Nerve Pathways in the Rectal Region of the Nerve of Remak of the Chicken

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Abstract. An electrophysiological investigation of nerve pathways was performed on the chicken's isolated rectal region of Remak's nerve trunk and isolated perfused rectum with attached Remak's nerve supply. Compound action potentials were recorded using the sucrose gap method. The rectal region of Remak's nerve trunk was found to consist of four groups of nerve pathways composing of B and C fibers, according to the conduction velocity of nervous impulses. Some of them involved synaptic transmission somewhere in the trunk. Present results also provided evidence for the existence of nerve pathways innervating the rectum: (1) that was to ascend or descend in Remak's nerve trunk, leave the trunk and run in the branches to the rectum, (2) that was to descend splanchnic lumbosacral nerve, enter Remak's nerve trunk, leave the trunk, and run in the branches to the rectum, and (3) that was to ascend the branches, enter Remak's nerve trunk, leave the trunk, and run in the branches to the rectum (a local reflex arch). It seemed very likely that these nerve pathways innervating the rectum were excitatory and mediate non-cholinergic, non-adrenergic responses in the rectum.

The rectum of the chicken is innervated by the extrinsic nerves via the nerve of Remak which is a unique autonomic nerve of fowls. The nerve of Remak is a ganglionated nerve trunk arising from the ganglia coli (G1) near the distal end of the caudal mesenteric artery and running in the mesentery rostrally in parallel to the intestine [16]. In the rectal region of the nerve, six ganglia are localized [9] and each of them gives off about ten fine branches running through the mesentery to the rectum.

Bartlet & Hassan [1] reported that the nerve of Remak contains adrenergic fibers, cholinergic fibers and non-cholinergic fibers which innervate excitatory the large intestine. Takewaki et al. [15] provided evidence for the presence of non-adrenergic fibers in the nerve of Remak which mediate relaxation of the rectum, in addition to the adrenergic fibers.

In the present study, compound action potentials were recorded from the trunk or branches of the nerve of Remak following electrical stimulation of the trunk at different regions, and they were analysed electrophysiologically and pharmacologically to show the types of nerve fibers, involvement of synaptic transmission and presence of afferent nerve pathways in addition to efferent ones. Furthermore, relationship between the evoked compound action potentials and the mechanical responses of the rectum to nerve stimulation was investigated. From the experimental results, the functional role of the nerve of Remak was discussed.

Materials and Methods

White Leghorn cocks, being more than 150 days-old, were stunned and bled, and the rectal region of the nerve of Remak was removed together with the
whole rectum, the caudal mesenteric artery and vein, and the mesentery.

*Remak nerve trunk preparation:* The trunk of the nerve of Remak on which six ganglia are located was dissected free from the surrounding connective tissues under a binocular microscope, and tied off with cotton thread at both cut ends. The isolated nerve trunk (1.0–1.2 mm in diameter and 60–80 mm in length) was used for the experiments. The six ganglia have been designated as $G_{I}$--$G_{VI}$ in their rostral order from the anal end of nerve of Remak [9].

*Remak nerve-rectum preparation:* The isolated rectum was perfused with Krebs solution via the caudal mesenteric artery at a constant rate of 3 ml/min by means of a roller pump. One of the branches which arise from $G_{II}$ or $G_{IV}$ and run to the rectum was dissected free from the mesentery, tied off with fine cotton thread at the distal end, cut off, and prepared for recording electrical responses. One more branch was prepared in the same way for stimulation of its central end.

*Recording of compound action potentials:* The sucrose-gap apparatus, as described by Kostelitz and Wallis [12] and Kostelitz *et al.* [13] (Fig. 1a) was used for recording compound action potentials from one end of the trunk of Remak's nerve. The apparatus consists of four chambers, which are hereafter referred to as stimulation chamber, test chamber, sucrose chamber and Krebs chamber in order from left to right. The sucrose chamber was isolated from adjacent chambers with thin rubber membrane. In the stimulation chamber (50×10×10 mm), three pairs of ring-shaped platinum electrodes (Ea, Eb and Ec) were placed at distances of 42, 23 and 13 mm, respectively, from the rubber membrane between the test and sucrose chambers.

