WITHDRAWAL REFLEX AND CONDUCTION BLOCK IN THE GIANT AXONS OF A SABELLID WORM (*PSEUDOPOTAMILLA OCCELATA*)

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SUMMARY

1. Body contraction in the sabellid worm, *Pseudopotamilla occelata*, during the rapid withdrawal reflex occurred only in the anterior half of the body. End-to-end shortening was never observed. The longitudinal muscles are well-developed in the anterior half, and poorly developed in the posterior half.

2. Conduction of action potentials along the giant axons was blocked at the mid-body, and was responsible for the anteriorly restricted body contraction.

3. Electrophysiological and histological studies excluded the possibility that conduction block resulted from a safety factor attributable to the special geometry of the axons.

4. Current injection across the giant axon membrane in the region of the conduction block indicated that changes in the properties of the membrane were responsible for the conduction block.

INTRODUCTION

The rapid withdrawal behaviour of sedentary polychaetes is their principal defensive reflex against predators. Almost synchronous segmental contractions result in end-to-end shortening and retraction of the feeding crown into the tube. This ability is correlated with the presence of giant axons in the ventral nerve cord. The enlarged diameter of the axon increases the rapidity of conduction and therefore facilitates simultaneous contractions of the segmental longitudinal muscles (see Nicol, 1948).

Although the giant axons achieve their greatest development among the tubicolous polychaetes, their arrangement and structure are far from uniform (Nicol, 1948). Many species of sabellid possess two giant axons which run continuously throughout

the entire body. In these, the rapid, symmetrical and synchronous body contractions are achieved not only by rapid conduction but probably also by the synchronous activities of paired giant axons *via* chemical (Bullock, 1953; Hagiwara, Morita & Naka, 1964) or electrical transmission (Mellon *et al.* 1980) between the paired giant axons.

The physiological properties of the giant axons in several species of sabellid worms have been thoroughly investigated (see Dorsett, 1978). In some species, an individual giant axon may be derived from more than one cell located in the segmental ganglia, after the segmental units have become aligned and function as a single conduction system. Yet, irrespective of their evolutionary origin, it seems that the giant axons show uniform physiological properties along their entire length.

In this paper we demonstrate that the conduction of action potentials along the giant axons is blocked at the mid-body in *Pseudopotamilla occelata*, and that this phenomenon causes the anteriorly restricted body contraction during the withdrawal reflex. We also consider the mechanisms of this conduction block.

MATERIALS AND METHODS

Specimens of *Pseudopotamilla occelata* were collected from Akkeshi Bay, Hokkaido, Japan. They were kept in an aquarium in circulating sea water (at 10–15°C) before use.

For observation of the body contraction during the withdrawal reflex, the worms were carefully removed from their tubes, and settled individually into glass tubes of suitable bore (about 8 mm) fixed perpendicularly in a small aquarium. Withdrawal movements were induced by direct mechanical stimulation or light shock to the aquarium, and observed visually or recorded with a ciné camera.

Specimens prepared for histological studies were fixed in Bouin's fluid. They were pinned to minimize the distortion of the body during fixation. The preparations were dehydrated in ethanol and embedded in paraffin wax. Thin sections (10 μm) were cut transversely and stained by either the Azan or the Haematoxylin–Eosin method for examination under a light microscope.

The electrophysiological experiments were carried out on semi-dissected worms in a chamber filled with natural sea water. Access to the giant axons was obtained by making an incision along the dorsal mid-line and pinning open the body wall from the latter half of the thorax to the middle of the abdomen. The paired giant axons in the dorsomedial position of the ventral cord were partially exposed by removing the overlying digestive tract. Complete visualization of the giant axons was, however, impossible because of the muscles and connective tissue surrounding the ventral cord.

The giant axon was stimulated at the thorax by rectangular current pulses (50–100 μs in duration) with paired silver electrodes. Action potentials were recorded intracellularly and successively with KCl-filled glass microelectrodes (10–20 MΩ in resistance) at several points along the giant axon. In some experiments, hyperpolarizing or depolarizing current pulses were delivered through a glass

microelectrode inserted near the recording electrode. By means of a switching device, the microelectrode for current injection could also be used for recording, so that successful insertion of this electrode was confirmed before and after current injection.

