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Abstract

In the extrinsically denervated smooth muscle esophagus of the hen anesthetized with urethane (1 g/kg, i. m.), it was studied whether peptidergic neurons in the intramural plexus are involved in the intrinsic reflex. Ascending and descending contractions, and descending relaxation were induced by electrical stimulation of a narrow segment of the esophagus. Naloxone (1 microM), desensitization to substance P (0.3 microM) and spantide (20 microM) inhibited the ascending and descending contractions, respectively. The degree of the inhibition of the contractile response by a combination of naloxone and substance P was nearly the same as that by a single administration of naloxone or substance P. The ascending and descending contractions were reduced to one-third of the control by hexamethonium (100 microM) and abolished by atropine (10 microM). The descending relaxation was abolished after desensitization to vasoactive intestinal peptide (0.3 microM). Taken together the results suggest that in the hen's esophagus, opioid- and substance P-containing neurons in the intramural plexus may act as preganglionic neurons of cholinergic motor neurons in the ascending and descending excitatory pathways and that vasoactive intestinal peptide-containing neurons are involved in the descending inhibitory pathway.

KEYWORDS: intrinsic reflex, peptidergic neuron, opioid, substance P, hen's esophagus

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Intrinsic Reflexes Mediated via Peptidergic Neurons in the Smooth Muscle Esophagus of the Hen

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In the extrinsically denervated smooth muscle esophagus of the hen anesthetized with urethane (1g/kg, i. m.), it was studied whether peptidergic neurons in the intramural plexus are involved in the intrinsic reflex. Ascending and descending contractions, and descending relaxation were induced by electrical stimulation of a narrow segment of the esophagus. Naloxone (1μM), desensitization to substance P (0.3μM) and spantide (20μM) inhibited the ascending and descending contractions, respectively. The degree of the inhibition of the contractile response by a combination of naloxone and substance P was nearly the same as that by a single administration of naloxone or substance P. The ascending and descending contractions were reduced to one-third of the control by hexamethonium (100 μM) and abolished by atropine (10 μM). The descending relaxation was abolished after desensitization to vasoactive intestinal peptide (0.3 μM). Taken together the results suggest that in the hen's esophagus, opioid- and substance P-containing neurons in the intramural plexus may act as preganglionic neurons of cholinergic motor neurons in the ascending and descending excitatory pathways and that vasoactive intestinal peptide-containing neurons are involved in the descending inhibitory pathway.

Key words : **intrinsic reflex, peptidergic neuron, opioid, substance P, hen's esophagus**

Peristalsis in the smooth muscle portion of the esophagus, corresponding to the secondary peristalsis during swallowing, is mediated by an intramural neuromuscular mechanism (1, 2), suggesting that an intrinsic reflex similar to that in the intestine is essential for initiation of peristalsis in the smooth muscle esophagus. In the intestine, ascending excitatory and descending inhibitory reflexes are elicited by mechanical, chemical and electrical stimulation of the mucosa (3-5). The motor neurons of the ascending excitatory and descending inhibitory pathways may be cholinergic excitatory and non-adrenergic inhibitory neurons, respectively. It has further been suggested that neurons containing opioid, substance P and vasoactive intestinal peptide may be involved in these pathways, probably as interneurons (5-8). These peptidergic neurons were immunohistochemically demonstrated in the intramural plexus of the esophagus (9-11) as well as in the small intestine. Muscle layers of the esophagus consist of smooth muscles along the whole length in the hen. In mammalian species, however, only the lower one-third of the esophagus consists of

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smooth muscles (*e. g.* cats, monkeys and human beings) or the whole length of the esophagus consists of striated muscles (*e. g.* rats, guinea pigs and dogs). Nakayama (12) reported previously that the intrinsic reflex was produced by mechanical and chemical stimulations of the mucosa in the hen's esophagus, and that motor neurons in the excitatory pathways may be cholinergic. However, the action of peptidergic neurons such as opioid-, substance P- and vasoactive intestinal peptide-containing neurons on the intrinsic reflexes has not been determined.

