Smooth Muscle Excitatory Substances from Remak Nerve of the Chicken and a Comparison of Their Pharmacological and Chemical Properties with Substance P

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Abstract—Active substances extracted from the Remak nerve of the chicken were subjected to chromatographic and electrophoretic separation followed by bioassay of contracting activities on the longitudinal muscle of the guinea-pig ileum (LMGPI) and on the isolated whole chick rectum (WCR). Gel filtration profiles on a Sephadex G-50 column showed two peaks of LMGPI-contracting activity and of WCR-contracting activity. No difference was seen in the enzymatic destruction between the LMGPI-contracting activity and substance P. Their similarities were also indicated by the parallelism of their elution curves in the gel chromatography on Sephadex G-25, their equal stability in acid solutions, and comparable antagonism and inhibition of the contractile effects on LMGPI by substance P antagonists and after desensitization of substance P receptors. Ion exchange chromatography revealed the existence of two main substances responsible for the LMGPI-contracting activity. One of them eluted at the same position as that for substance P, but differed in immunoreactivity and electrophoretic mobility from substance P. The WCR-contracting activity differed from the LMGPI-contracting activity in that it was pepsin-resistant and carboxypeptidase A-susceptible, and it eluted at a different position during ion exchange chromatography. It seems likely that the LMGPI-contracting activity in the extracts is attributed to a substance P-family of peptides, but the WCR-contracting activity is due to another substance of a peptide nature.

The rectum of the chicken receives an extrinsic innervation of non-adrenergic, non-cholinergic (NANC) neurones via the nerve of Remak which is a ganglionated nerve trunk running along the alimentary canal from the cloaca to the duodenum. NANC excitatory junction potentials have been recorded intracellularly from smooth muscle cells of the rectum in response to electrical stimulation of the extrinsic nerve or intramural nerves (1, 2). Kanazawa et al. (3) reported that cell bodies of NANC neurones are located in the ganglia associated with the nerve of Remak. However, the neurotransmitter is not known yet. In our previous study (4), crude extracts which had activity in contracting the longitudinal muscle strip of the guinea-pig ileum (LMGPI) and isolated rectum of young chick (WCR), were obtained from tissue samples of the Remak nerve. Approximately half of the contracting activity was attributable to acetylcholine. The remainder was not mediated by histamine, serotonin, angiotensin II, ATP or prostaglandins (E₁, E₂ and F₂α), but its susceptibility to a proteolytic enzyme, pepsin, suggested that it might be due to some substance of peptide nature. Moreover, gel filtration profiles of the extracts showing contractile effects on LMGPI and WCR...
suggested the presence of at least two active substances, one substance having a much higher contracting potency in LMGPI than in WCR, and the other having a reverse relation in contracting potency.

In an attempt to investigate further, we have tried further chromatographic and electrophoretic separation followed by bioassay of contracting activities on LMGPI and WCR, and we compared chemical and pharmacological properties of the LMGPI-contracting activity and WCR-contracting activity with one another and with those of substance P, since the peptide has been suggested to be involved in non-cholinergic excitatory synaptic transmission in sympathetic ganglia of the guinea-pig (5, 6).

Materials and Methods

Rectal regions of the chicken intestines together with Remak nerves were obtained within 20 min after slaughter and were kept in ice-cold Tyrode solution containing 20 μM indomethacin. Remak nerves were dissected within a few hours. Crude extracts of the tissue samples were prepared as described in the previous paper (4). The tissues were blotted dry, weighed, placed in a glass tube containing ice-cold 0.01 M HCl solution, heated at 100°C for 5–7 min, and homogenized. After centrifugation at 10000 × g at 4°C for 20 min, aliquots of the supernatant were defatted by washing 3 times with petroleum ether or ethyl acetate, lyophilized and stored in a deep freezer at -20°C.

Gel chromatography and ion exchange chromatography: The lyophilized extract was dissolved in 0.1 M acetic acid and applied to a Sephadex G-25 column (1.6×70 cm) or a Sephadex G-25 column (1.2×55 cm) equilibrated with the same acid solution. Elution was made with 0.1 M acetic acid, and the eluates were collected in 2 or 4 ml fractions and lyophilized. The lyophilized extract in each fraction was dissolved in 0.2 or 0.4 ml distilled water, and its smooth muscle contracting activity was bioassayed on the longitudinal muscle of the guinea-pig ileum (LMGPI) and the isolated whole chick rectum (WCR). The active region was pooled and lyophilized. Elution was monitored by a UV monitor at 254 or 280 nm. The void volumes (Vo) of the columns were determined with blue dextran.