RESULTS

The withdrawal reflex

Worms which were acclimatized to living in the glass tubes showed similar behavioural patterns to those in their natural dwelling tubes. Only the head of the worm emerged from the opening of the artificial tube, and the crown, consisting of many pinnate processes called radioles, spread out in the usual manner. When they were stimulated mechanically, they withdrew rapidly into the tubes.

Throughout the observations (about 20 preparations), no end-to-end shortening during withdrawal was ever observed. Body contraction was restricted to the anterior half of the body (Fig. 1A). 100 to 140 body segments were used in this study and the length of each segment was approximately 1 mm in its natural condition. The first nine segments form the head and thorax, and the remainder form the abdomen. There was almost no visible shortening in the regions of the head and thorax, perhaps on account of the considerable stiffness of epidermis in these regions. The actively contracting regions lay between the 10th to approximately the 30th segments, and there was no observable contraction in the posterior half of the body. This anteriorly restricted contraction of the body usually resulted in the head retracting several millimetres within the opening of the tube.

Structure of the longitudinal muscles

In polychaetes the withdrawal reflex is caused by contraction of the paired dorsal and ventral longitudinal muscles. In this worm, these muscles are well-developed in the anterior half of the body, approximately as far as the 30th segment, but are poorly developed in the posterior region (Fig. 1B). A schematic representation of the change in cross-sectional area of both dorsal and ventral longitudinal muscles is shown in Fig. 1C, where the cross-sectional area of ventral muscle can be seen to decrease gradually from head to mid-body region. The dorsal muscle, however, showed the greatest development in the 10th to the 15th segments – the initial segments of the abdomen. Gradual reduction in the cross-sectional area of the dorsal longitudinal muscle was observed from the 15th to the 40th segment. From the 40th segment posteriorly, the cross-sectional area of both dorsal and ventral longitudinal muscles was almost constant. These changes in the musculature occurred symmetrically and closely corresponded with the withdrawal behaviour: contraction during the withdrawal reflex was restricted to the region of well-developed musculature. It would thus appear that the dorsal longitudinal muscles in the 10th to the 30th segments are primarily responsible for the rapid contraction of the anterior half of the body during the withdrawal reflex.
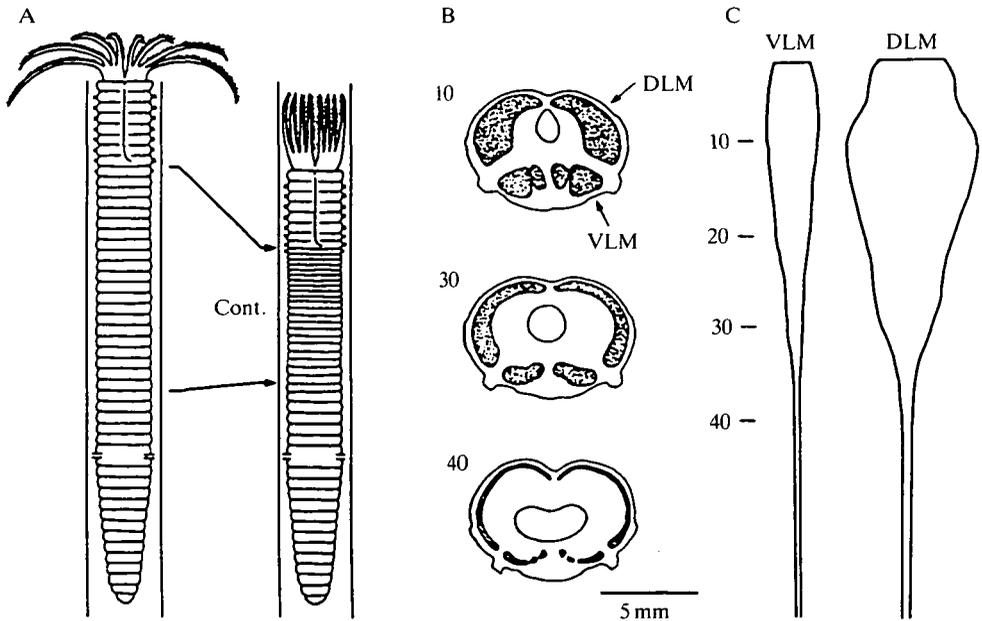
Fig. 1. (A) Schematic representation of the body contraction during the withdrawal reflex in *Pseudopotamilla occelata*. Left: usually only a part of the head with the expanding crown emerges from the opening of the tube. Right: rapid retraction of the head is performed by contraction restricted to the anterior half of the body. Cont., region of active contraction. Part of the posterior half is abridged in this figure. (B) Drawings of cross-sections of dorsal longitudinal muscles (DLM) and ventral longitudinal muscles (VLM). Drawings were traced from photographs of transverse sections of the whole body at the 10th, 30th and 40th body segments. (C) Schematic representation of the relative change in the cross-sectional area of VLM and DLM along the body length. Numerals at the left indicate the order of body segments.