In the present experiments, therefore, whether intramural peptidergic neurons are involved in the intrinsic reflex in the smooth muscle esophagus was studied in the hen.

Materials and Methods

Seventeen white leghorns weighing 1.5 to 2.0 kg were anesthetized with an intramuscular injection of urethane (1 g/kg). Experiments were carried out under artificial respiration. As shown in Fig. 1, after midline incision of the neck, the esophagus was exposed as far as the crop, and two balloons were introduced into the lumen of the esophagus 3 and 13 cm anal to the pharynx, respectively. To elicit an intrinsic reflex by electrical stimulation of the intramural nervous system (4, 5), a bipolar platinum ring electrode was introduced into the lumen at the midpoint between the balloons through a small incision made in the wall and attached to the mucosal surface. Electrical stimulation of 5–20 Hz, 0.5 msec and 4–10 V was applied for 30 sec. Motility of the esophagus was recorded with a low pressure transducer through a water manometer on a pen-recorder. Intraballoon pressure of the relax phase was set at 3 cmH_2O. A pool for holding Tyrode's solution was made around the esophagus using incised neck skin, and the drugs were administered in the pool. The drugs were washed out by changing the pool solution to fresh Tyrode's solution. Tyrode's solution had the following composition (mM): NaCl, 136.9; KCl, 2.7; $CaCl_2$, 1.8; $MgCl_2$, 2.5; $NaHCO_2$, 11.9; NaH_2PO_4, 0.4; and glucose, 5.6.

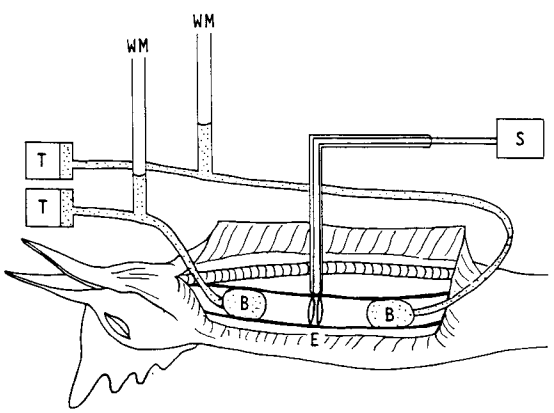

Fig. 1 Experimental arrangements. B: thin rubber balloons, T: pressure transducers to record motility of the segments oral and anal to the stimulated segment, E: bipolar platinum ring electrode in contact with the mucosal surface, S: stimulator, WM: water manometers.

Extrinsic nerve denervation of the esophagus was done by cutting the laryngoesophageal, esophagus and vagus nerves at the level of the larynx, bilaterally. In these birds no response was produced by stimulating the central cut end of one of the esophageal branches of the vagus nerve. In contrast, a peristaltic reflex was induced by afferent stimulation of the esophageal branch of the vagus in the innervated esophagus. It was, therefore, considered that the response evoked by stimulation of the mucosal surface should be intrinsic in origin.

Drugs used were urethane (Nacarai Tesque, Kyoto, Japan), atropine sulfate (Merck Co., Darmstadt, West Germany), hexamethonium bromide (Sigma Chem. Co., St. Louis, MO. USA), naloxone hydrochloride (Sigma Chem. Co.), substance P (Peptide Institute, Minoh, Japan), [D-Arg^1, D-Trp^{7,9}, Leu^{11}] -substance P, spantide (Peptide Institute), vasoactive intestinal peptide (Peptide Institute) and capsaicin (Sigma Chem. Co.). Final concentrations of the drugs administered were similar to those effective on the guinea-pig isolated ileum.

Statistical comparisons were made using a standard paired Student's *t*-test to test for the significance of the drug effect on the reflex responses. All the data are expressed as the mean ± SEM of the percentages of the response height to the control.

