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Waveform generation of the electric organ discharge in *Gymnotus carapo*

II. Electrophysiological properties of single electrocytes

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Summary. During in vitro experiments, different electrophysiological properties of single electrocytes were demonstrated in 3 portions (abdominal, intermediate, and tail) of the electric organ of the weakly electric fish *Gymnotus carapo*. Membrane potentials were measured with intracellular microelectrodes, while action currents were recorded by means of extracellular micropipettes facing the electrogenic surfaces (rostral and caudal). The nerve supplies to the caudal and rostral faces of abdominal, doubly innervated electrocytes (DIEs) could be independently stimulated, while this separation is not possible in other portions of the electric organ (EO). At the abdominal level, the rostral faces generate only postsynaptic potentials; the caudal faces give rise to action potentials. When the activation of both faces is properly timed, the action currents recorded at the rostral face reproduce quite faithfully the waveform generated by the whole EO, including the duration of the different phases.

Within the intermediate portion, neural activation of DIEs (tube 1 electrocytes) was performed via the posterior electromotor nerve. An action potential originates at the rostral face, invading secondarily the caudal membrane. In the most caudal 30% of the EO, the electrocytes of tube 1 are singly innervated on their caudal faces. Consequently, when activated via the posterior electromotor nerve, the caudal membrane gives rise to an action potential, which secondarily invades the rostral face. This electrical behavior is also observed in electrocytes belonging to tubes 2, 3, and 4 (caudally innervated) all along the EO. The number and distribution of spinal roots providing innervation to single DIEs at different levels of the EO were determined by electrophysiological methods.

Abbreviations: AEN anterior electromotor nerve; *DIE* doubly innervated electrocytes; *EO* electric organ; *EOD* electric organ discharge; *PEN* posterior electromotor nerve

Introduction

The cellular bases of EOD waveform generation in *Gymnotus carapo* were first studied by Bennett and Grundfest (1959), reviewed by Bennett (1971) and by Bass (1986). The existence of doubly innervated electrocytes (DIEs) (Szabo 1960, 1961; Trujillo-Cenóz et al. 1984) justifies, as stated by Bennett (1971), a reinvestigation of the electrophysiological properties of single electrocytes. Our previous in vivo experiments on abdominal electrocytes (Lorenzo et al. 1988) also support the need of new recordings from single electrocytes all along the electric organ (EO).

In this study, 4 different portions of the EO were prepared in vitro. The nerve supply to the electrocytes was stimulated, while the membrane potential was recorded by means of intracellular micropipettes; extracellular currents were recorded with microelectrodes placed close to the electrogenic (caudal and rostral) membranes (see Fig. 1). The results presented, along with the neurohistological and biophysical data reported in the two accompanying papers (Trujillo-Cenóz and Echagüe 1989; Caputi et al. 1989) lead to a comprehensive view of waveform generation in *G. carapo*.

Material and methods

Sixty-seven *G. carapo* 8–15 cm in length were used in experiments in which different portions of the EO were surgically isolated and maintained in vitro. Intra- and extracellular re-

cordings were obtained from electrocytes while stimulating appropriate nerve trunks.

Animals were anesthetized by cold, and the cranial CNS was mechanically destroyed. The selected portion of the EO was excised and placed in a Lucite-Sylgard chamber with *Electrophorus* Ringer's solution at room temperature (18–20 °C). Bubbling with 95% O_2, 5% CO_2 was maintained throughout the experiment. The preparation was transilluminated, and a surgical microscope was used to visualize the electrocytes.

The surgical technique varied according to the level of the EO to be studied:

1. In the case of the abdominal portion, the abdominal wall was placed flat in the chamber, with its peritoneal side up. This portion of the EO contains lateral rows composed of DIEs, and medial rows with electrocytes innervated only on their caudal faces (Trujillo-Cenóz et al. 1984). The nerves supplying innervation to the electrocytes were dissected and mounted on forceps electrodes for stimulation. A small hole was opened in the peritoneum overlying the electrocyte selected for recording.