The lyophilized residue after the gel filtration on Sephadex G-25 was dissolved in 50 ml of 0.1 M acetic acid, and it was applied at room temperature to a column of sulfopropyl-Sephadex C-25 (0.8×10 cm) previously equilibrated with this acid solution. The column was washed with 20 ml of 0.05 M pyridine-acetate buffer (pH 5.5) and developed with a linear gradient which was generated by using 180 ml of 0.05 M pyridine-acetate (pH 5.5) in the mixing chamber and 180 ml of 2 M pyridine-acetate (pH 5.5) in the reservoir at a flow rate of 20 ml per hr. The column eluates were collected in 5 ml fractions. Aliquots of each fraction were lyophylized, dissolved in distilled water and used for bioassays.

Bioassay preparations: Young male chicks, less than 2 weeks after hatching, were killed by stunning and were bled out. The rectal region of the large intestine was isolated, flushed clean with Tyrode solution and suspended in a 2.5 ml polypropylene organ bath. Guinea-pigs of either sex, weighing 300–500 g, were killed by a blow on the neck and bled. The longitudinal muscle strip (5 cm long) was obtained from the proximal to the terminal 10 cm of the ileum (7) and suspended in a 2.5 ml polypropylene organ bath. The assay bath contained Tyrode solution composed of (mM) NaCl, 136.9; KCl, 2.7; NaH₂PO₄, 0.4; CaCl₂, 1.8; MgCl₂, 2.1; NaHCO₃, 11.9 and glucose, 5.6, aerated with air and kept at 30–32°C. Isometric tension changes of the assay tissues were recorded by a force-displacement transducer (Nihon Kohden, TB-612T) and a potentiometric recorder (Hitachi, 056). Extracts and drugs were injected using a micropipette into the bathing solution in a volume less than 0.02 ml, i.e., less than 1% of the bath volume, unless otherwise stated. The added substances were removed by replacing the bathing medium with the fresh solution.

Enzymatic studies: All enzyme preparations, such as pepsin (EC 3.4.23.1), trypsin (EC 3.4.21.4, Type I), α-chymotrypsin (Type II), carboxypeptidase A (EC 3.4.12.2, Type II), carboxypeptidase B (Type II), pyroglutamate aminopeptidase (EC 3.4.11.8) and leucine
aminopeptidase (EC 3.4.11.1, Type V) were purchased from Sigma and used without further purification. These enzymes, except for pepsin, were dissolved in 0.05 M Tris-HCl buffer (pH 7.8) and pepsin was dissolved in 0.01 M HCl, at the desired concentrations and stored in a deep freezer at −20°C until used. Lyophilized extracts were usually reconstituted with 0.05 M Tris-HCl buffer (pH 7.8) for testing susceptibility to enzymes and to chicken serum, but they were reconstituted with 0.01 M HCl for testing susceptibility to pepsin. Each enzyme solution was added to different aliquots of the extract to give the following respective final concentrations in the reaction mixture: pepsin, 33 μg/ml; α-chymotrypsin, 20 μg/ml; trypsin, 20 μg/ml; carboxypeptidase A, 630 μg/ml; carboxypeptidase B, 250 μg/ml; pyroglutamate aminopeptidase, 8 μg/ml; and leucine aminopeptidase, 100 μg/ml. Incubation of the reaction mixture was made at 37°C for 30 or 60 min. Enzyme solutions boiled for 30 min were used as inactivated enzymes. Chicken serum was freshly prepared and added to an aliquot of the extract at the volume ratio of 1 to 4.

Stability: Extract solutions were boiled for 3 min with an equal volume of 2 M HCl or 2 M NaOH, neutralized and then used for bioassay.

Radioimmunoassay: The dried active material obtained after the gel filtration on Sephadex G-25 was dissolved in 1 ml of distilled water, and an aliquot of this solution was subjected to radioimmunoassay of substance P-like activity employing a rabbit antiserum directed towards bovine substance P (I 675, UCB-Bioproducts S.A.). Another aliquot in the same volume as that used for radioimmunoassay was bioassayed on LMGPI for its substance P-like activity.