Results

Electrical stimulation caused contractions of both oral and anal segments of the esophagus (ascending and descending contractions). The amplitude of the ascending contraction was always larger than the descending one. Because a

Table 1 Effects of various drugs on ascending and descending contractions evoked by electrical stimulation of intramural nerves

Drugs	Ascending contraction (%)	n	Descending contraction (%)	n
Naloxone (1 μM)	46.9 ± 8.5	9 (6)	53.7 ± 16.2	9 (6)
Substance P (0.3 μM)	65.0 ± 4.3	9 (6)	55.0 ± 8.3	9 (6)
Naloxone (1 μM) + substance P (0.3 μM)	43.6 ± 14.4	6 (4)	46.9 ± 13.6	6 (4)
Spantide (20 μM)	23.3 ± 0.8	5 (2)	33.3 ± 0.5	5 (2)
Atropine (10 μM)	8.7 ± 5.4	5 (5)	10.9 ± 7.3	6 (6)
Hexamethonium (100 μM)	27.2 ± 9.4	10 (6)	35.2 ± 6.2	9 (5)

The data are expressed as the mean ± SEM of the percentage of the amplitude of contractile responses to the control. n indicates the number of trials : The number in parentheses shows the number of birds. All the drugs inhibited both ascending and descending contractions, significantly (at least $p < 0.05$).

maximal response was produced at a frequency of 10 Hz, 10-Hz-stimulation was mainly used in the following experiments. Both ascending and descending contractions were abolished by tetrodotoxin (0.3 μM), an agent that blocks action potentials in nerves. In only one of the 17 birds, an inhibition of spontaneous motility was induced in the anal segment by the stimulation (descending relaxation).

Drug effects on the ascending and descending contractions are summarized in Table 1. There was no significant difference between the effects on the ascending and descending contractions. Substance P (0.3 μM), at a concentration which desensitizes substance P receptors, and naloxone (1 μM) significantly reduced contractions in both oral and anal segments. An example of the naloxone effect on the evoked response is shown in Fig. 2. In the presence of both naloxone and substance P, the ascending and descending contractions were reduced to about half of the control. There were no significant differences between the effects of naloxone and substance P, or between the individual effects of naloxone and substance P and the effect of both drugs in combination.

Contractions induced in the oral and anal segments were reduced to one-third or less of the control by spantide (20 μM), a specific antagonist of substance P. After replacement of Tyrode's solution the responses recovered.

Capsaicin (0.3 mM), which blocks the antidromic action of extrinsic sensory fibers containing peptide substances on the cholinergic motor neurons and intestinal smooth muscles (13), had

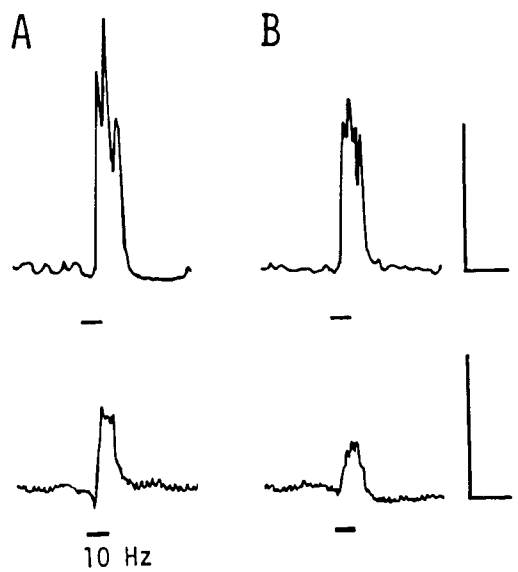

Fig. 2 Effect of naloxone (1 μM) on ascending (upper trace) and descending (lower trace) contractions (B). Bars under each trace show the period of electrical stimulation (10 HZ, 0.5 msec, 5 V). A: control, Cal.: 1 cmH_2O and 1 min.

no effect on the spontaneous motility or on the ascending and descending contractions (Fig. 3).

The descending relaxation was blocked after administration of vasoactive intestinal peptide (0.3 μM) which caused desensitization to its receptors (Fig. 4).

