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Measurement of the propelled liquid by isolated hamster ileum as a parameter to evaluate peristalsis

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Abstract

We present a method to measure the volume of liquid propelled by peristaltic movements of isolated hamster ileum as a novel means to assess peristaltic activity. The oral and aboral ends of the dissected ileum were attached to cannulas fixed horizontally. The application of intraluminal pressure by raising the level of liquid in the bottle connected to the oral end evoked peristalsis and intermittent propulsion of the intraluminal liquid. The inhibition of intrinsic neurons by tetrodotoxin stopped propulsion; this indicated that the liquid propulsion was correlated with neuron-regulated peristalsis. The volume of liquid propelled by one complete peristaltic movement was significantly greater than that by incomplete peristalsis, whereas recordings of pressure changes were indistinguishable. Inhibitors of nitric oxide synthase decreased the volume of liquid propelled by peristaltic movements, suggesting a role of nitrergic neurons in peristalsis. Our data show that the method described above might be suitable for analyzing peristalsis.

Keywords: Ileum; Nitrergic neuron; Nitric oxide; Peristalsis; Propelled liquid volume; (Hamster)

1. Introduction

Propulsion of the intestinal contents is a crucial part of digestion that depends on the coordinated activity of circular and longitudinal smooth muscles brought about by the peristaltic reflex (Olsson and Holmgren, 2001; Grider, 2003b). The peristaltic reflex is initiated by mucosal stimulation or stretching of the intestinal wall, which results in a circular contraction behind the stimulus and an area of relaxation in front of it (Kunze and Furness, 1999; Olsson and Holmgren, 2001; Hansen, 2003). The wave of contraction then moves in an oral-to-aboral direction, thereby propelling the luminal contents forward (Waterman et al., 1994a,b). The sequence of peristaltic events does not depend on extrinsic autonomic innervation but rather involves the activation of intrinsic sensory neurons, which are coupled via modulatory interneurons to excitatory and inhibitory motor neurons that project into the circular muscle layer (Olsson and Holmgren, 2001; Hansen, 2003). Therefore, an isolated segment of the intestine is the experimental preparation of choice for analyzing the neural circuitry of the enteric nervous system that regulates peristalsis (Ciccocioppo et al., 1994; Suzuki et al., 1994; Waterman and Costa, 1994; Lazar et al., 2001; Grider, 2003a; Onori et al., 2003; Ji et al., 2004).

Various parameters are used to assess the peristaltic activity of intestinal preparations, including intraluminal pressure, contraction frequency, latency after initiation stimulus, threshold pressure for initiation, and velocity of propulsion (Ciccocioppo et al., 1994; Suzuki et al., 1994; Waterman and Costa, 1994; Onori et al., 2003). In a few papers, the amount of propelled intraluminal contents has been measured as an additional parameter to assess intestinal peristalsis (Smith and Robertson, 1998). When we consider that the major function of peristalsis is the propulsion of intraluminal contents, measurement of the

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volume of intraluminal contents propelled by peristaltic movements may be a useful parameter for evaluating peristaltic activity. However, there has not been a comprehensive assessment of the relationship between the volume of liquid propelled from isolated ileum and peristaltic function.

Hamster intestinal segments have been used for studying neuronal regulation of intestinal motility (Matsuyama et al., 1999, 2002, 2003; El-Mahmoudy et al., 2003), in addition to those isolated from guinea pigs, rats and mice. Non-adrenergic, non-cholinergic excitatory (El-Mahmoudy et al., 2003) and inhibitory (Matsuyama et al., 1999) neurotransmissions in the hamster ileum have been reported. These reports indicate that the hamster ileum might be one of the materials suitable for researching the peristaltic activity.

In the present study, we established a method to measure the volume of liquid propelled from the aboral side of isolated hamster ileum. To evaluate the effectiveness of this novel method as a parameter to assess intestinal peristalsis, we compared the quantitative results to the changes in intraluminal pressure, which is used extensively to evaluate peristaltic function. In addition, we applied the propelled liquid volume method to examine the physiological role of nitric oxide (NO) in intestinal peristalsis. Our data show that propelled liquid volume might be a useful parameter to complement the analysis of peristaltic movements of isolated segments of intestine.

