J Physiol Sci (2016) 66:105–111
DOI 10.1007/s12576-015-0401-8

ORIGINAL PAPER

Does the capsaicin-sensitive local neural circuit constitutively regulate vagally evoked esophageal striated muscle contraction in rats?

Takeshi Shima[1] · Takahiko Shiina[1] · Kiyotada Naitou[1] · Hiroyuki Nakamori[1] · Yuuki Sano[1] · Yasutake Shimizu[1]

Received: 29 June 2015 / Accepted: 7 September 2015 / Published online: 30 September 2015

Abstract To determine whether a capsaicin-sensitive local neural circuit constitutively modulates vagal neuromuscular transmission in the esophageal striated muscle or whether the neural circuit operates in a stimulus-dependent manner, we compared the motility of esophageal preparations isolated from intact rats with those in which capsaicin-sensitive neurons had been destroyed. Electrical stimulation of the vagus nerve trunk evoked contractile responses in the esophagus isolated from a capsaicin-treated rat in a manner similar to those in the esophagus from a control rat. No obvious differences were observed in the inhibitory effects of D-tubocurarine on intact and capsaicin-treated rat esophageal motility. Destruction of the capsaicin-sensitive neurons did not significantly affect latency, time to peak and duration of a vagally evoked twitch-like contraction. These findings indicate that the capsaicin-sensitive neural circuit does not operate constitutively but rather is activated in response to an applied stimulus.

Keywords Capsaicin-sensitive neuron · Enteric neuron · Esophagus · Striated muscle · Vagus

Introduction

The external muscle layer of the mammalian esophagus contains not only smooth muscle but also striated muscle fibers [1, 2]. Movement of the esophageal striated muscle is known to be controlled by the vago–vagal reflex via the brainstem [3, 4]. We have previously reported that in addition to the vago–vagal reflex there is a local neural reflex in esophageal striated muscle, as demonstrated by the mechanical responses induced by electrical stimulation and the exogenous application of drugs in isolated esophageal segments from several mammals, including the rat [5–9]. This reflex circuit consists of capsaicin-sensitive primary afferent neurons and myenteric (intrinsic) neurons, which can inhibit the release of neurotransmitters from vagal motor neurons in the esophageal striated muscle [7, 9]. This inhibitory action can be induced by the application of afferent-stimulating agents, such as capsaicin and piperine [5–9].

We have also examined functional roles of the local reflex in esophageal peristalsis in vivo using rats in which the capsaicin-sensitive neurons had been destroyed by neonatal treatment with capsaicin [10, 11]. Subsequent examination demonstrated that the capsaicin-treated rat esophagus showed a multi-phasic rise in intraluminal pressure, possibly due to non-coordinated contractions of the esophageal muscles, whereas a mono-phasic response was observed in the intact rat esophagus. The results suggest that the local neural reflex consisting of capsaicin-sensitive neurons and intrinsic neurons contributes to coordinated contractions of the esophageal striated muscle through the inhibition of neuromuscular transmission. Notably, the in vivo experiments demonstrated that the absence of capsaicin-sensitive neurons disturbs the operation of the local neural circuit, although downstream intrinsic neurons would remain intact even after destruction of the capsaicin-sensitive neurons. Given that capsaicin-sensitive primary afferents can be activated spontaneously [12, 13], it is possible that the local neural reflex consisting of capsaicin-sensitive neurons and

✉ Takahiko Shiina
tshiina@gifu-u.ac.jp

[1] Laboratory of Physiology, Department of Basic Veterinary Science, The United Graduate School of Veterinary Sciences, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan

intrinsic neurons operates constitutively and can thus modify vagal neuromuscular transmission when vagal motor neurons are activated. Alternatively, it is also possible that the neural circuit does not operate under the basal condition but rather is activated in response to stimuli, including distention caused by intraluminal contents. The two hypotheses can be tested by comparing vagally evoked contractions in isolated esophageal preparations of capsaicin-treated rats with those of intact rats. If the former hypothesis holds up, then vagally evoked contractions in the capsaicin-treated rat esophagus would be greater than those in the intact rat esophagus. Alternatively, if the latter hypothesis is the case, vagally evoked contractions in the capsaicin-treated rat esophagus would be similar to those in the intact rat esophagus.