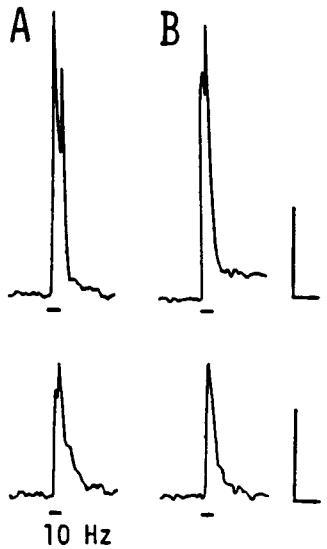

Fig. 3 Effect of capsaicin (0.3 μM) on ascending (upper trace) and descending (lower trace) contractions (B). Bars under each trace show the period of electrical stimulation (10 Hz, 0.5 msec, 5 V) A: control, Cal.: 1 cmH_2O and 1 min.

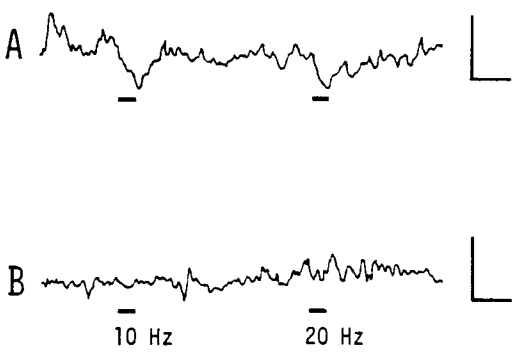

Fig. 4 Effect of vasoactive intestinal peptide (0.3 μM) on descending relaxation (B). The left-hand bars under each trace in A and B show the period of electrical stimulation at 10 Hz, 0.5 msec and 4 V, and the right-hand bars show the period of stimulation at 20 Hz, 0.5 msec and 4 V. A: control, Cal.: 0.4 cmH_2O and 1 min.

The ascending and descending contractions were reduced to one-third by administration of hexamethonium (100 μM). Both contractions resistant to hexamethonium were abolished by successive administration of atropine (10 μM). Atropine also abolished the ascending and descending contractions by itself.

Discussion

Electrical stimulation of intrinsic nerves of a narrow segment of the hen's esophagus caused the same patterns of responses as demonstrated by Nakayama (12) : Chemical and mechanical stimulations of the mucosa such as 0.1 N HCl and stroking elicited ascending and descending contractions. It was obvious in the present study that both responses were elicited by the intrinsic reflex mediated through the intramural plexus since the ascending and descending contractions were abolished by tetrodotoxin but unaffected by capsaicin.

In the present experiments, naloxone, an opioid antagonist, spantide, a substance P antagonist, and substance P desensitization greatly reduced ascending and descending contractions evoked by activation of intrinsic nerve. Both responses were sensitive to atropine, but partly sensitive and partly resistant to hexamethonium. These results suggest that peptidergic neurons containing opioid and substance P and cholinergic neurons in the excitatory pathways play a role as preganglionic neurons of cholinergic motor neurons. This idea is supported by the previous observations that opioid and substance P neurons exist in the myenteric plexus of the esophagus (10, 11). It has been suggested that substance P and opioid administered exogenously or myenteric neurons containing these peptides cause atropine-sensitive but hexamethonium-resistant cholinergic contractions in the canine small intestine (5, 14, 15).

Substance P administered exogenously may enhance stimulus evoked contractions of the

smooth muscle esophagus by potentiating cholinergic motor neurons or smooth muscle directly (11). However, this peptide causing the ascending and descending contractions may act on cholinergic motor neurons but not on the smooth muscles since atropine abolished reflex contractions.

No significant differences were observed among the inhibitions of the evoked contractions in the presence of naloxone or after desensitization to substance P and in the presence of both naloxone and substance P (at a concentration which would desensitize substance P receptors). Thus, it is probable that these peptidergic neurons connect sequentially with cholinergic neurons in the excitatory pathways.

Vasoactive intestinal peptide-containing neurons, which are found in the myenteric plexus of the esophagus (9) and may act to cause an inhibition of the lower esophageal sphincter tone (14), may be involved in the descending inhibitory pathway since desensitization to vasoactive intestinal peptide inhibited the descending relaxation. It is probable that this inhibitory reflex plays a role in attennuating the descending contraction. Thus, the descending contraction may always be smaller than the ascending contraction.

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