Neuromuscular Transmission in *Electrophorus electricus* (L.)

In many electric fishes the electric organ originates from muscle. Very little information as yet exists about the embryological development of the electric organ in *Electrophorus electricus*, in which the electrogenic process has been more fully investigated.

Concerning the mechanism of neuro-electroplate transmission in *Electrophorus*, there is general agreement that it is cholinergic in nature[1]. Nothing is known about its neuromuscular system. An investigation of the mechanism of neuromuscular transmission in this fish is not only valuable for comparative neuromuscular physiology but will provide an opportunity to seek possible differences in the mechanism of junctional transmission in muscle and in electric organ. In the present experiments, the neuromuscular transmission in *Electrophorus* was investigated.

The abductor muscle of the ventral fin was used. This muscle has an approximate diameter of 0.5 cm and its length is about 3 cm. One end of the muscle is attached to the ventral fin bone and the other is inserted into the skin of the body's side (Figure 1). Each motor nerve trunk supplies 2 neighbouring muscles before entering the muscle, and each nerve divides into a long and a short branch. The muscle was excised with its nerve trunk and was fixed in a perspex chamber at its resting length.

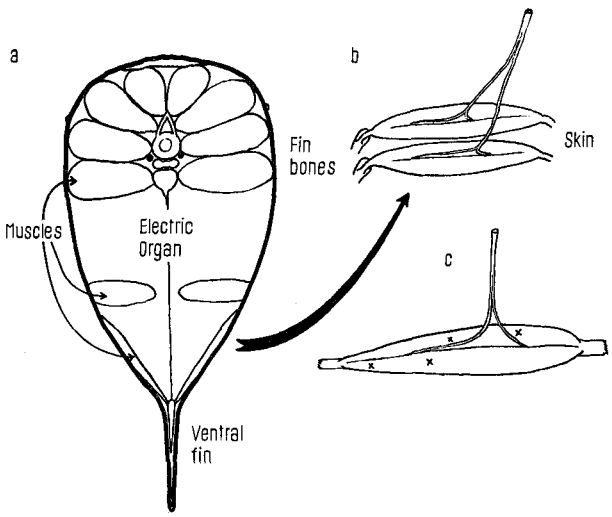

Fig. 1. (a) Cross section of the fish body behind anus. (b) Innervation of 2 abductor muscles of the ventral fin. (c) A single abductor muscle, showing the central groove and 4 focal points (crosses where the amplitude of the end-plate potential appears highest).

A saline solution with the following concentration was used: Na^+ 168.1 m*M*, K^+ 5 m*M*, Ca^{++} 6 m*M*, Mg^{++} 1.5 m*M*, Cl^- 185 m*M*, HCO_3^- 2.3 m*M*, and $H_2PO_4^-$ 0.8 m*M*. The pH of the saline solution was 7.4. Solutions with a high magnesium concentration (12–15 m*M*) were prepared by mixing appropriate amounts of isotonic Mg^{++} solution with a Mg^{++}-free Ringer solution. D-tubocurarine chloride diluted in normal saline solution was used in a concentration of $5 \cdot 10^{-7}$ w/v.

Measurements of transmembrane potentials were made with conventional KCl microelectrodes and the potential changes were recorded in a cathode-ray oscilloscope. Rectangular pulses of current delivered from an electronic stimulator and isolation unit were used to stimulate the enrve. All the experiments were performed at room temeprature (23 °C).

When the muscle was impaled with a microelectrode, an internal negativity of 93.4 mV (S.D. $\pm$ 1.5) was recorded.

By applying adequate stimulus to the motor nerve, action potentials (90–100 mV in amplitude and 1–1 1/2 msec in duration) were elicited in the muscle fibres (Figure 2). The action potential usually overshoots the zero membrane potential level by 4–6 mV.

In regions of the muscle which were found later to be neuromuscular junctions (Figure 1c), a step was seen at the foot of the action potential (Figure 2). As can easily be distinguished in Figure 2, the action potential is initiated when the step reaches a depolarization level of about 35 mV. This step was not observed in regions outside the neuromuscular junctions.

The addition of D-tubocurarine ($5 \cdot 10^{-7}$ w/v) to the solution bathing the muscle caused a complete blockade of the neuromuscular transmission in about 5 min in superficial fibres and in about 30 min in the whole muscle. The suppression of the action potential by curarization permitted us to record, in response to nerve stimulation, a