2. Materials and methods

2.1. Tissue preparation

Male Syrian hamsters (5–7 weeks old) were anesthetized with diethyl ether and were exsanguinated via the carotid arteries. The abdominal cavity was opened and the entire ileum (~6 cm long) was removed and immersed immediately in physiological salt solution (PSS, see below) at room temperature. The contents of the excised segment were flushed with PSS. The segment was cannulated at the oral and aboral ends and mounted horizontally in an organ bath (50 ml capacity) containing PSS. The PSS in the bath was oxygenated by bubbling with a 95% O_2/5% CO_2 gas mixture and maintained at 36±1 °C to eliminate any effects of hypoxia and abnormal temperature. Tissues were equilibrated for ~20 min before experiments were undertaken. All procedures of experiments and animal handling were performed in accordance with the Gifu University Animal Care and Use Committee and the Japanese Department of Agriculture guidelines.

2.2. Experimental preparation

To measure the volume of liquid propelled from the isolated ileum, we modified the methods of Tonini et al. (1981), Smith and McCarron (1998) and Smith and Robertson (1998). As shown in Fig. 1, the oral and aboral ends of the segment were attached to cannulas that were fixed horizontally within the organ bath; this prevented longitudinal shortening of the segment. The cannula attached to the oral side (inner (i.d.) and outer (o.d.) diameters were

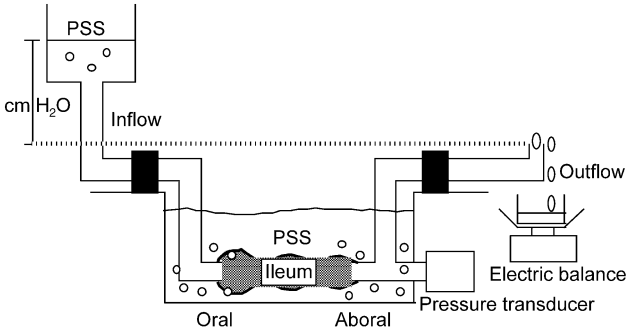

Fig. 1. Experimental preparation. An isolated segment of hamster ileum was cannulated at each end and placed in a bath that contained oxygenated, warmed physiological salt solution (PSS). Both ends of the segment were fixed to prevent longitudinal shortening of the ileum. PSS was infused by raising the inflow bottle that was connected to the cannula attached to the oral end of the segment. The volume of liquid outflow and changes in intraluminal pressure were measured using an electric balance and a pressure transducer, respectively.

2 and 3 mm, respectively) was connected to a bottle containing PSS that was oxygenated and warmed. The cannula attached to the aboral side (i.d.: 4 mm, o.d.: 6 mm) was connected to a plastic tube to collect the outflow of PSS. The volume of the PSS outflow from the aboral side was measured with an electric balance. A pressure transducer (MP5100, Baxter, Tokyo, Japan) was connected to the outflow line via a T connector and changes in intraluminal pressure were recorded with a chart recorder (RTA-1000, Nihon Kohden, Tokyo, Japan).

The peristaltic reflex of the isolated ileum was initiated by increasing the intraluminal pressure. The pressure stimulation was applied by gradually raising the height of the bottle containing PSS that was connected to the oral end of the ileum (about 1 cm/s up to 3 cm). The strength of the pressure against the intestinal wall was represented as liquid pressure (cm H_2O), which was equivalent to the height between the level of liquid within the bottle connected to the oral end of the ileum and the end of the outflow tube connected to the aboral end (Fig. 1). Peristaltic waves were expressed at least for 70 min in the presence of 3.0 cm H_2O of intraluminal pressure stimulation. Gross inspection revealed at least three different peristaltic movements of the isolated ileum; for convenience, we designed these movements as complete peristalsis, incomplete peristalsis, and other intestinal motility (see Results for details).

