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RAPID HEAT PRODUCTION ASSOCIATED WITH EXCITATION OF ELECTRIC ORGANS OF THE ELECTRIC EEL

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Summary: By constructing a new type of thermal detector, rapid production of heat was demonstrated during the rising phase of the action potential of the eel electric organs evoked by direct electric stimulation. Following the indirect (synapse-mediated) action potential of the organs, the existence of very large and variable production of heat of unknown origin was revealed.

The electric organs of the electric eel are a quite remarkable material for physiologists studying the origin of bioelectricity (1-3). A fully grown eel (1 - 2 m in length) can generate a potential difference of 400 - 600 volts between the head and tail of the fish. The electric organs, which are the sources of this potential difference, are composed of a large number of small quadrangular compartments, each enclosing one functional unit, electroplate. Together with gelatinous substance in the compartments, these electroplates are arranged in the form of compact columns, oriented in the anteroposterior direction. Because only the innervated (posterior) membranes of these regularly arranged electroplates are excitable, the organs are capable of generating a large potential variation when all the electroplates are excited simultaneously.

There is a marked similarity between electroplates and nerve fibers in the process of action potential production (2). In both tissues, a pulse of electric current passing outwards through the excitable membrane is effective in eliciting an action potential. The process of excitation is all-or-none. The time-course of the action potential of the electroplate is similar to that seen in amphibian nerve fibers; at room temperature, the duration of the action potential of the eel electroplate is 1.5 - 2 ms (2,3).

As is well known, nerve fibers produce extra-heat when they are excited (4,5). Therefore, one would expect that excitation of electric organs might

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also be associated with rapid heat production. However, because of the slowness of the response of heat-sensors employed in previous studies (6,7), it was not possible to detect any rise in temperature of the organ associated directly with the action potential. In fact, Aubert and Keynes (7), who employed a thermistor probe for heat detection, regarded the earliest rise in temperature observed in the eel electric organ as an artefact.

In the present study, we have constructed, using the synthetic pyro-electric material, polyvinylidene fluoride (PVDF), a sensitive thermal detector specially suited to studies of eel electric organs (cf. ref. 5). By shielding the PVDF heat-sensor extensively with a thin metal sheet, we were able to completely eliminate electric artefacts arising from strong stimulating shocks and large action potentials. We have obtained irrefutable evidence for the existence of rapid heat production during the action potential evoked by direct electric stimulation. In addition, we have found that, in association with indirectly evoked (i.e. synapse-mediated) action potentials, there is a large production of heat of which the time-course is extremely variable. We surmise that this large heat production takes place in the pre-synaptic nerve fibers.

MATERIAL AND METHODS

Electric eels, *Electrophorus electricus*, usually 40 to 100 cm in length, were purchased from Worldwide Scientific Animals, Fla. After quick decapitation, the fish was cut into about 10 cm long segments. The segments were skinned and the main electric organs and the organs of Sachs were separated from the spinal column, swimming muscles and Hunter's organ. Then, the segments were sliced further into about 4 cm long pieces and the portions of the organ on one side of the connective tissue partition in the middle were removed. The pieces were kept in normal eel saline solution which had the following composition: 190 mM NaCl, 5 mM KCl, 3 mM $CaCl_2$, 1.5 mM $MgCl_2$, 10 mM D-gluse and 1.5 mM Na-phosphate buffer (pH 7.4 - 7.6).

The heat-sensor employed was constructed using polyvinylidene fluoride film (PVDF), purchased from Pennwalt Corp., Pa. The design of the thermal detector is illustrated schematically in Fig.1. It consisted of two parts: a top plate carrying the PVDF heat-sensor and a plastic box housing amplifiers. The top plate was made of 3 mm thick polystyrene with a 5 mm diameter hole in the middle. A thin (2 or 5 μm) sheet of stainless steel was glued to the upper surface of the polystyrene plate. To the lower surface of the metal sheet in the middle of the hole in the plate, 3 mm square double layer of 6 μm PVDF film was glued with a thin layer of "5 min Epoxy". The top plate was kept 1 cm above the teflon top of the amplifier housing. Both aluminum surfaces of the PVDF film were connected to the input of the operational amplifier (OPA 128) using fine wires. The upper surface of the metal sheet was covered with a 100 μm thick mylar sheet which had a 1 cm square hole in the middle. Finally, the exposed portion of the metal sheet in the middle, which constituted the heat-sensitive area of the detector, was electrically insulated with a thin layer of polyurethane ("Humiseal", Chase Corp. N.Y.).

A piece of electric organ was placed on the thermal detector. Care was taken to bring the clean, intact surface of the organ in direct contact with the heat-sensitve surface of the detector. The piece of organ was excited with electric shocks applied by using a pair of large Ag-AgCl electrodes placed near the ends of the piece (e_1 & e_4 in Fig. 2, top). Stimulating shocks employed were 10 - 100 μs in duration and 15 - 30 V in amplitude.