Conduction block in the giant axon

In this worm, the giant axon (150–200 μm in diameter in the anterior half of the body) has a resting potential in the region of 80–90 mV, responding to depolarization of 15–20 mV with 100–110 mV action potentials, and has a conduction velocity of 5–7 m s^{-1}. When the two giant axons were stimulated in the thorax, either simultaneously or separately, the contractions of longitudinal muscles were seen to be restricted to the anterior half of the body, thus corresponding to the behavioural observations.

In the experiment illustrated in Fig. 2, action potentials were recorded in one of the two giant axons following stimulation of the giant axon with a stimulus interval of 1 s or more. Monitoring the effectiveness of the stimulation and recording the control responses were performed in the 13th segment which lies in the initial segments of the abdomen. Action potentials were simultaneously recorded in a more posterior region. At the 24th segment, the amplitude of action potentials was abruptly reduced, resulting in complete disappearance at the 26th segment. This suggests that a conduction block occurred around the 23rd or the 24th segment, and that reduced responses at the 24th and the 25th segments might result from the electrotonic spread
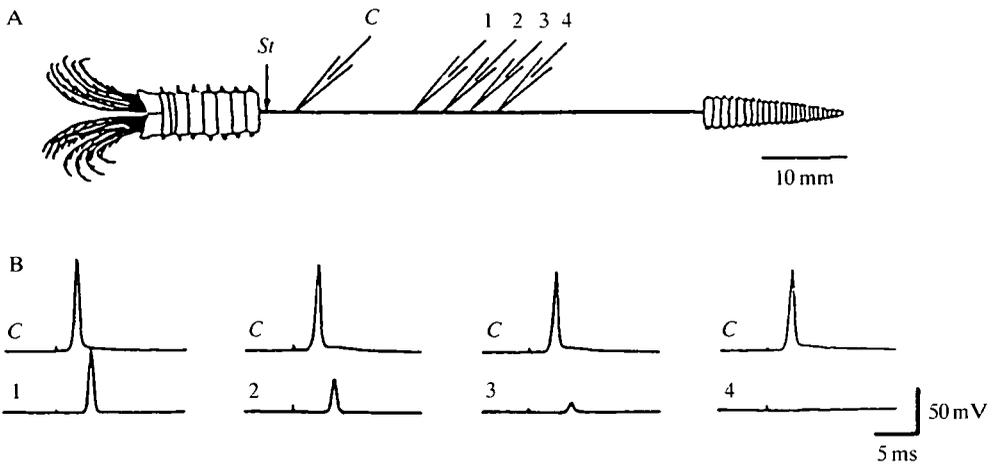
Fig. 2. Conduction block of the giant axon. (A) Schematic representation of the arrangement of external stimulation (*St*) and intracellular recordings. (B) Action potentials and reduced potentials of the giant axon. Control responses (*C*) were recorded at the 13th segment. Each pair of control response and the response at the 23rd (1), 24th (2), 25th (3) or 26th (4) segment was recorded simultaneously.

of blocked action potentials. Similar experimental results were obtained with all of the preparations used (about 50 preparations). It is significant that the region of the conduction block was effectively restricted to the area between the 20th and the 40th segments.

The conduction block and giant axon geometry

As known for the giant axon of the cockroach (Parnas, Spira, Werman & Bergmann, 1969) and for a crustacean axon (Grossman, Spira & Parnas, 1973), irregularities in the axonal structure may produce regions with a low safety factor for the conduction of action potentials. Experiments were, therefore, performed to investigate the relationship between the region of the conduction block and the geometry of the giant axon.