Drugs: Drugs used were atropine sulphate (Nakarai), pyrilamine maleate (Sigma), methysergide hydrogen maleate (Sandoz), indomethacin (Sigma), leucine-enkephalin (Peptide Institute Inc.), synthetic bovine substance P (Peptide Institute, Inc.), [D-Pro², D-Trp⁷,⁹] substance P (Peptide Institute, Inc.) and [D-Arg¹, D-Pro², D-Trp⁷,⁹, Leu¹¹] substance P (Peptide Institute, Inc.). Indomethacin was dissolved in 0.05 ml of 95% ethanol and then diluted with distilled water to the desired concentrations. All other drugs were dissolved in distilled water at high concentrations and stored in a deep-freezer at −20°C. The stock solutions were diluted with distilled water to the desired concentration and then kept at ice temperature until used.

High voltage paper electrophoresis: High voltage paper electrophoresis was performed on Whatman No. 3 MM paper at 80 volts per cm using an immersed strip type apparatus. The buffer solution was pH 1.9, formic acid-acetic acid-water (20:80:900). The Whatman paper was washed with 20% pyridine and then 20% acetic acid for 20 days before use. Serine (5 μg), substance P and angiotensin II (45 μg each) were spotted on one side of the samples as appropriate standard amino acid and peptides, which were located by staining with 0.2% ninhydrin in acetone. Strips (1 cm in width) of the sample side of the paper were each eluted with 0.1 M acetic acid, and the contracting activity was bioassayed.

High performance liquid chromatography: High performance liquid chromatography was performed using a pump (CCPD, Tōyō Sōda) and a UV detector (UV-8000, Tōyō Sōda). The column used (4.6 mm × 25 cm) was TSK-GEL (ODS-120T, Tōyō Sōda) equilibrated with 25% CH₃CN in 0.05% TFA. Samples were injected in 50 μl of the column buffer, and fractions of 40 sec were collected at a flow rate of 1 ml/min. UV adsorbance at 220 nm of the column eluate was monitored. Aliquots of each fraction were lyophilized, dissolved in distilled water, and bioassayed.

Results

1. Gel filtration of Remak nerve extract on Sephadex G-50

Gel filtration profiles of Remak nerve extract with a Sephadex G-50 column are shown in Fig. 1. The curves were obtained by measuring UV absorbance of the eluate at 254 nm and its effects on the tension of the longitudinal muscle of the guinea-pig ileum (LMGPI) and of the isolated whole chick rectum (WCR), treated with atropine (0.2 μg/ml), pyrilamine (1 μg/ml) and methysergide (1 μg/ml). However, some extracts showed only weak or almost no
activity in contracting WCR. The peak activity in contracting WCR was seen to elute slightly after the peak activity in contracting LMGPI, but both lay before the main peak UV absorbance at 254 nm, confirming the earlier report of Komori et al. (4). The values of Ve/Vo for WCR-contracting activity and LMGPI-contracting activity were 2.33±0.06 (n=6) and 2.29±0.06 (n=5), respectively. Frequently, one additional minor peak of LMGPI-contracting activity was seen to elute at the void volume side of the main LMGPI-contracting component.

Biologically active fractions from the Sephadex G-50 column were pooled, lyophilized, reconstituted in distilled water and used as samples for the following experiments.

2. Mechanical responses of LMGPI and WCR to samples

The samples caused a contraction of LMGPI which was frequently preceded by a small and fast relaxation. The time to the peak tension ranged from a few seconds to 10 sec depending on the concentrations used. While in WCR, the sample-induced contraction developed without relaxation. The time required to reach the peak tension ranged from 5 sec up to 20 sec. When activated alumina was added to the sample solution at pH 9.0, and then removed by centrifugation, complete

Fig. 1. Gel filtration profiles of Remak nerve extract on a Sephadex G-50 column. The curves show UV absorption at 254 nm of the eluate (solid line) and its contractile effects on the longitudinal muscle of the guinea-pig ileum (O······O) and isolated whole chick rectum (●······●) pretreated with atropine (0.2 µg/ml), pyrilamine (1 µg/ml) and methysergide (1 µg/ml). Column size, 1.6×70 cm; fraction size, 4 ml; eluent, 0.1 M acetic acid; flow rate, 40 ml per hr. Fractions were lyophilized; then each residue was dissolved in 0.4 ml distilled water, and the contracting activity was measured.