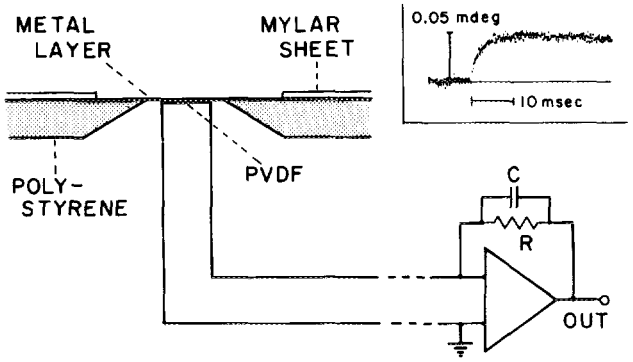

Fig. 1. Schematic diagram illustrating the design of the thermal detector employed. PVDF represents heat-sensor made of a 3 mm square double layer of 6 μm thick polyvinylidene fluoride film. The metal layer used was 2 or 5 μm thick stainless steel. The 10mmx10mm portion of the metal layer in the middle (not covered with the mylar sheet) was coated carefully with a thin layer of insulator. The values of R and C were either 1 GΩ and 0.12 nF (Fig.2) or 10 GΩ and 0.4 nF (Fig.3). The inset shows an example of records of temperature rise brought about by a brief (0.1ms) pulse of Joule heat.

The voltage variation at the output of the operational amplifier was registered photographically with a Data-6000 recorder after additional 30-fold amplification. Simultaneously, the action potentials evoked were recorded by using a pair of small electrodes placed close to the heat-sensitive area (e_2 & e_3). The thermal detector output was calibrated by utilizing Joule's heat generated by passing current pulses (10 ms duration) through a 3 mm thick 5% polyacrylamide gel (equilibrated beforehand with a 50 mM NaCl solution). The time-resolution of the heat-sensor was determined by using brief (0.1 ms) pulses of Joule heat. The sensors were found to respond to brief heat pulses with a 50% rise-time of 0.9-1.5 ms in the case of the sensors covered with 2 μm thick metal sheet (see Fig. 1, inset) and 1.5-2.0 ms for the sensors with 5 μm thick metal sheet. Most heat measurements were performed at room temperature, 22 - 23°C.

RESULTS

Fig. 2 furnishes an example of temperature changes (upper traces) in pieces of the main electric organ, recorded from the intact (either dorsal or lateral) surface, and the potential changes in the organ recorded simultaneously (lower traces). The left-hand traces in the figure were taken with the polarity and strength of the brief (75 μs) stimulating pulse adjusted to the level yielding maximal action potentials. The latency of the action potential observed was very brief (roughly 0.1 ms), indicating that the electroplates were excited directly, namely, without involving synaptic transmission. It is seen that, immediately after the delivery of the shock, there was a rapid rise, followed by a gradual fall, of the temperature of the organ. The right-hand record in the figure was taken using a stimulating current pulse of the same intensity but with its polarity reversed; in this case, the temperature rise observed was negligibly small. This indicates that the Joule heat associated with the stimulating current pulse brought about

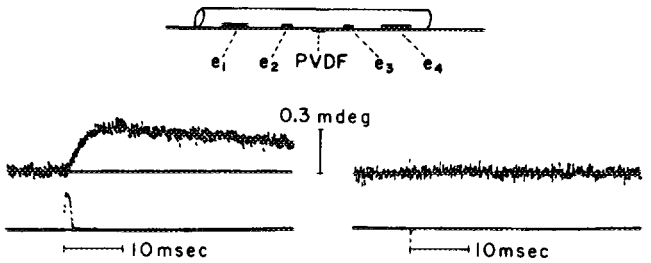

Fig. 2. Top: Diagram illustrating a piece of the main electric organ (about 4 cm in length) placed on the thermal detector. The dorsal surface of the organ was in direct contact with stimulating electrodes (e_1 & e_4), recording electrodes (e_2 & e_3) and the heat-sensitive area of the detector. Bottom left: Changes in the temperature (upper trace) and the potential difference between e_2 and e_3 (lower trace) evoked by application of a brief 30 volt pulse to the region of the electric tissue between e_1 and e_4 (-15V to e_1 and simultaneously +15V to e_4). The external (stimulating) circuit was open except during the 45μs period of stimulation. Action potential amplitude, 8.3V. The records on the right were taken from the same preparation with the polarity of the stimulating pulse reversed. 22°C.

practically no contribution to the temperature rise observed in association with action potential production. Under these conditions, no stimulation artefact was observed. (Note that the brief downward deflection of the lower right-hand trace represents the potential drop, evoked by the stimulating pulse, across the portion of the electric tissue between the recording electrodes.) Signal-averaging was not required to take these records.

