazol (right). The stimulus intensity and frequency were 0.01mA~0.5mA and 5 Hz, respectively. Asterisk show significant difference between before and after administration of imidapril ($P < 0.05$). Each symbol and vertical bar indicate mean and SEM.

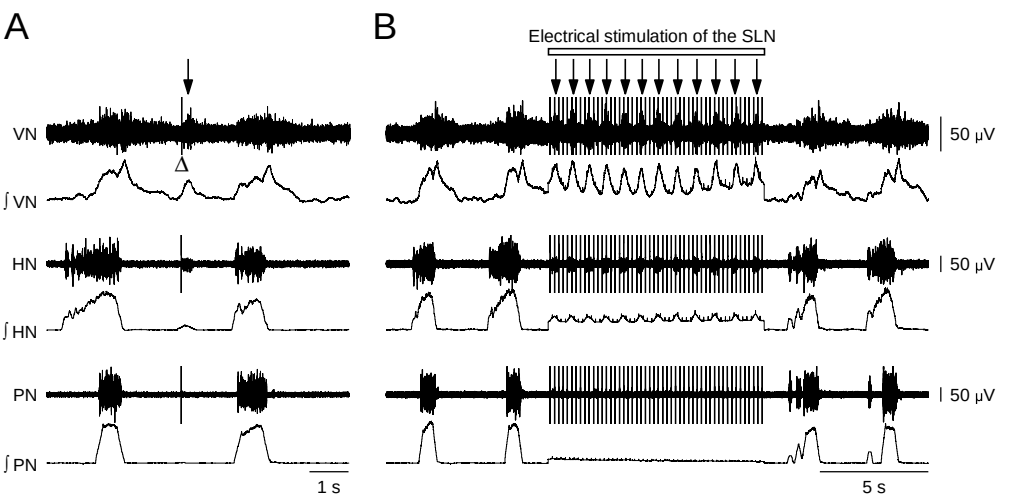

A
B
Oral injection of distilled water
VN
∫ VN
HN
∫ HN
PN
∫ PN
50 μV
100 μV
200 μV
5 s

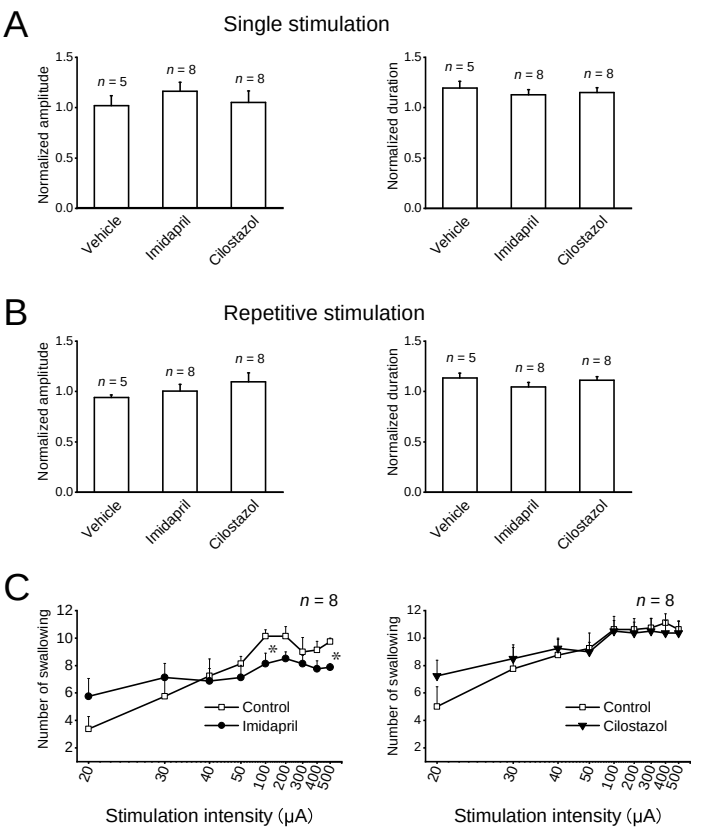

A
Control
Imidapril
VN
50 μV
50 μV
∫ VN
0.5 s
0.5 s
B
n = 9
Amplitude (μV・s)
10
8
6
4
2
0
*
Control
Imidapril
n = 9
Duration (ms)
800
600
400
200
0
Control
Imidapril


A
Control
Cilostazol
VN
50 μV
50 μV
∫VN
0.5 s
0.5 s
B
n = 8
Amplitude (μV・s)
10
8
6
4
2
0
Control
Cilostazol
n = 8
Duration (ms)
800
600
400
200
0
Control
Cilostazol


A
Control
Amantadine
VN
50 μV
50 μV
∫VN
0.5 s
0.5 s
B
n = 5
Amplitude (μV · s)
10
8
6
4
2
0
Control
Amantadine
n = 5
Duration (ms)
800
600
400
200
0
Control
Amantadine


A
VN
40 μV
∫ VN
0.5 s
B
Normalized amplitude
Normalized duration
†
†
n = 5
n = 9
n = 5
n = 5
Vehicle
Imidapril
Aprepitant
Aprepitant / imidapril
C
D
LE300
LE300 / imidapril
n = 6


A
B
Electrical stimulation of the SLN
VN
∫ VN
HN
∫ HN
PN
∫ PN
50 μV
50 μV
50 μV
1 s
5 s


A
Single stimulation
Normalized amplitude
Normalized duration
n = 5
n = 8
n = 8
Vehicle
Imidapril
Cilostazol
B
Repetitive stimulation
C
Number of swallowing
Control
Imidapril
Cilostazol
Stimulation intensity (μA)