2.3. Solutions and drugs

The PSS used in this study had the following composition (in mM): NaCl 135.9, KCl 2.68, $CaCl_2$ 1.8, $MgCl_2$ 1.0, NaH_2PO_4 0.41, $NaHCO_3$ 11.9, and glucose 5.6. Tetrodotoxin (Wako, Osaka, Japan), atropine sulfate monohydrate (Wako), hexamethonium chloride (Wako), acetylcholine chloride (Sigma, St. Louis, MO, USA), N^G-nitro-L-arginine methyl ester (L-NAME) (Sigma), and L-arginine hydrochloride (Wako) were dissolved in distilled water. N^G-nitro-L-arginine (L-NNA) (Sigma) and N^G-nitro-D-arginine (D-NNA) (Sigma) was dissolved in 0.1 M HCl solution.

2.4. Statistical analysis

Data are presented as mean±S.E.M. Sample sizes (n) represent the number of experiments performed using different tissue

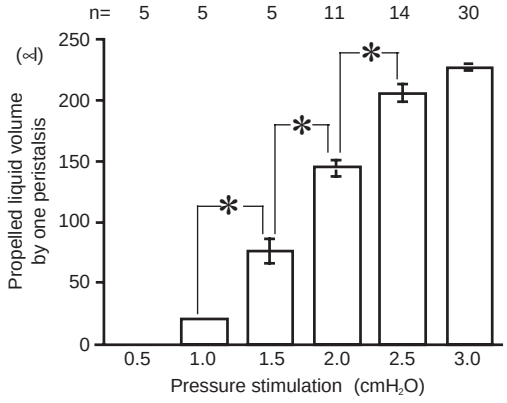

Fig. 2. Effects of intraluminal pressure stimulation on intermittent liquid propulsion owing to peristaltic movement. Intraluminal pressure was increased gradually by increasing the height of the PSS within the inflow bottle connected to the oral end of the isolated segment of the ileum. The volume of liquid propulsion owing to one peristaltic movement was measured. The pressure stimulus is represented as liquid pressure (cm H_2O). Values are mean ± S.E.M. *$P<0.05$. n indicates the number of preparations in each experimental group.

preparations from different hamsters. Data were analyzed using analysis of variance (ANOVA) and Duncan's multiple range test was used for the post hoc analysis. A P value of less than 0.05 indicated statistical significance.

3. Results

3.1. Characterization of peristaltic movements elicited by intraluminal pressure stimulation

An increase in intraluminal pressure evoked visible peristaltic movements in the isolated segment of ileum. The mean threshold pressure for the induction of ileal movements was ~1.0 cm H_2O. In gross inspection, two types of peristaltic movement were observed following suprathreshold intraluminal pressure stimulation (3.0 cm H_2O), namely, complete and incomplete peristalsis. In complete peristalsis, contraction of the circular muscle occurred at the oral end of the ileal segment and propagated completely to the aboral end. By contrast, in incomplete peristalsis, contraction of the circular muscle was either initiated in the middle part of the ileal segment or was not propagated completely to the aboral end. Complete peristalsis was observed in all preparations ($n=40$). Incomplete peristalsis was evident in 13 preparations. In addition to these two types of peristalsis, irregular segmentation was categorized for convenience as 'other intestinal motility' and was observed only in seven preparations.

3.2. Relationship between intraluminal pressure stimulation and propelled liquid volume

Intraluminal liquid was propelled from the oral to the aboral end intermittently following complete peristaltic movements evoked by application of intraluminal pressure. Fig. 2 illustrates the relationship between the intraluminal pressure versus the volume of the liquid propulsion following one complete peristaltic movement. Stimulation of the ileum with subthreshold pressure for the initiation of visible peristaltic movement (0.5 cm H_2O) did not promote the intermittent liquid propulsion from the aboral end of the segment, whereas pressure greater than 1.0 cm H_2O brought about the liquid propulsion. The volume of liquid propulsion resulting from one complete peristaltic movement increased as the stimulation pressure increased (Fig. 2).