The chambers were coated on their side-surfaces with vaseline and then clamped together with bolts and nuts. The rubber membranes were holed with a diameter fit for the nerve trunk. The nerve trunk was mounted in the sucrose-gap apparatus with the anal end in the stimulation chamber and one or more ganglia in the test chamber, as illustrated in Fig. 1a. The stimulation chamber was filled with 30°C liquid paraffin. The test chamber (5×10×5 mm) was perfused with Krebs solution or test solution, the sucrose chamber (10×5×5 mm) with isotonic sucrose solution, and the Krebs chamber (10×5×5 mm) with Krebs solution. Flow

![Diagram](image_url)

Fig. 1. Arrangements of sucrose gap for the trunk (a) and branch (b) of Remak's nerve. T, test solution; S, isotonic sucrose solution; K, Krebs solution; Ea, Eb and Ec, stimulating electrodes; R, recording electrodes; Rn, Remak's nerve trunk; Cl, cloaca; Cma, caudal mesenteric artery.

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rates were fixed at 1.5 ml/min for the test and Krebs chambers, and 5.0 ml/min for the sucrose chamber. Another sucrose-gap apparatus, as illustrated in Fig. 1b, was used for recording electrical responses from nerve branches. In comparison with the first apparatus, it has an organ bath (80×30×10 mm) instead of the stimulation chamber, and the test and sucrose chambers were reduced in width to 2 mm. The rectum of the preparation was pinned up on the black rubber board, sank in the organ bath, and perfused with Krebs solution or test solution. Flow rates were 0.6 ml/min for the test chamber and 2.0 ml/min for the sucrose chamber, respectively. A pair of Ag-AgCl electrodes were used for recording potential changes across the sucrose gap, which were connected via a preamplifier (Nihon Khoden, MZ-4) to an oscilloscope (Iwatsu DS-5016). A pair of flexible platinum electrodes were used for stimulation of the nerve trunk, and a bipolar glass suction electrode was used for stimulation of the nerve branch. Nerve stimulation was made with rectangular pulses of 0.1–0.5 msec duration. The stimuli were delivered by an electronic stimulator (Nihon Khoden, MSE-3R) and isolated from earth by means of an isolating unit (Nihon Khoden, MSE-JM). In some experiments, mechanical responses of the rectum of the preparation produced by nerve stimulation were recorded isometrically using a force-displacement transducer (Nihon Khoden, SB-1T) and a potentiometric pen recorder (Hitachi, 056).

Solutions and drugs: Krebs solution has the following composition (mM): NaCl 118.00, KCl 4.75, CaCl₂ 2.54, KH₂PO₄ 1.19, MgSO₄ 1.20, NaHCO₃ 25.00 and glucose 11.00. It was kept at 30°C and bubbled with a 5% CO₂ and 95% O₂ gas mixture. In low Ca²⁺ and high Mg²⁺ Krebs solution, CaCl₂ was reduced to 0.25 mM and MgSO₄ was increased to 12.00 mM. The concentration of the sucrose solution was 315 mM. Drugs used were hexamethonium bromide (Nakarai Kagaku), saccharose (Wako) and tetrodotoxin (Sankyo).