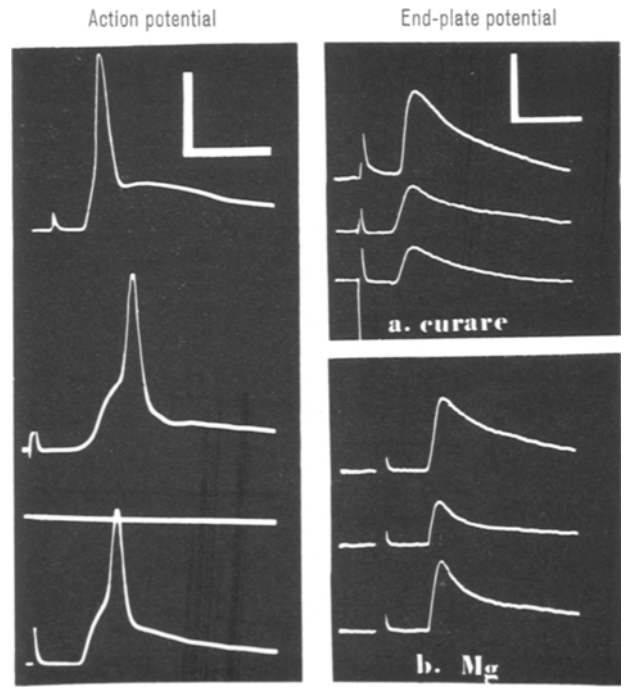

Fig. 2. Intracellular recorded action potentials in normal Ringer's solution and end-plate potentials in 2 different blocking agents. The action potential shown at the top was recorded outside the neuromuscular junction. The 2 others were recorded at the neuromuscular junction and show the 'end-plate component'. The horizontal line appearing on the action potential at the bottom indicates zero membrane potential. Calibration: horizontal, 4 msec; vertical, 40 mV. The end-plate potentials were recorded (a) in D-tubocurarine ($5 \cdot 10^{-7}$ w/v) and (b) in saline solution containing 12 m*M* of magnesium, from different fibres at various distances from the focus. Calibration: horizontal, 4 msec; vertical, 4 mV. Temperature 23 °C.

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transient decrease of the membrane potential whose characteristics are completely different from the action potential. The amplitude of this fall in membrane potential ranged from 1–10 mV in different fibres. At the neuromuscular junction its rising phase is about 1.2 msec and, after the summit, the potential falls by one half in about 3.7 msec. They are typical end-plate potentials similar to those described in the neuromuscular system of the frog[2]. Their amplitude falls and the potential changes are slowed down at points distant from the neuromuscular junction (Figure 2). During the process of curarization, it was possible to see that the step located at the foot of action potentials, recorded at the neuromuscular junctions, corresponds to the first part of the end-plate potential (end-plate component).

Summation of 2 end-plate potentials may give rise to propagated spike and twitches. The end-plate potential elicited by the second nerve volley was usually of larger amplitude than the first end-plate potential but of similar time course.

Blockade of neuromuscular transmission was also found when a saline solution containing a high magnesium concentration (12–15 m*M*) was used. Figure 2b shows end-plate potentials recorded from different muscle fibres after 5 min of equilibration in high magnesium solution. Similar results were described with magnesium in frog muscle[3]. The neuromuscular blockade observed with high magnesium solution or D-tubocurarine was completely reversible and no change on resting potential was found after the addition of these 2 blocking agents in a concentration strong enough to abolish the neuromuscular transmission. The average resting potential recorded in 20 muscle fibres in D-tubocurarine ($5 \cdot 10^{-7}$ w/v) was 94.7 mV (S.D. $\pm$ 1.7), and in high Mg^{++} (15 m*M*) 91.5 mV (S.D. $\pm$ 2.2). The average resting potential recorded in 60 muscle fibres in normal Ringer solution was 93.4 mV (S.D. $\pm$ 1.5). Calcium withdrawal also caused neuromuscular blockade, which suggests that in the neuromuscular system of *Electrophorus*, the cation also plays a facilitatory action on the release of the neuro-hormone by nerve impulse.

Although more detailed information is required about the neuromuscular system in *Electrophorus*, the results presented above seem to indicate that the mechanism of neuromuscular transmission in this fish is cholinergic and that the properties of the motor end-plate are, as a whole, very similar to those described in frog muscle.

The results also lead to the conclusion that the mechanism of neuromuscular and neuro-electroplate transmission in *Electrophorus* are similar. If the electric organ in *Electrophorus* originates from muscle as in other electric fishes, the process of differentiation does not include the mechanism of junctional transmission.

Résumé. Le système neuromusculaire dans *Electrophorus electricus* est étudié avec des électrodes intracellulaires. La transmission neuromusculaire est supprimée avec D-tubocurarine ($5 \cdot 10^{-7}$ *p*/v) ou avec un excès des ions Mg^{++} (12 m*M*) dans la solution.

W. C. DE MELLO and Y. C. CHANG

Instituto de Biofísica, Universidade do Brasil, Rio de Janeiro (Brasil), April 4, 1966.

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The Effect of Gibberellic Acid Treatment on the Alkaloid Content of Mature *Ipomoea violacea* L. Seeds

Several studies have shown that the alkaloid content of medicinal plants can be influenced markedly by treatment with gibberellic acid (GA). While in some cases such treatment has brought about an increase, it has, in others, led to a reduction of alkaloid concentration in various plant organs[1–6]. GA effects observed vary generally with the plant organ investigated and are, furthermore, dependent on such factors as the age of the plant and mode and concentration of the GA application. Studies of these effects on psychotomimetic constituents are particularly important since misuse of seeds of *Ipomoea violacea* has been reported[7–9]. The present study was initiated to investigate whether conditions for GA treatment could be found which would reduce the alkaloid content of the seeds. 11 plants of *I. violacea* L. (Heavenly Blue) were grown under greenhouse conditions (60–70 °F). The seeds were sown on March 10, 1965. The small plants were transplanted into pots and after they reached a height of 15 inches (April 13, 1965) 4 groups of 2 plants each were treated weekly with the following GA sprays[10]: 100 ppm for 2 weeks (GA [1]); 500 ppm for 2 weeks (GA [2]); 100 ppm for 9 weeks (GA [3]); 500 ppm for 9 weeks (GA [4]). 3 plants served as controls. After reaching maturity the seeds from each individual plant were collected and analysed for total alkaloids, lysergic acid amide, *iso*lysergic acid amide and clavine alkaloids by methods reported previously[11]. Morphological observations were also recorded and will be reported elsewhere. Flower production started during the first week of June, lasted for 5 weeks and reached a peak 12 weeks after the first GA treatment. The seeds were mature about 60 days after fertilization. 11 weeks following the first peak a second smaller peak in flower production was produced by all plants. The seeds from both peaks were collected separately and are referred to as first and second crop, respectively.

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