After observation of the conduction block and precise measurement of the re-cording electrode position according to the segmental structure, specimens were prepared for histological study, and a reconstruction of the giant axons around the region of the conduction block was performed. In approximately 80% of the giant axons investigated in this study (about 50 cases), an isthmus-like structure in the giant axon was situated near the level of the conduction block (Fig. 3A). The site of the conduction block, however, was consistently localized in the anterior region of the isthmus-like structure. In almost all of the other cases, the conduction block occurred in a region of uniform geometry (Fig. 3B). Although detailed studies of the effect of the isthmus-like structure on conduction are lacking, the possibility of a low safety factor, owing to a sudden increase of the axon diameter, can be excluded
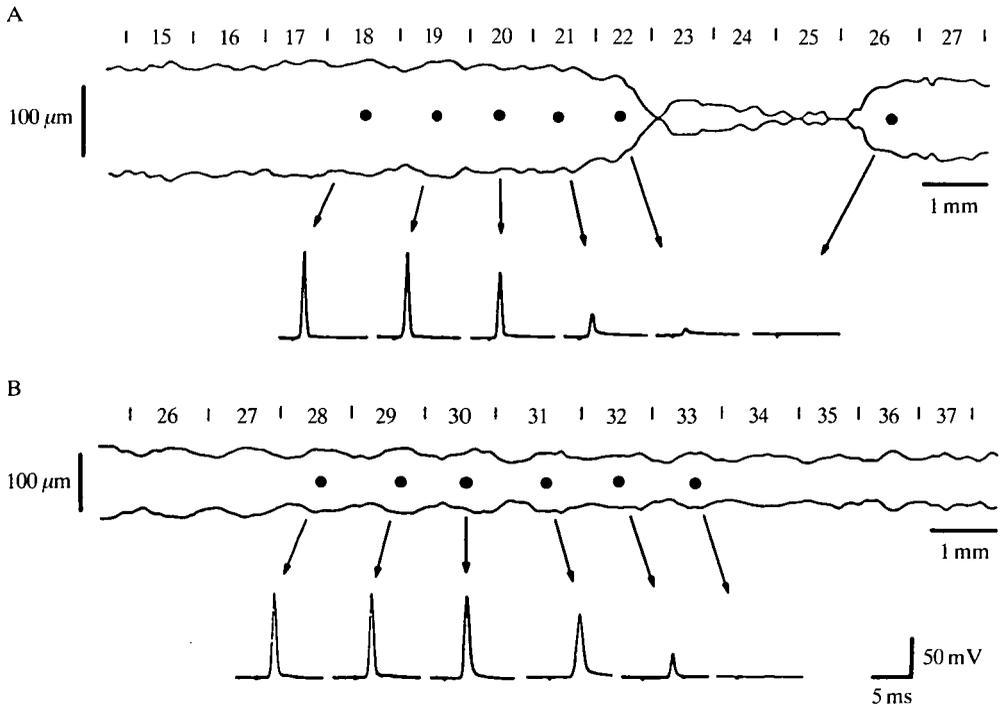
Fig. 3. Conduction block and giant axon geometry. Each potential was recorded at the segment indicated by closed circles and arrows. Numerals above the axon show the order of the body segment. (A) Conduction block near the isthmus-like structure. (B) Conduction block in the region of uniform geometry. A and B are recorded from different preparations.

from the possible factors causing conduction block in the giant axon, as the block occurs anterior to the isthmus-like structure.

Nevertheless, the participation of the isthmus-like structure in the conduction block cannot be excluded. Although the data are not shown, isthmus-like structures are consistently found in the giant axons in the mid-body segments (the 20th–40th segments), where the longitudinal muscles gradually reduce in thickness (see Discussion).

Voltage spread along the giant axon

In these experiments constant hyperpolarizing current pulses (100 ms duration) were passed through the giant axon of one segment. The resultant displacement of membrane potential was simultaneously recorded in the nearest segments to calculate the space constant. Between the 10th and the 15th segments, where the conduction block rarely occurred, the space constant was about 1·5 mm (1·55 ± 0·44 mm average in 10 preparations). In the region of the conduction block (Fig. 4), the space constant was also about 1·5 mm (1·50 ± 0·29 mm in six preparations). In addition, this value was approximately the same for the electrotonic spread of reduced potentials resulting from the blocked action potentials.

When some of the preparations included the isthmus-like structure, the space constant was greatly reduced, although the exact value could not be determined because of the great variety in the formation of the individual isthmus-like structures.

These experimental results suggest that the passive properties of the giant axon membrane may have nothing to do with the conduction block.