RESULTS

When the nerve trunk was stimulated at Ec (see Methods) with single pulses at supermaximal intensities, potential changes were recorded usually with four peaks at the more oral region (Fig. 2). These potentials were abolished by tetrodotoxin (10⁻⁷ g/ml) and reversed after removal of the neurotoxin, indicating composed action potentials. The four peaks were designated as P₁, P₁₁, P₁₁₁ and P₁ᵥ in order from the fastest one. The delay of each peak after delivering stimuli was less than 5 msec for P₁, 10–13 msec for P₁₁, 25–33 msec for P₁₁₁ and 40–65 msec for P₁ᵥ. The amplitudes of P₁ and P₁₁ were less than 0.5 mV. P₁₁₁ was always the largest response of 1.5–5.0 mV in amplitude. The amplitude of P₁ᵥ was 0.2–1.5 mV. In 5 out of 16 preparations, the interval between P₁ and P₁₁ was so short that they overlapped on the records. The responses were evoked constantly without any remarkable changes for a period of 4 hours.

Figure 3 shows compound action potentials evoked by nerve stimulation at

![Fig. 2. A typical compound action potential of Remak's nerve trunk evoked by stimulating the trunk at the more anal region.](image-url)
Fig. 3. Compound action potentials of Remak's nerve trunk evoked by stimulating the trunk at three different sites. Distances between the stimulating and recording sites in a, b and c are 13, 23 and 42 mm, respectively. See the delay of peak potentials in relation to the distance.

three different sites. It can be seen that when nerve stimulation was made at Ea, P_1 and P_III were absent, and P_III and P_IV were markedly reduced in amplitude. This suggests that nerve pathways responsible for P_1 and P_II may not run over 40 mm in Remak nerve trunk, and that those responsible for P_III and P_IV may reduce in number; that is, many nerve pathways may branch off and run to the rectum.

In 5 preparations which were mounted in the reverse way, nerve stimulation was made at the oral region and potential changes were recorded at the more caudal region. The evoked potentials had four peaks and substantially similar changes of them were observed when nerve stimulation was made at varied distances. Therefore, Remak nerve trunk contains ascending and descending nerve pathways which are similar properties in conducting the stimuli.

The graph as shown in Fig. 4 shows the relationship of the distance between the stimulating site and the recording site to the delay of each peak. It can be seen that the delay is directly proportional to the conduction distance. These relationships in 4 preparations gave conduction velocities of nerve pathways responsible for individual peaks as 3.7–4.4 m/sec for P_1, 1.0–1.2 m/sec for P_II, 0.4–0.5 m/sec for P_III and 0.2–0.3 m/sec for P_IV, respectively. Therefore, it is concluded that the nerve pathways consist of B and C fibers.

P_1 and P_II began to be evoked at volt-
Fig. 5. Relationship between amplitude of each peak (P1-P4) of compound action potentials and stimulus intensity. The compound action potentials, recorded from Remak's nerve trunk, evoked in response to single stimuli applied to the trunk at different intensities, are shown in left hand column. Intensity of applied stimuli was gradually increased for the records (from the top to the bottom).

Fig. 6. Effect of lowering Ca concentration of the external solution on the compound action potential of Remak's nerve trunk. Compound action potentials were recorded before (left) and 5 min after (right) external solution was changed from Krebs solution to a low-Ca (0.25 mM) and high-Mg (12 mM) Krebs solution. See depression of the fourth peak.

ages lower than 10 V and reached their maximal at 10–20 V, but P_{III} and P_{IV} were evoked at voltages higher than 20 V, as illustrated in Fig. 5.

To determine whether synaptic transmission is involved in the electrical responses, effects of lowering Ca^{2+} and increasing Mg^{2+} in Krebs solution on the compound action potentials were examined. In 10 out of 15 preparations, only P_{IV}, or P_{III} and P_{IV} were reduced in amplitude (Fig. 6). Similar results were obtained with a ganglion blocking agent, hexamethonium (10^{-5} g/ml). It is well known that post-tetanic potentiation (PTP) is observed on the response involving synaptic transmission [7]. Increased release of transmitter after intense repetitive stimulation of presynaptic fibers can produce excitation of a larger number of postsynaptic neurons [3]. Repetitive supramaximal stimulation (50 Hz, 30 sec) resulted in potentiation in amplitude of P_{III} and P_{IV} by 50–200% (5 preparations). The PTP decayed with time. Semilogarithmic plot of decaying phase of the PTP against time revealed that the PTP decay could be expressed as two exponential processes with respective time constants of about 40 sec and 80 sec. From these results it is certain that the nerve pathways mediating P_{III} and P_{IV} in Remak nerve trunk involve synaptic transmission.