Fig. 2. Comparison of contractile effects of Remak nerve extract (ERN, o) on the longitudinal muscle of the guinea-pig ileum (LMGPI) before and after treatment with alumina. A, Responses of LMGPI with normal tone; B, those of LMGPI with tone increased by 4 ng/ml substance P (SP, ↑). In A and B, a, ERN before treatment with alumina; b, ERN after treatment with alumina. Calibration of 0.25 g for A and that of 0.5 g for B. The active material obtained after gel filtration on Sephadex G-50 was used as ERN, and the experiment was performed on the muscle treated with atropine (0.2 µg/ml), pyrilamine (1 µg/ml) and methysergide (1 µg/ml). Note lack of the relaxing action in b.
loss of UV absorbance at 254 nm occurred in the sample solution. Simultaneously, the inhibitory activity was eliminated with almost no change in residual excitatory activity, as judged by its lack of relaxing action on LMGPI with normal tone or with tone increased by 4 ng/ml substance P (Fig. 2). Adenine nucleotides, especially ATP and ADP, had a relaxing effect on LMGPI in concentrations higher than 0.1 μg/ml and showed UV absorption spectra with a peak at 254 nm. Alumina treatment of their solutions under the same conditions as used for the sample resulted in complete loss of the biological activity and UV absorbance at 254 nm. These results could mean that the adenine nucleotides are suspected to be responsible for the relaxing action of the sample.

3. Enzymatic studies on the LMGPI-contracting activity and WCR-contracting activity

The susceptibility of the LMGPI-contracting activity and WCR-contracting activity of samples to some proteolytic enzymes are given in Table 1. Aliquots of samples were incubated with active enzymes or inactivated enzymes, and changes in their contracting activity were tested on LMGPI and WCR. The effects of these enzymes on synthetic bovine substance P (hereafter, substance P) are also presented for comparison. Substance P was bioassayed on LMGPI but not WCR. No difference was seen in the enzymatic destruction between the LMGPI-contracting activity and substance P. In contrast to the LMGPI-contracting activity, the WCR-contracting activity was destroyed by carboxypeptidase A but resistant to pepsin. This finding corroborates the hypothesis that the two activities are due to separate substances.

4. Comparison of the LMGPI-contracting material in samples with substance P

Enzymatic studies showed a close similarity between the LMGPI-contracting activity and substance P. A series of experiments was performed to study further if the LMGPI-contracting component in samples and substance P were identical.

a. Effects of substance P antagonists: In the presence of either of the substance P antagonists, [D-Pro2, D-Trp7-9]-substance P (8 μg/ml) and [D-Arg1, D-Pro2, D-Trp7-9, Leu11]-substance P (16 μg/ml), contractile responses of LMGPI to a sample and substance P were both markedly reduced, thus suggesting they both acted on the same receptor. However, the antagonistic effect was invariably and significantly larger on the

| Table 1. Susceptibility of smooth muscle-contracting activities of Remak nerve extracts to proteolytic enzymes |
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| **Enzymes** | **Extracts** | **LMGPI-contracting activity** | **WCR-contracting activity** | **Substance P** |
| Chymotrypsin | Destroyed | Destroyed | Destroyed |
| Trypsin | Destroyed | Destroyed | Destroyed |
| Pepsin | Not destroyed | Destroyed | Destroyed |
| Carboxypeptidase A | Destroyed | Not destroyed | Not destroyed |
| Carboxypeptidase B | Not destroyed | Not destroyed | Not destroyed |
| Pyroglutamate aminopeptidase | Not destroyed | Not destroyed | Not destroyed |
| Leucine aminopeptidase | Not destroyed | Not destroyed | Not destroyed |
| Chicken serum | Not destroyed | Not destroyed | Not destroyed |

WCR: isolated whole chick rectum. LMGPI: longitudinal muscle of the guinea-pig ileum. Contracting activity of substance P was tested on LMGPI. Note a close similarity between LMGPI-contracting activity and substance P.
sample-induced contractions and more sustained after removal of the antagonist (Fig. 3). When an alumina-treated sample, which showed practically no inhibitory activity, was used, similar results were obtained. This excluded the possibility that the difference may result from the simultaneous presence of some inhibitory substance in the sample.