Hence, the aim of the study reported here was to clarify whether the capsaicin-sensitive local neural circuit plays a constitutive role in modifying vagal neuromuscular transmission of the esophageal striated muscle. For this purpose, we performed an in vitro study in which we compared the motility of the esophagus of capsaicin-treated rats in which capsaicin-sensitive neurons had been destroyed with the motility of the esophagus of intact rats.

Methods

Animals

Male Sprague–Dawley rats (age 10–12 weeks, weight 300–350 g) were obtained from Japan SLC (Shizuoka, Japan). They were maintained in plastic cages at 22 ± 2 °C under a 12/12-h light/dark cycle, with free access to laboratory chow (MF; Oriental Yeast, Tokyo, Japan) and water. The experiments were approved by the Gifu University Animal Care and Use Committee and were conducted in accordance with the committee guidelines on animal care and use (permission number: 14100 and 14105).

Neonatal capsaicin treatment

To destroy the capsaicin-sensitive neurons, we subcutaneously injected neonatal rats with capsaicin (50 mg/kg) at 2 days after birth as described previously [14–16]. At 8–9 weeks after administering the capsaicin, we selected only capsaicin-treated rats that responded to no more than two wipes of capsaicin solution (0.01 %) applied to the cornea.

Esophageal tissue preparations

Animals were anesthetized with isoflurane and exsanguinated via the axillary arteries. A 1-cm-long segment from the thoracic part of the esophagus was dissected out and immediately immersed in Krebs' solution (see below) at room temperature; the intraluminal contents of the excised segment were flushed out using a small cannula containing Krebs' solution.

Recording of mechanical activity in the esophageal segments

The entire dissected segment was placed in an organ bath (capacity 10 mL) filled with Krebs' solution (pH 7.4) through which a 95 % O_2 + 5 % CO_2 gas mixture was continuously bubbled; the temperature was maintained at 35 °C. For recording contractile responses in the longitudinal direction, we tied one end of the esophageal segment to the organ bath with a silk thread and secured the other end to an isometric force transducer (model T7-8-240; Orientec, Tokyo, Japan) also with a silk thread. Isometric responses were filtered and amplified through an amplifier (model AS1202; NEC, Tokyo, Japan) and recorded using a PowerLab system (AD Instruments, Bella Vista, Australia). An initial resting tension of 1.0 g was applied to the preparations, which were subsequently allowed to equilibrate for at least 30 min.

Electrical stimulation

For inducing the muscle contractile response, we applied electrical stimulations to esophageal preparations. In experiments using vagal stimulation, the end of the right-side vagus nerve trunk was drawn into a bipolar suction electrode and the electrode then immersed together with the esophagus preparation in the organ bath. The vagus nerves were stimulated using an electronic stimulator (model SEN-3201; Nihon Kohden, Tokyo, Japan) connected to the electrode. For stimulation of the vagus nerves to evoke contractile responses, we applied single square-wave pulses ranging in intensity from 1 to 80 V and in duration from 10 to 500 μs at intervals of 1 min; alternatively, we applied multiple square-wave pulses with an intensity of 80 V and duration of 100 μs at 1–20 Hz for 5 s.

Solutions and drugs

During the experiments, tissues were maintained in Krebs' solution (in mM: NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25; glucose, 11.7). D-tubocurarine, capsaicin, N^G-nitro-L-arginine methyl ester (L-NAME) and *N*-acetyl-1-tryptophan 3,5-bis (trifuluorometyl) benzyl ester (L-732,138) were obtained from Sigma-Aldrich (St Louis, MO). D-tubocurarine and L-NAME were dissolved in distilled water, capsaicin was

dissolved in ethanol and L-732,138 was dissolved in ethyl acetate. The highest concentration of vehicles for the drugs alone had no effect on the basal tone and contractile responses at the concentrations used. The concentrations of drugs given here are the final concentrations in the organ bath solution.

Statistical analysis

Data are presented as mean ± standard deviation (SD). *n* indicates the number of separate preparations. The significance of differences between mean values was determined by one-way analysis of variance followed by Dunnet's test. A *P* value less than 0.05 denotes the presence of a statistically significant difference.