2. In other set of experiments, the portion of EO underlying the swim bladder was studied. The abdominal wall was prepared as in (1), but in this case the excised portion was lengthened caudally. At this level the EO contains 4 rows of electrocytes at each side of the midline; the most dorsal tube of electrocytes (tube 1) is composed of DIEs. The nerves supplying the EO, which run just below the peritoneum, were dissected and mounted on forceps electrodes. Unlike the abdominal region, muscles had to be removed to visualize the electrocytes. No records from electrocytes other than those of tube 1 could be performed at this level of the EO.

3. A more caudal portion of the EO (proximal tail) was approached through the lateral wall of the animal. The caudal half of the fish was placed in the chamber lying on its side. The skin was removed, and the muscles covering the electrocytes were excised. The posterior electromotor nerve (PEN; Trujillo-Cenóz and Echagüe 1989) and the spinal roots from which it originates were exposed for stimulation. Electrocytes of all tubes were recorded at this level.

4. At the most caudal portion of the EO (distal tail), electrocytes of tube 1 lack rostral innervation (Trujillo-Cenóz and Echagüe 1989). Surgical procedures were similar to those described for portion (3).

In a different group of experiments, the number and distribution of spinal roots supplying innervation to a single DIE were studied by electrophysiological methods. These experiments were performed at two levels of the EO: the abdominal portion and the intermediate portion. The experimental approach consisted of recording intracellularly from a DIE and stimulating successively 10 to 18 spinal roots, corresponding to different metameric levels with respect to the recorded electrocyte. To verify the responsiveness of the latter, control action potentials were obtained by stimulating the AEN or the PEN.

Electrical stimulation. In (1) and (2) the nerves, mounted on forceps electrodes, were lifted out of the Ringer's solution for stimulation; the stimulating current flowed between the forceps and a second electrode in the chamber. In (3) and (4), a concentric electrode 250 μm in outer diameter was gently applied on the selected nerve or spinal root. Square pulses of 0.5 ms duration and 4–40 V in amplitude were used. In all cases, care was taken to avoid spread of the stimulating currents.

Recording procedures. Intracellular recordings were obtained by means of 3 *M* KCl-filled glass micropipettes (10–15 MΩ impedance). Extracellular recordings were performed with 3 *M* NaCl-filled micropipettes placed as close as possible to different surfaces of the impaled electrocyte (Fig. 1). A large Ag–AgCl electrode in the Ringer's solution was used as reference. The recorded activity was amplified by conventional methods, displayed on a storage CRO, and photographed.

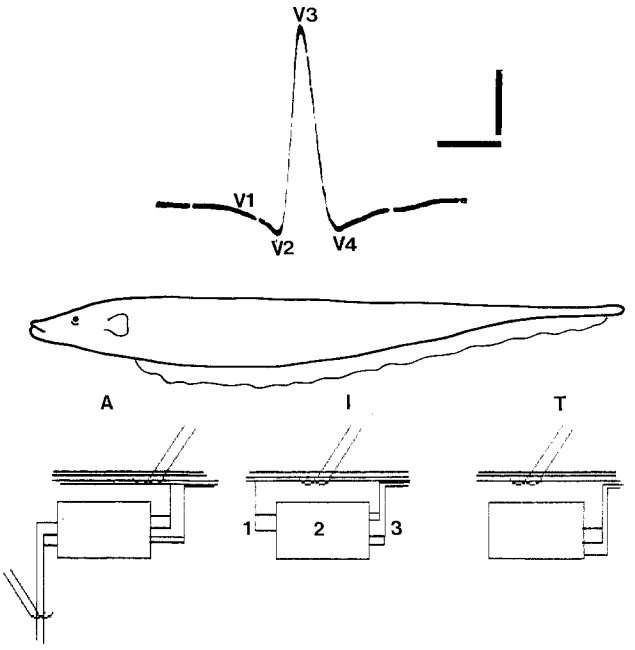

Fig. 1. Upper row: monopolar recording of the EOD (active electrode facing the animal's head) with labelled phases. *Bars*, 10 mV, 1 ms. Lower diagrams: experimental procedures at 3 portions of the EO positioned at the corresponding portions of the fish's body: *A*, abdominal; *I*, intermediate; *T*, tail. Hooks: stimulating electrodes located on the segmental and anterior electromotor nerves in A. For the 2 other portions, the stimulating electrodes were positioned on the posterior electromotor nerve. *1*, *2*, and *3* indicate the location of rostral extracellular, intracellular, and caudal extracellular recording micropipettes