3.3. The effects of tetrodotoxin, atropine, hexamethonium and acetylcholine on propelled liquid volume

To determine whether the intermittent liquid propulsion following peristaltic movements is associated with neuronally controlled peristalsis, the effects of tetrodotoxin, atropine and hexamethonium were examined. The drugs were applied 5 min after intraluminal pressure stimulation was applied (3.0 cm H_2O) and the peristaltic waves were elicited. Addition of tetrodotoxin (100 nM), atropine (100 nM) or hexamethonium (10 μM) to the organ bath completely eliminated the peristaltic movement elicited by increased intraluminal pressure; the intermittent liquid propulsion was also abolished (3 preparations for each; data not shown). Further increments in intraluminal pressure (4–6 cm H_2O) failed to promote any peristaltic movement in drug-treated preparations. These data indicated that the intermittent liquid propulsion in this experimental preparation was related exclusively to the neuronal peristaltic activity of the ileum. On the other hand, application of acetylcholine facilitated the volume of liquid propelled by one complete peristalsis; however, this increase was not significant (221 ± 6.5 versus 232 ± 10.2 μl) (5 preparations).

3.4. Comparison of propelled liquid volume and changes in intraluminal pressure

To assess the appropriateness of the propelled liquid volume as a parameter to evaluate the peristaltic activity of the isolated ileum, it was compared with the changes in intraluminal pressure. In this series of experiments, the pressure stimulus was 3.0 cm H_2O and the motility of the ileum was categorized as complete peristalsis, incomplete peristalsis, or other intestinal motility. Representative recordings of changes in intraluminal pressure are presented in Fig. 3. A transient increase in intraluminal pressure corresponded

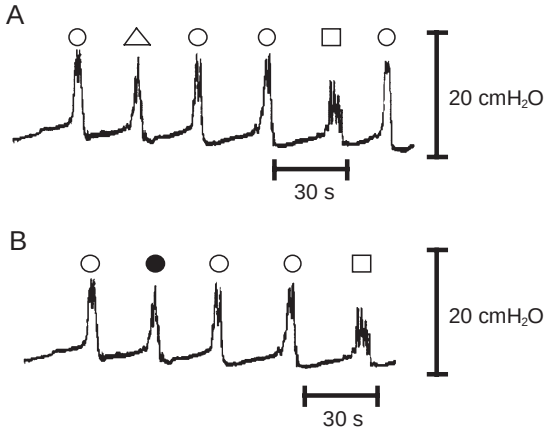

Fig. 3. Representative recordings of changes in intraluminal pressure produced by peristaltic waves. Two representative recordings of changes in intraluminal pressure in two different preparations (A and B). The pressure stimulus was 3.0 cm H_2O. Open circles, open triangles, open squares, and closed circles indicate pressure changes owing to complete peristalsis, incomplete peristalsis, other intestinal motility, and antiperistalsis, respectively.

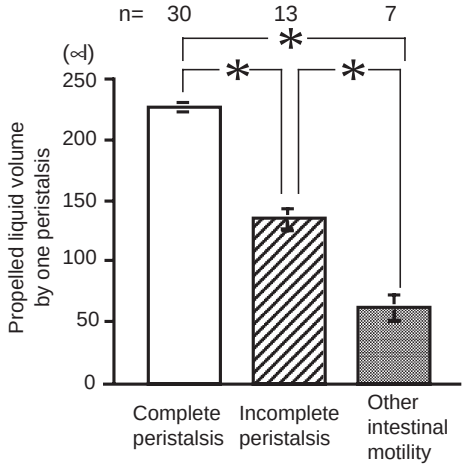

Fig. 4. Liquid propulsion resulting from three types of peristaltic movement. Bar graphs show the volume of liquid propelled by one peristaltic movement (μl) for each type of peristaltic movement. Values are mean±S.E.M. *$P<0.05$: comparison was made among three types of peristaltic movement. *n* indicates the number of preparations in each experimental group.

to each complete peristaltic movement (Fig. 3, open circles). In the case of incomplete peristalsis (Fig. 3, open triangles), changes in intraluminal pressure were similar to those that occurred during complete peristalsis. In addition, the maximal amplitude of the pressure change induced by incomplete peristalsis (12.0±0.7 cm H_2O; 13 preparations) was not significantly different from that owing to complete peristalsis (12.2±0.9 cm H_2O; 30 preparations). Therefore, recordings of intraluminal pressure could not be used to distinguish between complete and incomplete peristaltic movements. The pressure changes that resulted from the other intestinal motility (Fig. 3, open squares) were substantially different from those related to peristaltic movements, but the amplitude of such pressure changes (10.8±1.4 cm H_2O; 7 preparations) was not significantly lower than the pressure changes associated with the other types of peristalsis. By contrast, the liquid propulsion in response to incomplete peristalsis and other intestinal motility was significantly lower than that resulting from complete peristalsis (Fig. 4). In a few preparations, antiperistalsis was observed (intraluminal liquid was propelled from the aboral to the oral end of the segment). In such cases, there was no propulsion of intraluminal liquid from the aboral end, although typical intraluminal pressure changes during antiperistalsis (Fig. 3, closed circles) resembled those that occurred during anterograde peristalsis.