Results

Effects of capsaicin on contractile responses induced by electrical stimulation of the vagus nerve in intact and capsaicin-treated rat esophageal segments

No spontaneous responses occurred without application of electrical stimulation in the dissected rat esophagus. Electrical stimulation of the vagus nerve trunk with single pulses evoked monophasic (twitch-like) contractile responses in the esophagus of both the intact and capsaicin-treated rats (Fig. 1). To investigate the activity of capsaicin-sensitive neurons that modulate striated muscle contractions in the rat esophagus, we examined the effect of capsaicin, an activator of primary afferent neurons, on twitch contractions elicited by vagus nerve stimulation. Application of capsaicin (30 μM) inhibited the vagally mediated twitch contractions of esophageal segments in the intact rat esophagus (Fig. 1a). We confirmed that the inhibitory effect of capsaicin was attenuated by pretreatment with the nitric oxide (NO) synthase inhibitor L-NAME (200 μM) or with the selective tachykinin NK1 receptor antagonist L-732,138 (2 μM), (data not shown), as demonstrated in our previous study [9]. In contrast, an inhibitory effect of capsaicin was not observed in the esophagus isolated from a capsaicin-treated rat (Fig. 1b).

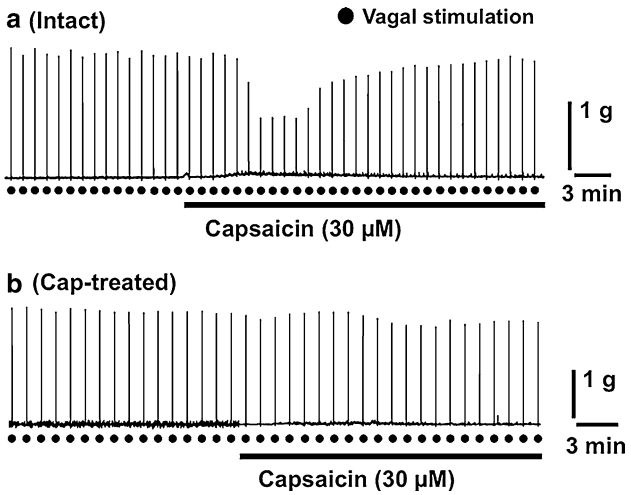

Fig. 1 Effect of capsaicin on vagally evoked contractions in the rat esophagus. Representative tracings of the effect of capsaicin (30 μM) on vagally mediated contractions in the esophagi of the intact rat (**a**) and capsaicin (*Cap*)-treated rat (**b**), respectively, are shown ($n = 4$). Electrical stimulations were applied to the vagus nerve using single pulses of 80 V (pulse duration 100 μs; between-pulse interval 1 min), and longitudinal mechanical responses were recorded isometrically. *Filled circles under tracings* Points of application of single-pulse electrical stimulation. Electrical stimulation of the vagus trunk induced monophasic (twitch-like) contractions of esophageal striated muscle. Capsaicin was added to the organ bath at a final concentration of 30 μM

Stimulus–response relationships of vagally evoked contractions in intact and capsaicin-treated rat esophageal segments

To clarify whether the capsaicin-sensitive local neural circuit constitutively suppresses the release of neurotransmitters of vagal motor neurons, we examined the stimulus–response relationships of vagally evoked contractions. Vagally evoked twitch-like contractions were augmented with increasing intensity (Fig. 2a, b) and duration (Fig. 2c) of the pulse stimuli. There was no significant difference in the twitch-like contractions between the intact rat and the capsaicin-treated rat esophagi, respectively. Increases in frequency of pulses in electrical stimulation evoked tetanic contractions in the esophagus isolated from a capsaicin-treated rat in a manner similar to that in the esophagus from a control rat (Fig. 2d). Maximum amplitudes of tetanic contractions were not significantly different: 4.27 ± 0.87 g in intact rats ($n = 5$) versus 4.24 ± 0.96 g in capsaicin-treated rats ($n = 3$).

Inhibitory effects of an antagonist of muscular nicotinic acetylcholine receptors, D-tubocurarine, on vagally mediated contractions of esophageal striated muscle

To determine the pharmacological properties of neuromuscular junctions on the intact and capsaicin-treated rat esophagi, respectively, we examined the effects of D-tubocurarine, an antagonist of the muscular nicotinic acetylcholine receptor. This blocker inhibited contractile responses in a dose-dependent manner (Fig. 3), and no obvious differences were observed in its effects on the intact and capsaicin-treated rat esophagi, respectively.