Following stimulation of the nerve pathways...
trunk with single supramaxinal pulses, compound action potentials were recorded from the branch of $G_{II}$. The amplitude of the compound action potential varied according to the stimulating sites of the nerve trunk. When nerve stimulation was made at $G_{II}$, the amplitude was maximal. As the stimulating site was moved away from $G_{II}$ toward oral or anal direction, the compound action potential became smaller and disappeared if the stimulating sites were more oral than $G_{V}$. Similar results were obtained for the compound action potential in the branch of $G_{IV}$. The relationship between the stimulating site and amplitude of the compound action potential in the branch of $G_{IV}$ was shown in Fig. 7. It is apparent from this figure that amplitude reduction per unit distance is larger for the oral direction. These results show that some nerve pathways ascending or descending in Remak nerve trunk leave the trunk and run in the branches, and that the ascending pathways run longer distances in the trunk than the descending pathways. In addition, nerve pathways descending from the site more oral than $G_{VI}$ do not connect with any nerve pathways in the branch.

When splanchnic lumbosacral nerve was stimulated with single supramaximal pulses, potential changes like a compound action potential of about 1 mV were recorded from the branch (Fig. 8). Thus, it is clear that there are some nerve pathways running from splanchnic lumbosacral nerve to the branch via Remak nerve trunk.

Reflex discharges of less than 0.5 mV were recorded from the nerve branch, when its neighbouring branch was stimulated. The amplitude of the reflex response was reduced as the distance between two ganglia from which the

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**Fig. 7.** Relationship between peak amplitude of compound action potentials recorded from a nerve branch of $G_{IV}$ and stimulating site of Remak's nerve trunk. Ordinate, percent amplitude of the compound action potentials; abscissa, location of six ganglia on Remak's nerve trunk which was determined by mean relative distances of other ganglia from $G_{IV}$ obtained from 5 preparations.

**Fig. 8.** Effect of lowering Ca concentration of the external solution on the compound action potential recorded from a nerve branch of $G_{II}$ in response to stimulation of splanchnic lumbosacral nerve. Compound action potentials were recorded before (left) and 5 min after (right) external solution was changed from Krebs solution to a low-Ca (0.25 mM) and high-Mg (12 mM) Krebs solution.

**Fig. 9.** Potential changes of a nerve branch of $G_{II}$ following stimulation of the central cut end of nerve branches of $G_{V}$ (a), $G_{IV}$ (b), $G_{III}$ (c) and $G_{I}$ (d).
stimulating and recording branches arise increased, and no reflex potentials were evoked if more than three ganglia were present between them (Fig. 9), indicating the presence of local reflex pathways from one branch to another branch via Remak nerve trunk.

Hexamethonium \((10^{-5} \text{ g/ml})\) or the low \(\text{Ca}^{2+}\) and high \(\text{Mg}^{2+}\) Krebs solution was applied to the ganglia on the nerve of Remak via the caudal mesenteric artery [8]. Application of these solutions resulted in reduction of the compound action potential amplitude in response to stimulation of the nerve trunk and abolition of the electrical responses to stimulation of splanchic lumbar-sacral nerve and the central cut end of branches (Fig. 8 and Fig. 10). The degree of reduction of the compound action potential amplitude was greater with distance of the ganglion from which the recording branch arose to the stimulating electrode. These results indicate that all of these nerve pathways involve ganglionic transmission somewhere in the nerve of Remak.