Effects of hyperpolarization

Hyperpolarization, by current injection through another microelectrode inserted in the vicinity of the recording electrode, revealed a low safety factor for conduction around the region of the conduction block. In the preparation illustrated in Fig. 5A, we were able to record full-size action potentials up to the 24th segment, their amplitude being reduced after the 25th. Injection of hyperpolarizing current at the 24th segment increased the amplitude of the action potential. However, slight hyperpolarization appeared to prevent the invasion of action potentials into this region. In another preparation (Fig. 5B), in which full-size action potentials were recorded up to the 29th segment, a similar and pronounced effect of hyperpolarization was observed. The action potentials were completely blocked by hyperpolarization of the 29th segment. Although the records are not given, 100 mV or more hyperpolarization was insufficient to block conduction between the 10th and the 15th segments.
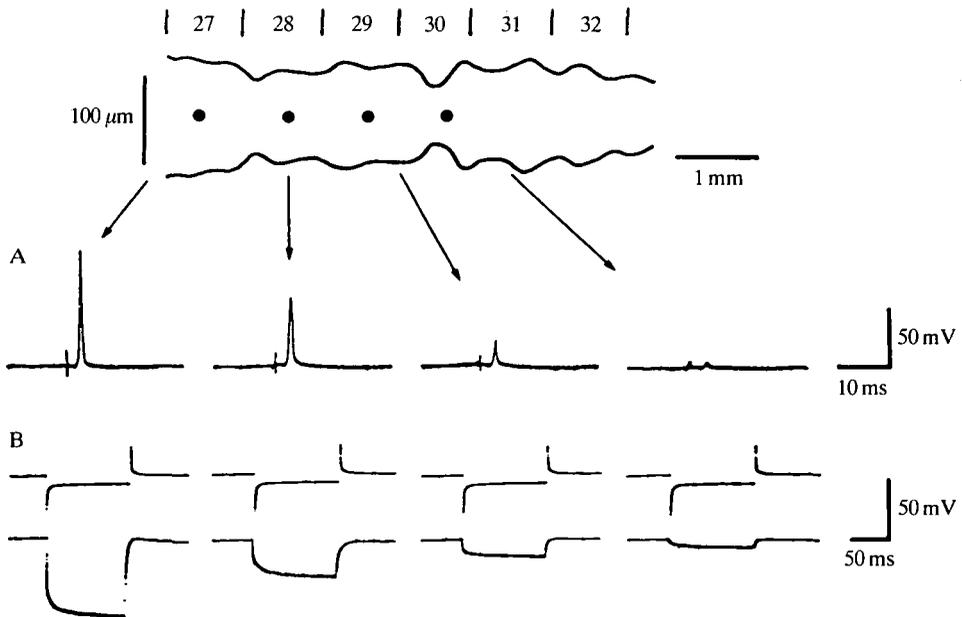

Fig. 4. Voltage spread around the region of the conduction block. (A) Action potential and reduced potentials recorded in a similar experiment to Fig. 3. (B) Responses, recorded in segments corresponding to those in A, to constant hyperpolarizing current pulses injected at the 27th segment.
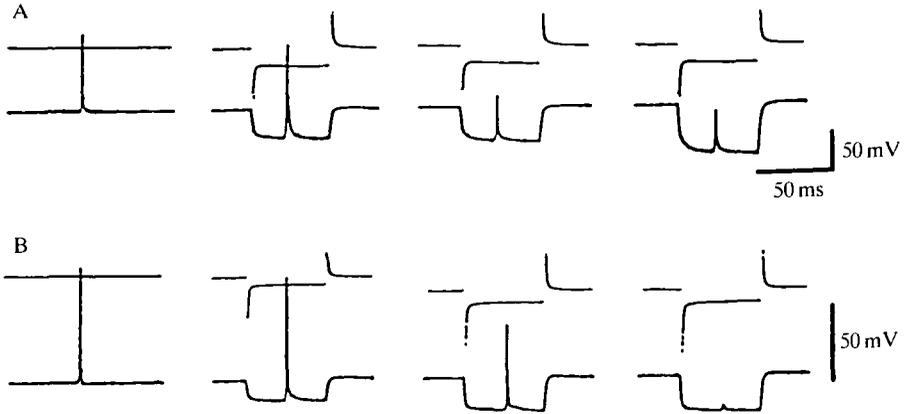
Fig. 5. Effect of hyperpolarization on the action potentials around the region of the conduction block in two different preparations. (A) Hyperpolarization reduced the amplitude of action potentials. (B) Action potentials were abolished by hyperpolarization.