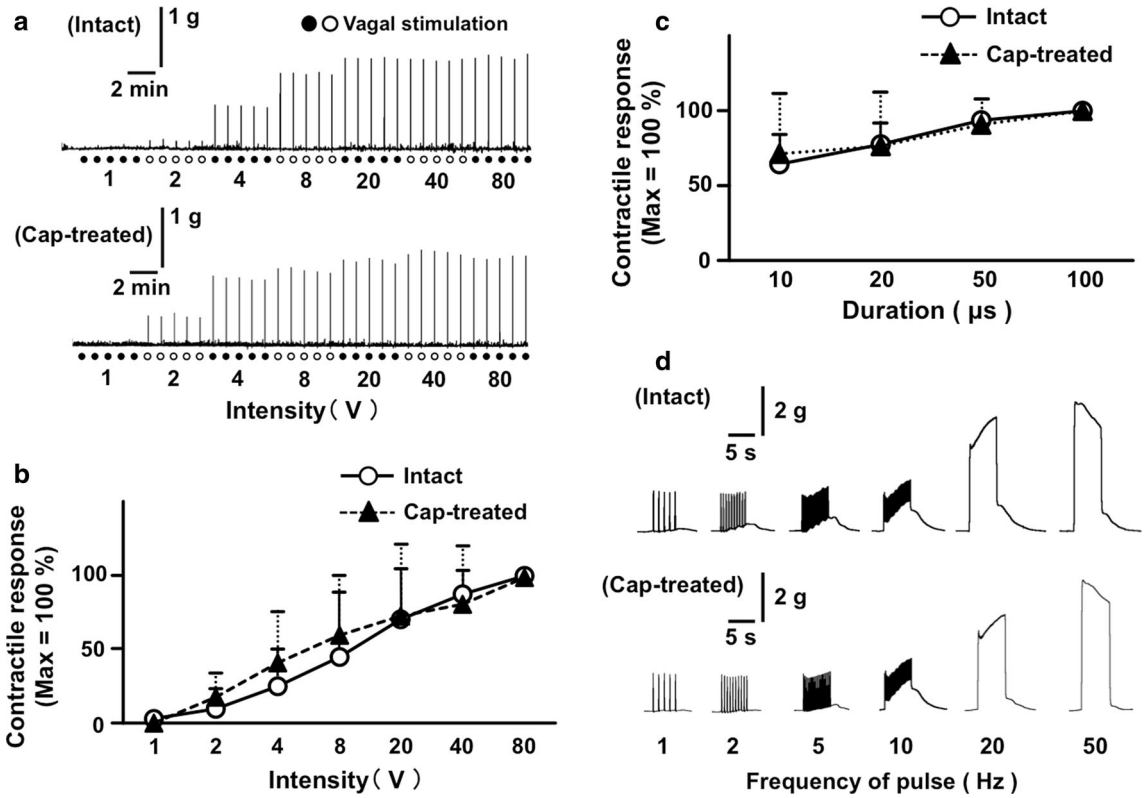

Fig. 2 Effects of elevations in the strength of electrical stimulation to the vagus nerve on esophageal striated muscle contractility in the rat esophagus. **a** Representative tracings of vagally mediated contractions in the intact and capsaicin (*Cap*)-treated rat esophagi, respectively, are shown. Electrical stimulations were applied to the vagus nerve using single pulses (pulse duration 100 μs; between-pulse interval 1 min) at intensities ranging from 1 to 80 V. *Filled circles* points of application of the single-pulse electrical stimulation (intensities 1, 4, 20, and 80 V). *Open circles* points of application of the single-pulse electrical stimulation(intensities 2, 8, and 40 V). **b** Intensity–response curves (maximal contraction 100 %) of the intact (*open circle*) and the Cap-treated (*filled triangle*) rat esophagi. Each *data point* represents the mean ± standard deviation (SD) ($n = 4$). **c** Electrical stimulations were applied to the vagus nerve using single 80-V pulses (pulse duration 10–100 μs; between-pulse interval 1 min). Duration–response curves (maximal contraction 100 %) of the intact (*open circle*) and the Cap-treated (*filled triangle*) rat esophagi are shown. Each *data point* represents the mean ± SD (intact $n = 5$, Cap-treated $n = 4$). **d** Representative tracings of contractions when electrical stimulations were applied to the vagus nerve using multiple 80-V pulses (pulse duration 100 μs) at a frequency of 1–50 Hz for 5 s in the intact and Cap-treated rat esophagi are shown. Increases in the frequency of pulses in the electrical stimulation evoked tetanic contractions