Takewaki et al. [15] reported that contraction of the isolated chicken rectum produced by electrical stimulation of the Remak nerve trunk was non-cholinergic in nature and abolished by the ganglion blocking agents, such as hexamethonium. The electrical responses recorded from the branch were also sensitive to \(\text{Ca}^{2+}\) deficiency and hexamethonium. Therefore, it is possible that the electrical responses mainly are due to excitation of nerve pathways involved in the non-cholinergic contraction. After all branches other than one branch of \(G_{II}\) or \(G_{IV}\) entering the rectum had cut, electrical stimulation of the nerve trunk at the various sites between \(G_{I}\) and \(G_{IV}\) still produced contractions of the rectum.

Fig. 10. Effect of lowering \(\text{Ca}\) concentration of the external solution on compound action potentials of a nerve branch of GIV evoked by stimulation of Remak's nerve trunk. The trunk was stimulated at the middle point between \(G_{II}\) and \(G_{IV}\) (a), between \(G_{II}\) and \(G_{III}\) (b) and between \(G_{I}\) and \(G_{III}\) (c). Compound action potentials were recorded before (left in each pair) and 5 min after (right in each pair) external solution was changed from Krebs solution to a low-\(\text{Ca}\) (0.25 mM) and high-\(\text{Mg}\) (12 mM) Krebs solution. See larger depression of the potentials with greater distance between \(G_{IV}\) and stimulating site.

Fig. 11. Contractile responses of the rectum to stimulation of Remak's nerve trunk with single pulses at different sites after all nerve branches other than one nerve branch of \(G_{III}\) entering the organ were cut. I, II and VI show \(G_{I}\), GIII and GVI. S indicates stimulating site.
that there are no synapses in the nerve pathways. The synaptic delay in the ganglion of the frog sympathetic trunk has been found to be shorter than 4 msec [10, 14]. If this is also the case for Remak ganglia, synaptic delay, if any, of conduction of the nerve impulses along the nerve pathway would not lead to another conclusion.

Nerve pathways mediating $P_1$ and $P_4$ should be B fibers, since the potentials were elicited with the short delay and lower threshold. They did not show PTP, this indicating no involvement of synaptic transmission. The B fibers appear to be smaller in number and do not run in the trunk over the distance of 40 mm, since $P_1$ and $P_4$ were smaller in amplitude than $P_{III}$ and $P_{IV}$ and not elicited at the site moved away 40 mm or more from the recording site. It is possible that the B fibers mediating $P_1$ and $P_4$ are preganglionic fibers terminating on Remak ganglia.

Nerve pathways mediating $P_{III}$ and $P_{IV}$ fall in the conduction velocity of C fibers, and they were suppressed by hexamethonium, lowering $[Ca]_o$ and showed PTP, indicating involvement of synaptic transmission. However, it is still possible that the nerve pathways may consist of preganglionic B fibers and postganglionic C fibers only when the former fibers are very short. As the stimulating site was moved away from the recording site, $P_{III}$ and $P_{IV}$ were reduced in amplitude, but not prolonged in duration, this indicating a gradual decrease in the number of nerve pathways mediating these potentials. Probably, most nerve pathways leave the trunk and extend toward the rectum in the nerve branches. As the length of the trunk conducting nerve impulses increases, the sensitivity of $P_{III}$ and $P_{IV}$ to hexamethonium and lowering $[Ca]_o$ increased. This may be explained.

Fig. 12. Contractile responses of the rectum to stimulation of Remak's nerve trunk, the central cut end of nerve branches of $G_1$ and $G_{III}$, and stimulation of splanchnic lumbosacral nerve after all nerve branches other than one nerve branch of $G_1$ entering the organ were cut. Single stimuli were used for nerve stimulation. I, II and VI show $G_1$, $G_{III}$ and $G_{IV}$. $S_1$, stimulation of Remak's nerve trunk at two sites just below the mark; $S_{III}$ and $S_{III}$, stimulation of nerve branches of $G_1$ and $G_{III}$; $S_5$, stimulation of splanchnic lumbosacral nerve.