Results

Anatomical studies (Trujillo-Cenóz and Echagüe 1989) have shown that the innervation of tube 1 electrocytes is not homogeneous along the EO. The results to be presented here indicate that some electrophysiological properties of these electrocytes also change at different levels of the EO. Taking into account these differences, the results regarding the physiological characteristics of electrocytes will be presented, dividing the EO in 3 portions. Within each portion, the anatomical and physiological properties of electrocytes are homogeneous. A further section is devoted to the study of the number and distribution of spinal roots supplying innervation to single DIEs located at different levels of the EO.

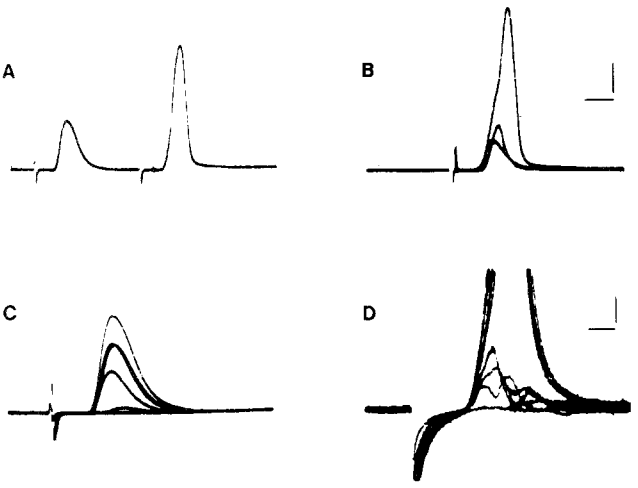

Fig. 2A–D. Intracellular responses of abdominal, doubly innervated electrocytes to stimulation of segmental (SN) and anterior electromotor (AEN) nerves. **A** The early, smaller deflection was evoked by a supramaximal stimulus to SN; the late one is an action potential elicited via the AEN. **B** Three superimposed traces are shown: the small responses are PSPs obtained successively by SN and AEN stimuli. When applied simultaneously they elicit the action potential. **C** Superposition of graded responses obtained by increasing intensities of stimulation to SN. **D** Same as in **C** but stimulating the AEN. Note that the large stimuli generate action potentials (out of the screen). **A**, **C** and **D** are from the same electrocyte. Bars in **B**, 20 mV, 1 ms; in **D**, 20 mV; 1 ms for **A**; 10 mV, 0.5 ms for **C**; 5 mV, 0.5 ms for **D**

Abdominal portion

The anatomical characteristics of this portion of the EO (Trujillo-Cenóz et al. 1984) allow us to stimulate separately the innervation of the rostral and caudal faces of DIEs (see Fig. 1).

Intracellular recordings obtained from an abdominal DIE are shown in Fig. 2. The resting potential varies between 80 and 95 mV in the 110 cells recorded. As shown in Fig. 2A, the supramaximal activation of the AEN innervating the caudal face elicits an action potential of 80 mV in amplitude. Lower intensities of stimulation to the AEN (Fig. 2D) reveal the caudal face postsynaptic potential (PSP), in which several steps of amplitude (from 5 to 7) could be recognized by changing the stimulus intensity. When the PSP attains a critical value, an action potential is generated.

On the other hand, the response to supramaximal stimulation of the segmental nerve, supplying the rostral face (Fig. 2A), consists of a depolarizing potential of 2 ms in duration and 30–40 mV in amplitude with the characteristics of a PSP. A stepped gradation in the amplitude of the rostral response (Fig. 2C) is provoked by lower intensities of segmental nerve stimulation. In this case, however, regardless of the intensity of the stimulus, we never observed a response with the characteristics of an action potential. Usually, the stimulation of one segmental nerve causes PSPs in 3 adjacent DIEs; the largest response is observed in the DIE located at the same metameric level as the stimulated nerve. Simultaneous supramaximal stimulation of the 3 segmental nerves supplying the rostral face innervation of a DIE also elicits only PSPs.