Properties of twitch-like contractile responses induced by electrical stimulation of the vagus nerve in intact and capsaicin-treated rat esophageal segments

To compare contractile properties in the capsaicin-treated rat esophageal striated muscle with those in the intact one, we analyzed the parameters of vagally mediated twitch-like contractions in the esophageal preparations. Destruction of the capsaicin-sensitive neurons did not significantly affect latency, time to peak and duration of a vagally evoked twitch-like contraction (Fig. 4).

Discussion

The aim of this study was to determine whether the capsaicin-sensitive local neural circuit operates constitutively to modulate vagal neuromuscular transmission in esophageal striated muscle or whether the neural circuit operates in a stimulus-dependent manner. For this purpose, we compared vagally evoked contractions in the esophagi of rats in which capsaicin-sensitive neurons had been destroyed with those in the intact esophagi. The results obtained in this study showed that there was no significant difference in esophageal striated muscle contractility

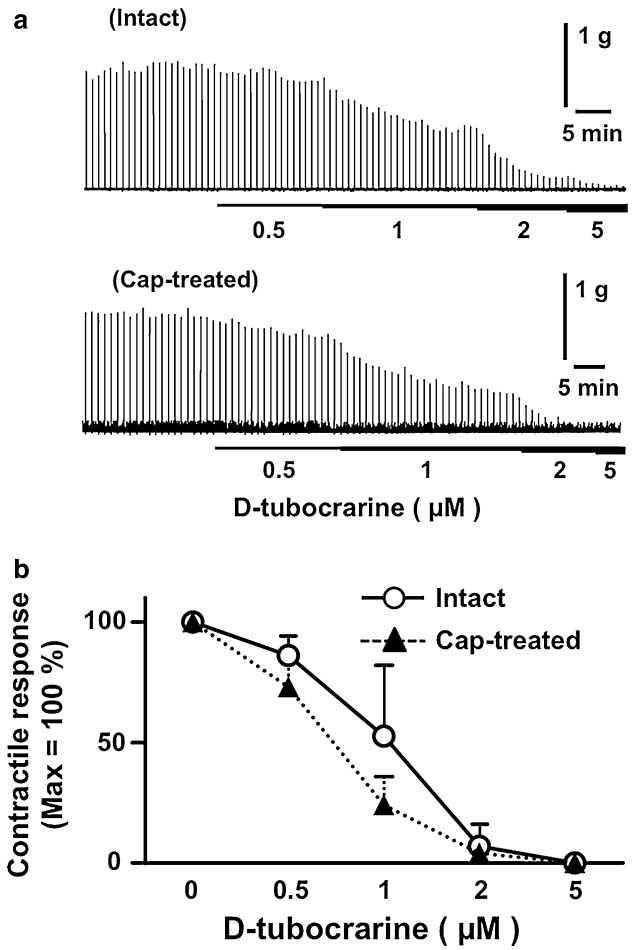

Fig. 3 Effect of D-tubocurarine on vagally mediated contractions in the rat esophagus. **a** Representative tracings of vagally mediated contractions in the intact and capsaicin (*Cap*)-treated rat esophagi, respectively, in the presence or absence of D-tubocurarine (0.5–5 μM) are shown. Electrical stimulations were applied to the vagus nerve using single 80-V pulses (pulse duration 100 μs; between-pulse interval 1 min). **b** Summary graphs of the effects of D-tubocurarine on vagal stimulation-evoked contractions in the intact (*open circles*) and the Cap-treated (*filled triangles*) rat esophagi, respectively (contraction after application of vehicle 100 %). Each *data point* represents the mean ± SD (intact $n = 5$, capsaicin-treated $n = 3$)