The magnitude of the contractile responses varied at different stimulating sites and were markedly reduced if the stimulating site was moved somewhere more oral than $G_1$ (Fig. 11). Stimulation of splanchnic lumbosacral nerve or the central cut end of branches (Fig. 12) also produced contractions. These similarities between the electrical responses of the branch and the contractile responses of the rectum strongly suggest that the nerve pathways are mainly excitatory ones innervating the rectum.

**DISCUSSION**

Present results provided evidence that the nerve pathways in Remak nerve trunk are composed of B and C fibers according to the conduction velocity and threshold for excitation. The conduction velocity was calculated by assuming
by assuming that the nerve pathways mediating \textit{P}_{II} and \textit{P}_{IV} change neurons in Remak ganglia and the postganglionic axons usually leave the trunk at the ganglion in which they change neurons, but some postganglionic axons run in the trunk through one or more ganglia before they leave the trunk.

Present results also provided evidence for the presence of the following nerve pathways to the rectum via the rectal region of the nerve of Remak: (1) that is to ascend or descend in Remak nerve trunk, leave the trunk and run in the branches to the rectum, (2) that is to descend the lumbosacral splanchnic nerve, enter Remak nerve trunk, leave the trunk, and run to the rectum, and (3) that is to receive afferent fibers in one branch and connect with efferent fibers in the other branches, local reflex arches whose centers may be located in Remak ganglia. All these nerve pathways contain synaptic transmission somewhere in Remak ganglia.

It seems very likely that the nerve pathways mentioned above are responsible for the non-cholinergic, non-adrenergic excitatory responses in the rectum. It has been shown that the e.j.p.s and contraction of the rectum in response to stimulation of the rectal region of the nerve of Remak contain ganglionic transmission so that they are blocked by hexamethonium [8, 11]. On the other hand, relaxation of the rectum in response to nerve stimulation remained almost unchanged after application of hexamethonium.

The efferent pathways in the lumbosacral splanchnic nerve change neurons and the postganglionic neurons are non-cholinergic and non-adrenergic, excitatory.

The interesting finding is that there are reflex arches from one branch to another. The reflex center is located on Remak ganglia. De Groat & Krier [4] demonstrated the sacral parasympathetic reflexes to the large intestine mediated via a spinal pathway, and suggested an essential role in initiation of propulsive activity during defaecation. It is well known that local reflex mechanisms including intramural ganglia play an important role in peristalsis of mammalian intestines [2, 5, 6, 17]. However, this local reflex mediated via extrinsic autonomic ganglia is the first evidence, which would play some role in controlling the rectal function.

References


要約

鶏の直結腸部 Remak 神経の神経節: 長崎正明・武藤義・大橋秀法 (岐阜大学解剖生理学講座) ——藤野から摘出した Remak 神経と直結腸をつかったままの Remak 神経を用いて、刺激に応じて神経に発生する電位変化を測定し、腸管の機械的反応を等尺性張力変換表を用いて測る。Remak 神経は神経インパルスの伝導速度からみて、B 細維と C 細維で構成されていることがわかる。Remak 神経中の神経節にはシナプスを含むものがあった。さらに、Remak 神経を上行あるいは下行してから神経枝を経て直結腸へ走向する径路、脇仙骨内臓神経から Remak 神経に入り神経枝を経て直結腸へ向う径路、ならびに直結腸から神経枝中の神経を経て Remak 神経に入り、近傍の神経枝の遠心路を経て直結腸にいたる反射回路が検出した。これらの神経路には、いずれも直結腸にいたる途中にシナプスが介在していた。Remak 神経などの刺激に応じて神経枝で記録される電位変化と直結腸の収縮反応との関には、薬物感受性において一致が多かったので、本報で明らかにした神経路は主として興奮性支配に関与しており、非コリン非アドレナリン作動性神経であると考えられた。