b. Desensitization of receptors for substance P: Applications of substance P in concentrations up to 10 ng/ml at intervals of 4-6 min produced consistent contractions of LMGPI with no sign of tachyphylaxis. When substance P in 400 ng/ml was applied to LMGPI, it produced desensitization of receptors for this peptide, as judged by its lack of contractile response to subsequent application of 4 ng/ml substance P. In most preparations, during exposure to substance P of 400 ng/ml, the increased tension of the muscle gradually subsided, but did not decrease to the initial level. However, the sustained tension was small enough to allow for the demonstration of desensitization to substance P. The desensitization resulted in abolition of contractile responses to samples as well as to substance P, 4 ng/ml (Fig. 4). The muscle in which tension had been increased to the same extent by 4 ng/ml substance P could respond with a large contraction to subsequent application of the same concentration of this peptide or the sample. Thus, the observed inhibitory effect was not attributable to the sustained tension. After removal of the substance P used for desensitization, recovery of the contractile responses to substance P and samples took place with a similar time course, as shown in Fig. 5, in which percentage recovery of the response is plotted against the time after the

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Fig. 3. Inhibitory effects of a substance P antagonist on contractile responses to substance P (SP, •) and to Remak nerve extract (ERN, ○) of the longitudinal muscle of the guinea-pig ileum. [D-Arg², D-Pro⁵, D-Trp⁷,⁹, Leu¹¹] substance P (SP antag.) was applied during the period shown († †) at 16 μg/ml. The active material obtained after gel filtration on Sephadex G-50 was used as ERN, and the experiment was performed on the muscle treated with atropine (0.2 μg/ml), pyrilamine (1 μg/ml) and methysergide (1 μg/ml).

Fig. 4. Effects of substance P and Remak nerve extract on the longitudinal muscle of the guinea-pig ileum (LMGPI) before and after desensitization of substance P receptor. A, Control responses to Remak nerve extract (ERN, ○) obtained after gel filtration on Sephadex G-50 and pretreated with alumina, and to 4 ng/ml substance P (SP, •): B, in the presence of 400 ng/ml substance P († †): C, in the presence of 4 ng/ml substance P († †). The experiment was performed on the muscle treated with atropine (0.2 μg/ml), pyrilamine (1 μg/ml) and methysergide (1 μg/ml). Note abolition of the contractile effects of ERN and substance P on substance P-desensitized muscle.
removal of agonist.

c. Stability: When the sample was boiled in 2 M HCl, the contracting activity was little reduced, but boiling in 2 M NaOH produced rapid loss of activity. Similarly, substance P was stable in the acid solution and unstable in the alkali solution.

d. Gel filtration: With a Sephadex G-25 column, samples and substance P were taken up in an appropriate volume of 0.1 M acetic acid and run under the same conditions, and their elution curves were compared. The sample gave an elution curve that was parallel to the elution curve for substance P, when the elution curves were determined by the effect of the eluate on the tension of LMGPI (Fig. 6).

It was found that when a sample prepared from Remak nerve of 2.6–3.0 g wet weight was applied to the Sephadex G-25 column, the contractions of LMGPI at the peak activity matched contractions produced by substance P concentrations in the range of 2–4 ng/ml. Such concentrations of substance P were found to be too low to contract WCR. In fact, concentrations higher than 100 ng/ml were required to cause a detectable rise in tension of WCR. Thus, substance P is about 100 times less potent in contracting WCR than LMGPI.

e. Ion exchange chromatography: When the active material obtained after the second gel filtration of a sample on Sephadex G-25 was subjected to ion exchange chromatography on sulfopropyl-Sephadex C-25, the elution profiles shown in Fig. 7 were obtained. The LMGPI-contracting activity was found to fractionate into two major components (the first, 70% and the second, 30%). The elution position for the second component was similar to that obtained when substance P was chromatographed by this ion exchange procedure. Ion exchange chromatography also yielded one main peak of WCR-contracting activity eluting at a region different
from those for LMGPI-contracting activity. The two components with LMGPI-contracting activity showed similar pharmacological properties which were characteristic of substance P. However, as shown in Fig. 8, when the second component was subjected to high voltage paper electrophoresis, the position at which it was recovered from the paper was different from the position of the ninhydrin-stained substance P spot. During high performance liquid chromatography, the two components had different retention times from one another and from substance P (Fig. 9).

f. Radioimmunoassay: An estimation by radioimmunoassay of the substance P-like activity in an aliquot of the active material obtained after gel filtration on Sephadex G-25 gave a result, 1.3 ng, which was much
lower than the value of 35 ng determined by bioassay for its substance P-like activity in another aliquot of the same origin and amount as that used for radioimmunoassay.