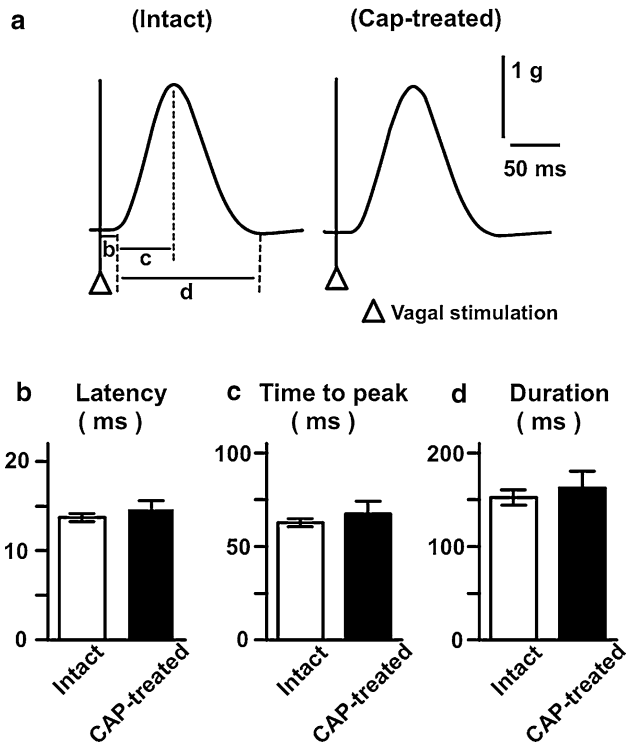

Fig. 4 Properties of twitch-like contractile responses elicited by electrical stimulation of the vagus nerve in the rat esophagus. **a** Representative tracings of vagally mediated contractions in the intact and capsaicin-treated rat esophagi, respectively. Electrical stimulations were applied to the vagus nerve using single 80-V pulses (pulse duration 100 μs). *Arrowhead* Point of a single-pulse electrical stimulation, *b* latency, *c* time to peak, *d* duration. **b–d** Summary graphs of latency (ms; **b**), time to peak (ms; **c**) and duration (ms; **d**) of a vagally mediated twitch-like contraction. Data are presented at the mean [*bars* ± SD (*error bars*) (intact $n = 4$, capsaicin-treated $n = 3$)]

between esophagus preparations isolated from normal and capsaicin-treated rats. Destruction of the capsaicin-sensitive neurons by neonatal capsaicin treatment was successful, since capsaicin applied to esophageal preparations isolated from capsaicin-treated rats had no inhibitory effect on vagally evoked twitch contractions. We therefore conclude that the local neural reflex is not constitutively active under the basal condition but rather is likely to be activated in response to stimuli.

We previously demonstrated that capsaicin inhibits vagally mediated twitch contractions of the esophageal striated muscle portion and that this inhibition is reversed by application of an NO synthase inhibitor or a tachykinin NK1 receptor antagonist [9]. We have also reported that capsaicin can suppress acetylcholine release from the esophageal segments evoked by vagus nerve stimulation [7, 9]. These findings suggest that capsaicin-sensitive afferent neurons act on myenteric nitrergic neurons via tachykinin NK1 receptors, resulting in the release of NO that subsequently inhibits the release of neurotransmitters from vagal motor neurons in the striated muscle portion of the esophagus [7, 9]. In the light of these results, we assumed that if the capsaicin-sensitive neural circuit is constitutively active, a lower amount of neurotransmitters would be released from vagal motor neurons in the intact esophagus than from those in the esophagus isolated from a capsaicin-treated rat. Based on this assumption, we examined the stimulus–response relationships of vagally evoked contractions since the release of neurotransmitters from vagal motor neurons would be increased with increasing stimulus strength. However, in the present study, contractile responses increased similarly in both the intact and capsaicin-treated rat esophagi with increasing intensity

and/or duration of the electrical stimuli. This result indicates that the presence of capsaicin-sensitive neurons itself is insufficient to suppress the release of neurotransmitters from vagal motor neurons. This notion is supported by the results of our experiments using a blocker of nicotinic acetylcholine receptors, D-tubocurarine. Because D-tubocurarine is a competitive inhibitor of acetylcholine, a difference in inhibitory pharmacological kinetics may imply a difference in the amount of released acetylcholine that induces contractile responses in the esophageal striated muscle [17]. However, we observed that D-tubocurarine inhibited vagally evoked contractions of two groups of esophageal preparations with similar dose dependencies, suggesting that the amount of acetylcholine released from vagal motor neurons are likely not suppressed constitutively by the capsaicin-sensitive local neural circuit.