In order to study the possible interaction between the activity of both innervated faces, paired stimulation of segmental and AE nerves was used (Fig. 2B). The small depolarizations shown in the figure were elicited successively by a submaximal stimulus to the AEN and a supramaximal stimulation of the segmental nerve, each of them producing a PSP. When both stimuli were applied simultaneously, an action potential was generated, indicating spatial summation of both synaptic currents.

Extracellular recordings were performed to identify the active faces of tube 1 electrocytes during these responses. The cells adjacent to the recorded one were surgically destroyed, eliminating their contribution to the extracellular flow of currents. In some experiments the AEN was also cut just distal to the recorded electrocyte. Figure 3 shows intracellular voltage and extracellular currents obtained from a DIE while stimulating supramaximally the AEN (caudal face innervation). The intracellular activity (Fig. 3A2) consists of an action potential of 70 mV. In Fig. 3A3 the extracellular micropipette was placed facing the caudal membrane of the electrocyte. A negative-positive wave was obtained; the initial negativity indicates that the action potential is generated by the caudal membrane. During the development of the action potential the extracellular field changes its polarity from negative to positive; this can be interpreted as indicating that the regenerative response originating at the caudal membrane has secondarily invaded the rostral face of the electrocyte. The opposite picture was observed when the extracellular microelectrode was placed facing the rostral membrane (Fig. 3A1). The biphasic flow of currents is not always observed when recording from DIEs at the abdominal level. Figure 3B2 shows the results of another experiment in which an action potential was obtained in a DIE after the stimulation of the AEN. In this case the extracellular recording at the caudal membrane (Fig. 3B3) showed a large negative wave, followed by a small positivity. It is possible that the late positivity could correspond to the recharging current of the caudal membrane previously depolarized by the action potential.

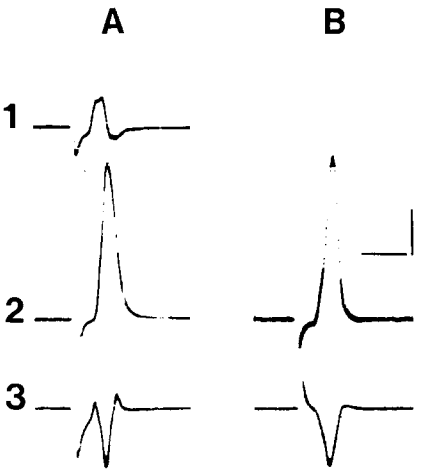

Fig. 3A, B. Intracellular action potentials (**2**) and extracellular currents (**1** and **3**) from two different, abdominal, doubly innervated electrocytes after stimulating the anterior electromotor nerve. Trace **1** was obtained by a microelectrode facing the rostral membrane (position 1 in Fig. 1); **3** corresponds to the caudal face. Positive is *up* in all extracellular records. The initial negativity observed at the caudal membrane (**A3**, **B3**) indicates that the action potential arises in this face. The late positive phase observed in **A3** (corresponding to a late negativity in **A1**) could indicate the invasion of the rostral face by the action potential. This invasion is not always present (**B3**). Bars, 20 mV for intracellular, 5 mV for extracellular, 2 ms