**Discussion**

The gel filtration profiles on Sephadex G-50 suggest that extracts of Remak nerve contain at least two separate smooth muscle excitatory substances, one mediating the LMGPI-contracting activity and the other mediating the WCR-contracting activity. This view is supported by the findings of resistance to pepsin and susceptibility to carboxypeptidase A of the WCR-contracting activity but not the LMGPI-contracting activity, and of their different behavior during ion exchange chromatography. Moreover, as discussed below, substance P, which is one of the possible peptides responsible for the LMGPI-contracting activity, is clearly not the excitatory substance mediating the WCR-contracting activity, because of the selectivity of the WCR assay. The minimum effective concentration of substance P in WCR was found to be about 100 ng/ml. Thus, the WCR-contracting activity of substance P in WCR was found to be about 100 ng/ml. However, in their pharmacological properties, both components were again indistinguishable from substance P. These results suggest that the active principles of the two components may be slightly different in structure from substance P, but may be a substance P-family of peptides serving physiological roles in chicken, or their fragments produced during the extraction. It is interesting that the biologically active substances were apparently not recognized by the antiserum directed toward bovine substance P, since species differences among other active peptides have been demonstrated.

It has been described that substance P is not destroyed by trypsin (8, 9). In the present experiments, the enzyme showed activity in destroying substance P. This disagreement may result from a possible contamination of traces of other enzymes having activity in destroying substance P, since Sigma enzyme preparations (EC 3.4.23.1, Type I) were used without further purification. However, the disagreement does not invalidate our conclusion, since the enzyme produced parallel effects on both LMGPI-contracting activity and substance P.

Using a bioassay employing substance P as the standard, the concentration of substance P-like peptides in the ganglionated nerve trunk of the Remak nerve was estimated to be 700–1000 ng/g wet weight of tissue. The presence of substance P in relatively high concentrations has been demonstrated in sympathetic ganglia of mammals by immunohistochemical (10) and radioimmunoassay (5, 11) methods. Especially in the inferior mesenteric ganglia of the guinea-pig, it has been suggested that substance P may function as the neurotransmitter of nerve fibres arising from neurones in the dorsal spinal ganglia and elicits the slow non-cholinergic excitatory synaptic potential in the post synaptic neurones. Immunohistochemical study on the Remak nerve has not been performed yet,
but substance P-like immunoreactivity has been observed in nerve fibres and neurones in the myenteric and submucosal plexuses of the chicken (12) and mammalian intestines (13, 14). The Remak nerve is a ganglionated nerve trunk, and the presence of nerve bundles connecting Remak ganglia with the spinal ganglia and intestinal wall have been demonstrated in the chicken morphologically (15) and electrophysiologically (3, 16). In this context, the substance P-like peptides might function as substance P in mammals, and some of them present in the Remak nerve would originate from axon terminals of the peptidergic neurones located in the spinal ganglia and/or the myenteric and submucosal plexuses.

The active principle of the WCR-contracting activity would be present in this tissue in a low concentration, and frequently, it was not obtained in sufficient amounts to permit investigation of its chemical and pharmacological properties. In the future it will be necessary to prepare tissue extracts containing this substance in sufficient amounts for its isolation and identification. Useful extracts from the chicken rectum for this purpose will be described in the following paper.

Another aim of the present study was to obtain a smooth muscle excitatory substance as a possible non-adrenergic, non-cholinergic (NANC) neurotransmitter. The cell bodies of NANC excitatory neurones innervating the smooth muscle of the chicken rectum are located in Remak ganglia (3). The neurotransmitter of NANC nerves in the chicken rectum, like other neurotransmitters, would be synthesized in the cell bodies and thus it would be expected to be distributed in the Remak nerve. In this respect, the substance P-like peptides and the active principle of the WCR-contracting activity may be possible candidates for the NANC neurotransmitter. To establish this, their possible neurotransmitter role should be examined in the chicken rectum. Recently, it has been suggested that a smooth muscle-contracting substance, which is chymotrypsin-susceptible but not substance P, is present in the extracts of plexus-containing longitudinal muscle sheets of mammalian intestines (17, 18).

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