We also addressed the possibility that the capsaicin-sensitive neural circuit modifies downstream intracellular events of receptors on the muscle cells. If the capsaicin-sensitive neural circuit affects downstream intracellular events, latency, time to peak and/or duration of a vagally evoked twitch-like contraction would be changed by the destruction of capsaicin-sensitive neurons. The tetanic contractions evoked by multi-pulse electrical stimulations would also be changed since the summation of contractions is dependent on the accumulation of intracellular Ca^{2+} [18]. However, neonatal treatment with capsaicin did not significantly affect any property of a vagally mediated twitch-like and tetanic contractions. Taken together, these findings indicate that the capsaicin-sensitive neural circuit may not operate constitutively to modify vagally mediated contractile responses in the esophageal striated muscle.

It also seems unlikely that the capsaicin-sensitive neural circuit constitutively operates to modify the motility of esophageal striated muscle. Alternatively, it is probable that the neural circuit can exert its regulatory effects on the esophageal striated muscle when capsaicin-sensitive neurons are activated. However, this does not necessarily mean that the trigger stimulus of the neural circuit is always capsaicin. Rather, capsaicin may just be a tool for stimulating the local circuit but not a physiological activator of the capsaicin-sensitive neural circuit. In fact, an in vivo study showed the capsaicin-sensitive neurons play an important role in the coordination of esophageal peristaltic motility even in the absence of capsaicin stimulus [10, 11]. We speculate that capsaicin-sensitive afferent neurons can be activated by physical and/or chemical factors in vivo. For example, physical stimulations to the esophagus, such as distention by intraluminal foods, influence the activity of capsaicin-sensitive afferents, which in turn can modify the regulation of esophageal motility by the central nervous system. This notion is supported by the results of a number of studies showing that c-fibers can be excited in response to distention or pressure stimulation [19–21]. It has recently been shown that inhibitory nitrergic motor neurons of the esophageal myenteric plexus are mechanosensitive [22]. However, such neurons may not be related to the capsaicin-sensitive neural circuit. This possibility is based on the findings that although intrinsic inhibitory neurons would be alive even in the capsaicin-treated rat [23], the action of the neural circuit is not expressed in the absence of capsaicin-sensitive neurons [10, 11]. Further study is needed to clarify the functional activators of the capsaicin-sensitive neural circuit in the esophagus.

In conclusion, we have addressed the question of whether the capsaicin-sensitive neural circuit, which is involved in regulation of motor activity of the esophageal striated muscle, can operate constitutively or whether it is activated when several stimuli are applied in rats. Our results demonstrate that the neural circuit does not operate under the basal condition but can exert its regulatory functions in a stimulus-dependent manner. The physiological stimulus that triggers the activity of the neural circuit remains to be elucidated.

Acknowledgments This work was supported in part by Grants-in-Aid for Scientific Research (KAKENHI) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan [Research Project Numbers: 20780206, 23780291, 26292164]. The authors are indebted to Ms. Ayako Takahashi (Gifu University, Japan) for her technical support.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Neuhuber WL, Raab M, Berthoud HR, Wörl J (2006) Innervation of the mammalian esophagus. Adv Anat Embryol Cell Biol 185:1–73
2. Wörl J, Neuhuber WL (2005) Enteric co-innervation of motor endplates in the esophagus: state of the art ten years after. Histochem Cell Biol 123:117–130
3. Goyal RK, Chaudhury A (2008) Physiology of normal esophageal motility. J Clin Gastroenterol 42:610–619
4. Lu WY, Bieger D (1998) Vagovagal reflex motility patterns of the rat esophagus. Am J Physiol 274:R1425–R1435
5. Boudaka A, Wörl J, Shiina T, Neuhuber WL, Kobayashi H, Shimizu Y, Takewaki T (2007) Involvement of TRPV1-dependent and -independent components in the regulation of vagally induced contractions in the mouse esophagus. Eur J Pharmacol 556:157–165
6. Boudaka A, Wörl J, Shiina T, Shimizu Y, Takewaki T, Neuhuber WL (2009) Galanin modulates vagally induced contractions in the mouse oesophagus. Neurogastroenterol Motil 21:180–188
7. Izumi N, Matsuyama H, Ko M, Shimizu Y, Takewaki T (2003) Role of intrinsic nitrergic neurones on vagally mediated striated muscle contractions in the hamster oesophagus. J Physiol 551:287–294