These results show that the safety factor for conduction has already decreased at the anterior region of the apparent site of the conduction block. In almost all of the preparations used, hyperpolarization of the anterior region of the apparent site of the conduction block was effective by itself. There was, however, a great divergence in the effective amount of hyperpolarization among the preparations. At present it is uncertain how widely such vulnerable regions extend, but it may be expected that the safety factor for conduction gradually lessens towards the apparent site of the conduction block.

Responses to depolarizing current injection

The responses of the giant axon to a depolarizing current injection around the region of conduction block are shown in Fig. 6. In this preparation the conduction of full-size action potentials was observed up to the 24th segment, and was blocked posteriorly (Fig. 6A). Anteriorly, the giant axon membrane responded to the depolarizing current with constant threshold depolarization and constant amplitude of action potential (Fig. 6B). At the 25th segment, however, the depolarization threshold rose and the amplitude of the action potential fell. The threshold for depolarization and the action potentials were enhanced at the 26th segment. This shows a gradual decrease of membrane excitability around the region of the conduction block. In this preparation, the excitability recovered at the 28th segment, indicating a localization of membrane excitability. Recordings at the 27th segment were unsuccessful, possibly because of the narrowing of the axon at the isthmus-like structure, which was observed in the histological preparation after the electrophysiological experiments.

Low electrical excitability of the giant axon membrane was a common characteristic at the region of the conduction block in all of the preparations used in this type of experiment (10 cases), whether the isthmus-like structure was present in this
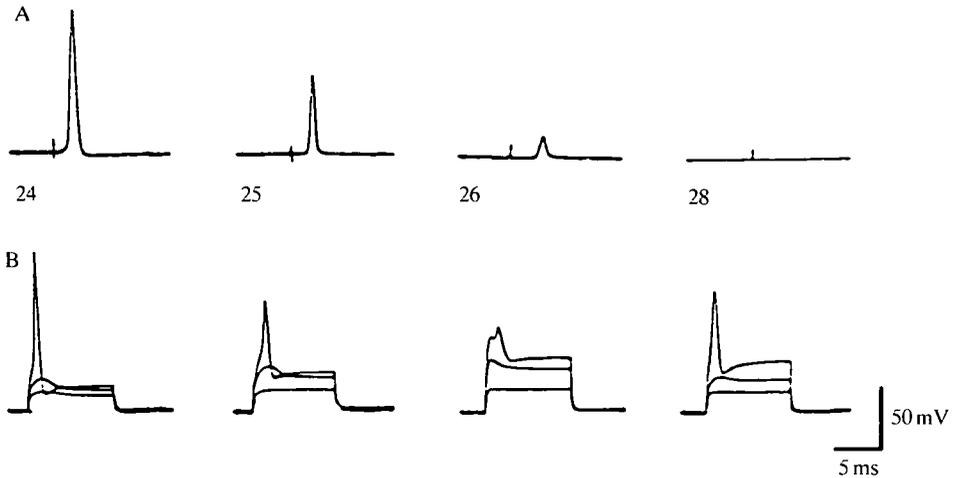
Fig. 6. Responses to depolarizing current pulses around the region of the conduction block. (A) Action potential and reduced potentials in each segment, whose order is indicated by numerals, obtained in a similar experiment to that in Fig. 3. (B) Responses to depolarizing current pulses injected in each segment corresponding to those in A.

region or not. These observations suggest that the low excitability of the giant axon membrane at certain regions of the mid-body may be one of the major factors responsible for the conduction block.

DISCUSSION

The present results demonstrate a new type of conduction block of action potentials. In several other experimental preparations in which a conduction block has been demonstrated, two common characteristics have been shown. The first is the special geometry of the axons. The structure of the axons at such regions shows a step up or a gradual increase in the membrane surface area (Eyzaguirre & Kuffler, 1955; Coombs, Curtis & Eccles, 1957; Parnas *et al.* 1969; Mellon & Kaars, 1974; Castel, Spira, Parnas & Yarom, 1976; Spira, Yarom & Parnas, 1976) or a bifurcation (Parnas, 1972; Grossman *et al.* 1973; Hatt & Smith, 1976; Raymond & Lettvin, 1978; Smith, 1980*a,b*; Theophilidis, 1984). The second is an ionic shift following axon activity at high frequency (Spira *et al.* 1976; Parnas, Hochstein & Parnas, 1976; Smith, 1977, 1980*a*). In the preparations used in this study, however, the conduction block did not involve either of these characteristics.