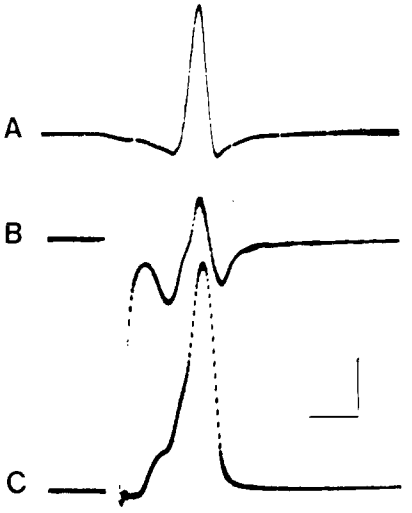

Fig. 4. **A** Monopolar recording of the electric organ discharge (EOD) in an intact *G. carapo*. The active electrode was placed just in front of the animal's head. **B**, **C** Simultaneous rostral extracellular (**B**) and intracellular (**C**) recordings obtained from an abdominal doubly innervated electrocyte. Stimuli to the anterior electromotor and segmental nerves were timed in order to obtain an intracellular response similar to that observed during spontaneous activation of the electrocyte. In this condition, the extracellular triphasic wave recorded at the rostral face of the electrocyte resembles the EOD waveform of the intact fish. *Bars*, 10 mV for **A**, 2 mV for **B**, 20 mV for **C**; 1 ms

Therefore, the secondary invasion of the rostral membrane by the caudal action potential could be an inconstant phenomenon.

Extracellular recordings were also performed during the activation of the rostral membrane via the segmental nerve; in this case the intracellular PSP is accompanied by an extracellular negativity at the rostral face (not shown). A biphasic flow of currents was never observed in this situation.

In vivo intracellular recordings from abdominal DIEs (Lorenzo et al. 1988) have shown that during the spontaneous discharge of the electrocyte, the activation of the rostral face precedes that of the caudal membrane by 0.54–1.0 ms. The extracellular flow of currents generated by this sequence of events in a single abdominal DIE was studied in vitro. In Fig. 4C, the stimulation of the segmental and AE nerves was timed so as to obtain a delay of 0.6 ms between the activation of the rostral and caudal faces. The resulting intracellular waveform was similar to that observed in intact animals during the spontaneous EOD. In these conditions the extracellular recording at the rostral face of the electrocyte (Fig. 4B) consists of a triphasic potential. The waveform and duration of this response is similar to the EOD of an intact fish (Fig. 4A) recorded monopolarly from the head using as a reference electrode a large metal mesh located under the fish's body.

Intermediate portion

This portion of the EO comprises segments (2) and (3) described in Methods, i.e., the EO underlying the swim bladder and that of the proximal tail. Although the methodological approach was different at each one of these levels, the electrophysiological properties of the DIEs were the same.

Intra- and extracellular recordings from a DIE located at the proximal portion of the tail organ are shown in Fig. 5 (similar results were obtained from 5 other DIEs). The activation of the electrocyte was provoked by electrical stimulation of the posterior electromotor nerve 0.5 cm rostral to the recorded cell. Adjacent electrocytes were surgically destroyed. Because of the complexity of the innervation at this level, separate stimulation of rostral and caudal innervation was not possible. In Fig. 5, traces 1, 2, and 3 correspond, respectively, to extracellular recording at the rostral face, intracellular activity, and extracellular recording at the caudal membrane.In Fig. 5A, a submaximal stimulus was delivered to the PEN, obtaining a small PSP in the intracellular record (Fig. 5A2). This response has a late change of slope in its decaying phase,

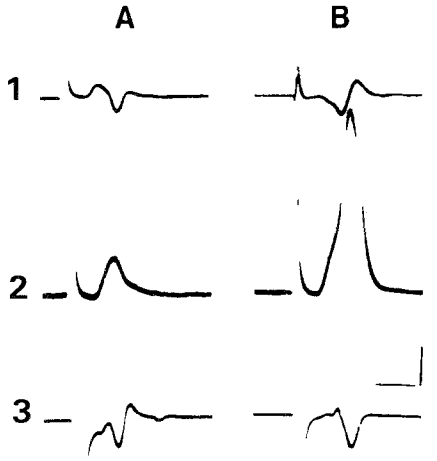

Fig. 5A, B. Intracellular potentials (**2**) and simultaneous extracellular currents (**1** and **3**) from a doubly innervated electrocyte of the intermediate portion of the electric organ, stimulated via the posterior electromotor nerve (PEN). Rostral face currents are shown in traces **1** and **3** correspond to caudal face currents. **A** Stimulation of the PEN generated an postsynaptic potential in the electrocyte; the extracellular records show that under these experimental conditions the caudal face was stimulated earlier. **B** Higher intensity of stimulus elicited an action potential. This response is generated at the rostral face, where a negative-positive extracellular flow of current was always observed. Bars, 2 mV in **A1** and **A3**, 10 mV for **A2**, **B1**, **B3**, 20 mV for **B2**; 1 ms