8. Shiina T, Shima T, Suzuki Y, Wörl J, Shimizu Y (2012) Neural regulation of esophageal striated muscle in the house musk shrew (*Suncus murinus*). Auton Neurosci 168:25–31
9. Shiina T, Shimizu Y, Boudaka A, Wörl J, Takewaki T (2006) Tachykinins are involved in local reflex modulation of vagally mediated striated muscle contractions in the rat esophagus via tachykinin NK1 receptors. Neuroscience 139:495–503
10. Shiina T, Shima T, Naitou K, Nakamori H, Shimizu Y (2014) Esophageal peristalsis is regulated by capsaicin-sensitive intrinsic neural circuit in rats. J Physiol Sci 64:S235
11. Shima T, Shiina T, Naitou K, Nakamori H, Shimizu Y (2014) Functional roles of capsaicin-sensitive intrinsic neural circuit in the regulation of esophageal peristalsis in rats: in vivo studies using a novel method. Am J Physiol Gastrointest Liver Physiol 306:G811–G818
12. Banik RK, Brennan TJ (2009) Trpv1 mediates spontaneous firing and heat sensitization of cutaneous primary afferents after plantar incision. Pain 141:41–51
13. Rong W, Hillsley K, Davis JB, Hicks G, Winchester WJ, Grundy D (2004) Jejunal afferent nerve sensitivity in wild-type and TRPV1 knockout mice. J Physiol 560:867–881
14. Jancso G, Kiraly E, Jancso-Gabor A (1977) Phramacologically induced selective degeneration of chemosensitive primary sensory neurons. Nature 270:741–743
15. Tsuda M, Koizumi S, Kita A, Shigemoto Y, Ueno S, Inoue K (2000) Mechanical allodynia caused by intraplantar injection of P2X receptor agonist in rats: involvement of heteromeric P2X2/3 receptor signaling in capsaicin-insensitive primary afferent neurons. J Neurosci 20:RC90
16. Yamashita H, Wang Z, Wang Y, Furuyama T, Kontani Y, Sato Y, Mori N (2008) Impaired basal thermal homeostasis in rats lacking capsaicin-sensitive peripheral small sensory neurons. J Biochem 143:385–393
17. Jonsson Fagerlund M, Dabrowski M, Eriksson LI (2009) Pharmacological characteristics of the inhibition of nondepolarizing neuromuscular blocking agents at human adult muscle nicotinic acetylcholine receptor. Anesthesiology 110:1244–1252
18. Nogueira L, Hogan MC (2010) Phenol increases intracellular [Ca^{2+}] during twitch contractions in intact Xenopus skeletal myofibers. J Appl Physiol (1985) 109:1384–1393
19. Aizawa N, Igawa Y, Nishizawa O, Wyndaele JJ (2011) Effects of nitric oxide on the primary bladder afferent activities of the rat with and without intravesical acrolein treatment. Eur Urol 59:264–271
20. De Groat WC, Booth AM, Milne RJ, Roppolo JR (1982) Parasympathetic preganglionic neurons in the sacral spinal cord. J Auton Nerv Syst 5:23–43
21. Yu S, Gao G, Peterson BZ, Ouyang A (2009) TRPA1 in mast cell activation-induced long-lasting mechanical hypersensitivity of vagal afferent C-fibers in guinea pig esophagus. Am J Physiol Gastrointest Liver Physiol 297:G34–G42
22. Dong H, Jiang Y, Dong J, Mittal RK (2015) Inhibitory motor neurons of the esophageal myenteric plexus are mechanosensitive. Am J Physiol Cell Physiol 308:C405–C413
23. Forster ER, Southam E (1993) The intrinsic and vagal extrinsic innervation of the rat stomach contains nitric oxide synthase. NeuroReport 4:275–278