The isthmus-like structure of the giant axons in *Pseudopotamilla* seems to be related to the conduction block and, in fact, the action potentials were blocked near the isthmus-like structure in many of the preparations. It may be particularly significant that the conduction block occurs in the anterior region of such a structure, but not in the posterior region. Although no efforts were made to investigate the effects of the gradual or sudden decrease of the axon diameter towards the isthmus-like structure, the reduction of the axon membrane surface area might be expected to increase the current density from action potentials. Moreover, the conduction block

occurred in a region without any sign of an isthmus-like structure. It therefore seems necessary to explain the conduction block in terms of factors other than the geometrical one.

In the case of other experimental preparations, for example in crayfish axon branching (Smith, 1980a) and the cockroach giant axon (Spira *et al.* 1976), several hundred or more action potentials are required to induce the conduction block, and it may be expected that during such numerous discharges the accumulation of extra-cellular potassium would reduce the potassium gradient across the membrane, resulting in depolarization and eventually in the creation of the conduction block. However, in the present study, no preceding action potentials were required to induce the conduction block. The conduction block consistently occurred during the first action potential of the experiment. The influence of ionic shift can, therefore, be excluded.

The synaptic input of the cockroach giant axon was effective in the modulation of the propagation of action potentials (Spira *et al.* 1976). This possibility may, however, be excluded from our preparations. Preliminary experiments have shown that a persistent conduction block occurred even when the giant axon was stimulated selectively by an intracellular microelectrode. It seems that there are no differences in the case of gross stimulation by an external electrode or selective stimulation by an intracellular electrode. Again, most of the possible sensory fibres of peripheral origin may have been removed from the area where the giant axons are exposed. It is still just possible that unknown connections between the giant axons and adjacent nerve fibres in the segmental ganglion may be related to the conduction block.

Observation of the voltage spread around the region of conduction block indicates that there is no change in the passive properties of the axon membrane in such a region. It is most likely that, in this preparation, areas of low safety factor or low electrical excitability around the region of conduction block are relatively large. We know nothing about the factors which cause the apparent electrical inexcitability. In a study of the conduction block in the giant axon of the crayfish, Smith (1977) commented briefly that the altered density of the sodium channels or other mem-brane properties as well as non-uniform cable properties might cause the conduction block. In addition, it has been suggested that the density of sodium channels may be lower in the region of low safety factor (Raymond & Lettvin, 1978). It is difficult to determine whether such a hypothesis can be applied to our preparation. It is probable, however, that a morphological change, which causes the formation of the isthmus-like structure, may involve a change of membrane properties affecting electrical excitability over a wide area. Alternatively, it might be that the isthmus-like structure is a secondary structure resulting from a change of membrane structure which is responsible for the membrane properties.

It should be noted that the results of this study are probably not specific to this animal. Although little is known about the development of polychaete giant axons, it has been suggested that the giant axons of this animal arise from special cells in each segment and that the separate giant axons then fuse together longitudinally (see Nicol, 1948). Such syncytial giant axons occur widely in sabellid worms and other

polychaetes. In the giant axons of several species of sabellid worms, variations in diameter and in form of isthmus are also reported (Nicol, 1948). There are at present few data about the hypothetical relationship between the process of development of the giant axons and the membrane properties around the region of the conduction block.

The rapid withdrawal behaviour of tube dwellers may be the only defensive reflex against their many predators. When the worm withdraws its anterior end into the tube, the radioles are rolled up. A retraction of several millimetres is, therefore, sufficient for the worm to conceal itself completely from predators. The behaviour patterns of this worm are very limited. Intermittently occurring, slow withdrawal (without apparent external stimulation and rotation in the tube) appears to be the only distinct form of behaviour other than rapid withdrawal (observation in this study). The anteriorly restricted development of the longitudinal muscles seems, therefore, to be specially related to the rapid contraction of the anterior half of the body during the withdrawal reflex.

Although the action potentials are blocked in the mid-body, the giant axons in the posterior half of the body have normal excitability and conductivity (T. Higuchi, H. Nakamura, K. Sawauchi & H. Okumura, unpublished observation). There may be a functional differentiation in both longitudinal muscles and giant axons between the anterior and posterior half of the body, although the function of the giant axons in the posterior part is at present unknown.

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