3.5. Application of the propelled liquid volume method

To assess the validity of the propelled liquid volume method, we applied this method to re-examine the contribution of NO to peristaltic movement. The drugs were applied 5 min after intraluminal pressure stimulation was applied (3.0 cm H_2O) and the peristaltic waves were elicited. Consistent with the previous findings that NO inhibits peristaltic activity in the ileum (Suzuki et al., 1994), we found that the NO synthase (NOS) inhibitor, L-NNA (10 μM), significantly increased the frequency of peristaltic waves (Fig. 5). Interestingly, the volume of liquid propelled by one peristaltic movement was reduced in the presence of L-NNA; D-NNA (10 μM) was not effective (Fig. 6). The inhibitory effect of L-NNA on the amount of the propelled liquid was abolished by pre-application of L-arginine (5 mM) (Fig. 6), indicating that the effects of L-NNA were specific to NOS. Considering that the amount of liquid that flowed from the bottle containing PSS in each min at 3 cm H_2O pressure without mounting an ileal segment was almost constant (298±5.3 μl/min), the inhibitory effect of L-NNA on the propulsion volume could reflect the attenuation of peristaltic activity. The total volume of liquid propelled per 5 min was dose-dependently inhibited by L-NNA. Application of 10 and 100 μM of L-NNA significantly reduced the propelled volume from 1463±149 μl (control; 12 preparations) to 1089±194 μl (10 μM; 6 preparations) and 596±35 μl (100 μM; 6 preparations; $p<0.05$), respectively. Similar results were obtained when L-NAME was used as a NOS inhibitor (data not shown).

4. Discussion

In this study, a simple method was devised to measure the volume of liquid propelled from the aboral end of isolated ileum of the hamster. To be a functional index for peristalsis, the propulsion volume should be correlated with peristaltic movement. We have provided the following evidence that this is the case. First, an increase in the intraluminal pressure elicited intermittent liquid propulsion that coincided with peristaltic waves, whereas pressures that were below the threshold required to stimulate peristaltic movement did not produce any propulsion. Second, the inhibition of intrinsic neuronal activity by drugs interrupted peristaltic movements and completely eliminated the intermittent liquid propulsion. We found also that the measurement of the propelled liquid volume had an advantage over recording of changes in intraluminal pressure, as different propulsion volumes were clearly associated with different

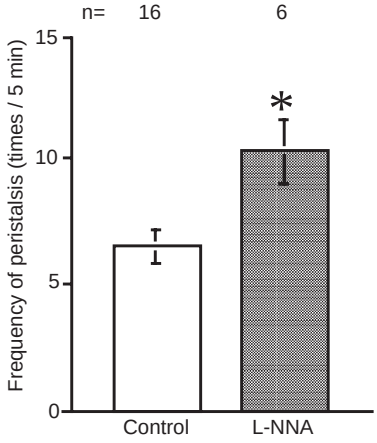

Fig. 5. Effects of N^G-nitro-L-arginine (L-NNA) on frequency of peristalsis. Bar graphs show the frequency (number of peristaltic movements per 5 min) of peristalsis in the control condition and during the application of 10 μM L-NNA. The drugs were applied 5 min after intraluminal pressure stimulation was applied (3.0 cm H_2O) and the peristaltic waves were elicited. Values are mean±S.E.M. *$P<0.05$ compared to control. *n* indicates the number of preparations in each experimental group.