suggesting that it could be the result of the activation of at least two sets of presynaptic fibers. The simultaneous extracellular record at the rostral face (Fig. 5A1) shows a biphasic positive-negative sequence; this indicates that the recorded PSP is the result of the activation of both (caudal and rostral) innervations, the former slightly preceding the latter. In Fig. 5B, the stimulus intensity was increased, and an action potential occurred. In this case the currents recorded at the rostral face (Fig. 5B1) show a negative-positive waveform, demonstrating that the action potential arises in the rostral membrane, invading secondarily the caudal face. When the extracellular electrode was placed at the caudal membrane (Fig. 5A3, B3) the results confirmed that the action potential is generated in the rostral face. Although caudal activation under these conditions of stimulation precedes the excitation of the rostral membrane (Fig. 5A), it cannot be concluded that the sequence of activation is the same during the spontaneous discharge of the electrocytes. Summarizing, at this level of the EO rostral and caudal membranes are able to generate action potentials. The rostral face has a lower threshold than the caudal membrane. These results are in agreement with those reported by Bennett and Grundfest (1959).

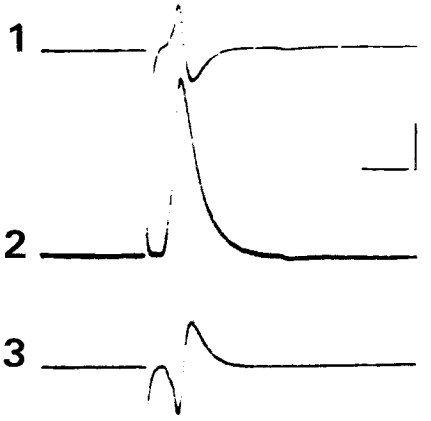

Fig. 6. Intracellular potential (**2**) and simultaneous extracellular currents (**1** and **3**) from a tube 1 electrocyte of the caudal portion of the EO. Stimulus via PEN. **1** corresponds to the rostral face and **3** to the caudal one. Note that in this case, the action potential arises first at the caudal membrane (negative-positive record). Bars, 20 mV for intracellular, 10 mV for extracellular; 1 ms

The tail portion

Intra- and extracellular records from a tube 1 electrocyte (singly innervated on its caudal face at this level of the EO) are shown in Fig. 6. An action potential was obtained by supramaximal stimulation of the PEN (Fig. 6-2). The extracellular flow of currents measured at rostral (Fig. 6-1) and caudal (Fig. 6-3) faces shows that as expected the regenerative response arose on the caudal face; the action potential later invaded the rostral membrane.

Electrocytes belonging to tubes 2, 3, and 4 are singly innervated on their caudal faces all along the EO. Intracellular recording from these electrocytes was performed at almost all levels of the EO (see Methods). Extracellular records performed on these cells show the same characteristics as tube 1 electrocytes of the tail portion. Action potentials are generated by the caudal membrane, invading secondarily the rostral face.

Spinal root stimulation

Abdominal organ. This portion of the EO was studied in two animals. In the experiment shown in Fig. 7, the 3rd abdominal DIE was impaled and 18 successive spinal roots were stimulated. Depolarizing potentials (PSPs) were obtained from the first three roots, corresponding to the segmental nerves supplying rostral innervation to the DIE (Trujillo-Cenóz et al. 1984). The third root gives the largest amount of rostral innervation. Roots