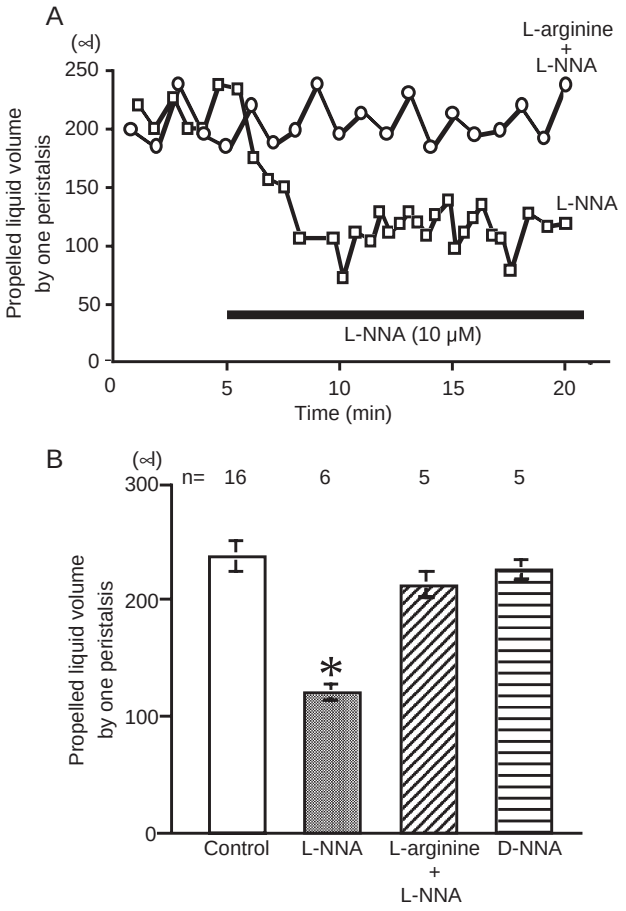

Fig. 6. Effects of L-NNA on volume of liquid propelled by peristaltic movements. Curves show the time course of changes in volume of liquid propelled (μl) owing to one peristaltic movement before and after application of 10 μM L-NNA with (open circles) or without (open squares) pre-treatment of 5 mM L-arginine in an ileal segment (A). Bar graphs show the volume of liquid propelled (μl) owing to one peristaltic movement in the control condition and during the application of 10 μM L-NNA, 5 mM L-arginine and 10 μM L-NNA or 10 μM D-NNA (B). Values are mean ± S.E.M. $*P<0.05$ compared to control. *n* indicates the number of preparations in each experimental group.

types of peristaltic movement, which were not apparent from changes in intraluminal pressure. Moreover, the propelled liquid volume method revealed a role of NO in regulation of peristalsis. Collectively, our results suggest that measurement of the volume of liquid propelled from isolated ileum may be a useful method to evaluate peristalsis.

To prove whether the measurement of propelled liquid volume from ileal segment is appropriate as a functional index of peristalsis, we have provided the following findings. An application of intraluminal pressure elicited intermittent liquid propulsion that coincided with peristaltic waves, whereas pressures that were below the threshold required to stimulate peristaltic movement did not produce the intermittent liquid propulsion. The propelled liquid volume was increased as the applied pressure increased. Considering that the activation of intrinsic neuronal networks and subsequent smooth muscle contraction depend on the strength of stretch stimuli (Smith and McCarron, 1998; Smith and Robertson, 1998; Spencer et al., 1999; Grider, 2003a), the findings may support the view that the liquid propulsion is correlated with neuronally controlled peristalsis. This was further supported by evidence that the inhibition of intrinsic neuronal activity by tetrodotoxin, atropine or hexamethonium, which inhibited peristaltic movements through blocking the neuronal pathway or neuro-muscular coupling, completely eliminated the pressure-induced intermittent liquid propulsion that coincided with peristaltic waves, even when the applied pressure was high enough to induce maximal peristaltic movement in the absence of these drugs. Taking into the consideration the major function of peristalsis, i.e. propulsion of intraluminal contents, measurement of the propelled intraluminal volumes by peristaltic movements may be valuable to evaluate roles of the neuronal network controlling the peristaltic activity.