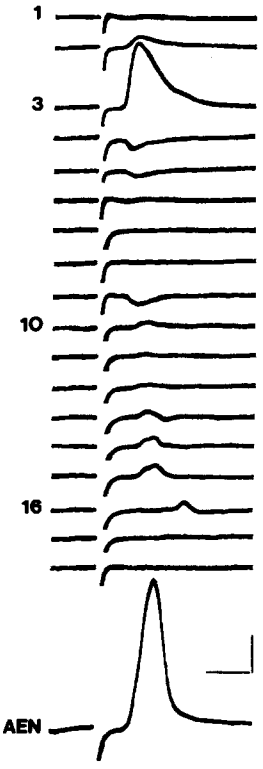

Fig. 7. Intracellular records from a doubly innervated electrocyte of the abdominal portion of the electric organ. Successive stimulation of 18 spinal roots. The largest postsynaptic potential (PSP) (**3**) was obtained by stimulation of the spinal root providing rostral face innervation. Much smaller PSPs were also obtained when roots **10** to **16** (supplying caudal face innervation) were stimulated. Note that intermediate roots (**4** to **9**) gave no response in the electrocyte; small negative deflections in these cases are produced by the activation of electrocytes adjacent to the impaled one. In the bottom trace, control stimulation of the anterior electromotor nerve elicited an action potential. Bars, 20 mV, 1 ms

4–9 give no innervation to the recorded DIE; small negative responses observed in 4, 5, 6, and 9 are interpreted (on the basis of previously shown extracellular records) as corresponding to extracellular currents generated by DIEs adjacent to the recorded one. The stimulation of the 7th root caudal to the metameric level of the electrocyte (numbered 10 in Fig. 7) again produces a small PSP. The available anatomical data (Trujillo-Cenóz et al. 1984; Trujillo-Cenóz and Echagüe 1989) leads us to interpret this response as originating via the AEN innervating the caudal face of the electrocyte. Small PSPs are also obtained by stimulation of roots 11–16. More caudal roots (17, 18) give no innervation to the recorded electrocyte. To test the physiological condition of the electrocyte, the action potential shown in the lower part of Fig. 7 was evoked at the end of the series, by applying the electrical stimulus to the AEN.

These electrophysiological data confirm the anatomical feature reported by Trujillo-Cenóz et al. (1984) regarding the shift of 7 segments between the spinal sources of rostral and caudal innervation of abdominal DIEs.

They also demonstrate that at least seven spinal roots contribute to the innervation of the caudal face via the AEN in these electrocytes. However, none of them is able to cause an action potential by itself. Therefore, the caudal face action potential observed by Lorenzo et al. (1988) in intact animals depends on the simultaneous activation of several spinal segments.

Intermediate portion. This experiment was performed in two animals. Although tube 1 electrocytes at this level are also doubly innervated, there is no anatomical information on the distribution of the spinal roots giving rostral and caudal innervation to a single electrocyte. Unlike what occurs at the abdominal level, the 9 or 10 roots contributing to the innervation of a single DIE form a continuum, without a gap of 'unresponsive' roots. Furthermore, action potentials could be evoked in the recorded electrocyte by stimulation of single roots (the one located at the metameric level of the electrocyte and the following two in the rostral direction). Small PSPs (5–7 mV) are caused by stimulation of the remaining roots.

Discussion

Our results allow us to distinguish 3 subdivisions of the EO of *G. carapo* in correspondence with those described by Trujillo-Cenóz and Echagüe (1989) on the basis of anatomical features.

At the abdominal portion, rostral and caudal innervation of DIEs can be stimulated separately. The synaptic properties observed by stimulation of the caudal innervation are very similar to those of the neuromuscular junction, except that several presynaptic fibers converge on a single electrocyte. By stimulating individual spinal roots, it was demonstrated that 7–8 roots provide the caudal innervation of a single DIE (Fig. 7). Also, when the AEN is stimulated with increasing intensities, up to 7 steps in the caudal PSP amplitude could be demonstrated (Fig. 2D). As in the neuromuscular junction, an action potential is generated when the caudal PSP reaches a critical value. At this level of the EO, caudal innervation of tube 1 electrocytes is able to provoke action potentials. Spontaneously occurring action potentials have also been recorded in vivo in these electrocytes after surgical section of the rostral innervation (Lorenzo et al. 1988).

Supramaximal stimulation of the segmental nerve, on the other hand, evokes a depolarizing potential with a maximal amplitude of 35–40 mV (Fig. 2). These potentials increase stepwise with the intensity of stimulation, but we never observed a response with the characteristics of an action potential. These depolarizing responses, interpreted as PSPs, are the physiological activity sustained by rostral membranes of abdominal DIEs during the spontaneous EOD (Lorenzo et al. 1988).

The PSPs obtained from rostral and caudal faces frequently present differences in their time courses (Fig. 2C, D). This could be related to the electrical excitability demonstratable in caudal faces and absent in rostral membranes (at least within the observed levels of depolarization). The possibility, however, of interference by electric currents originating in other electrocytes during AEN stimulation cannot be ruled out.

Summation of synaptic currents of both innervated faces could be demonstrated by simultaneous stimulation of rostral and caudal innervation (Fig. 2B); however, the physiological role of this feature is uncertain. Both in vivo (Lorenzo et al. 1988) and in vitro recordings have shown that caudal innervation alone is sufficient to generate action potentials. Furthermore, during the spontaneous EOD the activation of rostral faces precedes that of the caudal membrane by 0.5–1.0 ms (Lorenzo et al. 1988), making it unlikely that the summation of both synaptic currents occurs. Contribution to the EOD shaping seems to be the main physiological role of rostral PSPs of abdominal DIEs (Trujillo-Cenóz et al. 1984).

Our data cannot conclusively establish whether the rostral membrane of these electrocytes is electrically inexcitable, like the membrane of marine fish electrocytes (Grundfest and Bennett 1961; Bennett 1971). Extracellular recordings performed at the rostral membrane during a caudal action potential have occasionally shown a biphasic waveform suggesting that the caudal action potential could, under some conditions, actively invade the rostral membrane (Fig. 3A). There are no differences on anatomical grounds (by electron microscopy) that could explain the dissimilar electric behavior of both innervated faces (Trujillo-Cenóz and Echagüe 1989).

When rostral and caudal faces are activated, preserving the physiological delay between them, a triphasic extracellular flow of currents is observed (Fig. 4); under these conditions, a single DIE can approximately reproduce the EOD waveform of the whole fish.

At the intermediate portion of the EO, our results are similar to those described by Bennett and Grundfest (1959). The stimulation of the nerve supply of a DIE generates an action potential which arises on the rostral membrane (Fig. 5); the extracellular flow of current generates a biphasic waveform indicating that the action potential secondarily invades the caudal face. Even when the activation of the caudal face precedes that of the rostral membrane (as is the case in Fig. 5), the caudal action potential follows that of the rostral face. This indicates, as shown by Bennett and Grundfest (1959), that the rostral membrane has a lower threshold for action potential generation. The complexity of the neural network from which rostral and caudal innervations of DIEs originate at this level of the EO makes it unfeasible to stimulate them separately. As a consequence, the kind of interactions between the activities of both innervated faces could not be established. It cannot be ruled out that under physiological conditions caudal PSPs could play an important role facilitating the invasion of the caudal membrane by the rostral action potential.

At the tail portion, tube 1 electrocytes show the simplest functional properties. They are exclusively innervated at the caudal face; consequently, PSPs and action potentials originate at the caudal membrane, the rostral face being secondarily invaded by the regenerative response (Fig. 6).

The same physiological properties are observed in electrocytes belonging to tubes 2–4, all along the EO.

These data demonstrate that caudal membranes of all electrocytes (singly or doubly innervated) are able to generate action potentials. Caudal action potentials can be originated by two mechanisms, according to the type of electrocyte and portion of the EO considered. In the case of all singly innervated electrocytes and in doubly innervated cells from abdominal and tail organ, the neural activation of caudal faces generates the action potentials. In DIEs of the intermediate portion of the EO, the caudal action potential originates secondarily, by invasion of the rostral active response.

On the other hand, rostral membranes of DIEs show dissimilar electrogenic properties at different portions of the EO. They are able to generate action potentials in the body-tail portion. At the abdominal region, the neural activation of the rostral membrane generates a PSP, but the capability of this membrane to substain an action potential is uncertain.

These data, together with morphological aspects (Trujillo-Cenóz and Echagüe 1989) and biophysical measurements concerning the activity of different portions of the EO (Caputi et al. 1989), lead to a new proposal about the generation of the EOD in *G. carapo* (see Discussion in Caputi et al. 1989).

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