Changes in the intraluminal pressure of the ileal segment are the most widely used parameter to analyze neuronal network controlling the intestinal motility (Trendelenburg, 1917; Waterman and Costa, 1994; Waterman et al., 1994a; Smith and Robertson, 1998). For instance, an inhibitory role of intrinsic nitrergic neurons on peristaltic movement has been demonstrated by measuring the intraluminal pressure in the presence or absence of NOS inhibitors (Waterman and Costa, 1994; Waterman et al., 1994a). Intraluminal pressure is sensitive with respect to detecting the movement of a segment of intestine because the strength of contractile movement can be converted to the increment of intraluminal pressure (Trendelenburg, 1917; Smith and Robertson, 1998). In the present study, however, we have shown that changes in intraluminal pressure resulting from complete peristalsis were indistinguishable from that by incomplete (partial) peristalsis. The maximal amplitude of the pressure change induced by other intestinal motility also was not significantly different from that by other types of peristalsis. These findings may indicate that a slight change in the activity of intrinsic neuron, which is reflected by change in the movement, after application of drugs, would be overlooked when the parameter alone is used in the pharmacological studies. Moreover, both anterograde and retrograde peristaltic movements produced similar changes in intraluminal pressure, even though different neuronal network would be responsible for these movements. Therefore, intraluminal pressure alone would appear to be inadequate for the assessment of neuronal basis for peristaltic movement. By contrast, the volume of liquid propelled by the isolated ileum was sensitive enough to detect relatively slight changes in the movement, i.e. complete and incomplete peristalses. Furthermore, it is to be noticed that no outflow occurred in response to antiperistalsis. These observations demonstrate that measurement of the propelled liquid volume

would be an appropriate parameter to estimate roles of several specific neurons in controlling the peristaltic movement. In fact, in the present study, this method revealed a regulatory role of nitrergic neurons in peristalsis (see below).

It is generally accepted that nitrergic neurons play inhibitory roles in peristaltic movement. This view was derived from lines of evidence that L-NNA increased the frequency of peristalsis and the velocity of propulsion, shortened the latency of peristaltic wave initiation and reduced the threshold volume needed to initiate peristalsis in the isolated ileum or colon (Ciccocioppo et al., 1994; Suzuki et al., 1994; Waterman and Costa, 1994). The enhancement of peristalsis by NOS inhibitor could be explained on the basis of evidences that activation of nitrergic neurons and exogenous application of NO inhibit release of excitatory neurotransmitters (acetylcholine and tachykinins) (Lefebvre et al., 1992; Hryhorenko et al., 1994; Smith and McCarron, 1998; Smith and Robertson, 1998; Onori et al., 2003). In accordance with this, we also observed that L-NNA increases the frequency of peristaltic movements. However, the unexpected finding in the present study was that L-NNA decreased the volume of liquid propelled by one peristaltic movement or per 5 min. This suggests that nitrergic neurons might be necessary elements for completing peristalsis. Considering that NO is a potent relaxant of gastrointestinal smooth muscle (Mashimo and Goyal, 1999; Matsuyama et al., 1999, 2002; Ogulener et al., 2001), this parameter confirmed that intrinsic nitrergic neurons might be essential components of the descending relaxation pathway that regulates the intestinal peristaltic reflex because descending relaxation would bring about an increase in the volume of liquid propelled. The method used in this study was constructed to prevent longitudinal shortening of the segment (see Materials and methods), a role of NO in the movements of circular muscle for descending relaxation seemed to be evaluated especially. To our knowledge, such functional demonstration has rarely reported to show a contribution of intrinsic nitrergic neurons to the descending relaxation pathway, although it has been proposed on the basis of histological studies (Olsson and Holmgren, 2001; Takahashi, 2003).

It should be noted, however, that we do not advocate the exclusion of recordings of intraluminal pressure. Because the propelled liquid volume and other parameters such as intraluminal pressure could be assessed simultaneously in the same specimens, the combination of measurements of the propelled liquid volume and other established parameters might be a useful approach to elucidate the nature of the neuronal regulation of peristalsis.

To conclude, in the present study we found that the volume of liquid propelled from the isolated ileum is correlated with neuron-regulated peristalsis. Measurement of the volume of propelled liquid is a simple and valuable method by which neuronal regulation of intestinal peristalsis can be investigated.

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