The peak value of the temperature rise in the main organ evoked by direct stimulation was found to vary greatly depending on the physiological state of the electric organs. (In smaller fish, the organs appeared to be more susceptible to injury during surgery). In freshly isolated pieces of the main electric organ, the values of 0.1 - 0.3 times 10^{-3} degree centigrade were commonly observed.

When single pulses of stimulating current were applied to pieces of Sachs organ, action potentials were frequently evoked in response to pulses of both polarities (see ref.8). The latency of the action potential evoked was brief (0.1 ms or less) with current pulses of one polarity and long (1.5 - 2 ms) with pulses of reversed polarity. Long latency action potentials are brought about by indirect excitation of the electroplates involving synaptic transmission (8,2,3). We found that these indirectly evoked action potentials are accompanied by a large change in the temperature, of which the time-course varies considerably from preparation to preparation, as well as during the course of observation on one preparation. Frequently, an abrupt increase in the rate of temperature rise was seen at about the time when the action potential came to its end (see the left-hand record in Fig. 3). During the following period, the temperature may gradually fall below the level before stimulation or remain at a high, slowly varying level. On many occasions,

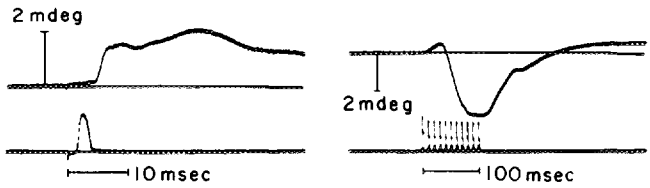

Fig. 3. Left: Record of temperature change (top) in a piece of Sachs organ, observed in association with an indirectly evoked action potential (bottom). The voltage pulse employed was 90μs in duration and 30 V in amplitude. The external circuit was open except during stimulation. Action potential amplitude, 2.5V. Right: Record taken from a different Sachs organ preparation using 100Hz repetitive stimulation. Action potential amplitude, 2V. 23°C.

temperature changes of 1 - 3 mdeg above or below the initial, resting level were encountered following the delivery of a single shock. The effect of repetitive stimulation (at frequencies between 20 and 100 Hz) was variable also. In many cases, the temperature of the tissue was seen to fall below the initial level during repetitive stimulation (see the right-hand record).

Under the present experimental conditions, it was not easy to determine the effects of pharmacological agents on heat production in the organ. Penetration of large molecules into blocks of the organ was slow and uncertain. The physiological state of the organ appeared to deteriorate gradually during a six hour period after isolation (2). Nevertheless, we could examine the effect of 0.1 mM d-tubocurarine on Sachs organ preparations of which the size was surgically reduced to about 1.5 g. After 1 - 2 hr immersion in physiological saline containing the drug, some pieces of Sachs organ could generate large heat without being preceded by a long-latency action potential. It is known that curare blocks synaptic transmission in the eel electric organ (8). On the assumption that d-tubocurarine suppresses the action of the chemical transmitter, acetylcholine, on the post-synaptic membrane, we surmise that the production of large, variable heat occurs in or near the presynaptic nerve terminals.

DISCUSSION

The thermal conductivity of stainless steel is known to be 0.035 $cal.deg^{-1}.cm^{-1}.sec^{-1}$. Undoubtedly, conduction of heat through the metal layer in our detector proceeds with great rapidity. However, since the introduction of a metal layer increases the heat capacity of the sensor and since the thermal conductivity of the electric tissue is expected to be close to that of water (0,0014), the response of the thermal detector is slightly slowed down by the introduction of a metal layer in the pathway of heat-transfer. It is to be noted, however, that the response time of the thermal detectors employed by previous investigators (6,7) is roughly two orders of magnitude longer than that of the present detector. Consequently, there is

very little in common between the experimental findings presented in this communication and those reported previously.

Quite recently, we have presented new experimental findings strongly suggesting that the heat generated during excitation of nerve fibers is derived from the Ca-Na ion-exchange process taking place in the superficial gel layer of the fibers (9). Based on the similarity in physiological behavior between nerve fibers and eel electric organs, it appears reasonable to suggest that the same explanation can be applied to the heat produced in association with the directly evoked action potential of the electric organs. Further studies are required to elucidate the origin of the heat associated with indirectly evoked action potentials of the eel electric organs.

REFERENCES

1. Albe-Fessard,D., Chagas,C., Couceiro,A., and Fessard,A. (1951) J. Neurophysiol. 14. 243-252.
2. Keynes,R.D., and Martins-Ferreira,H. (1953) J. Physiol. 119.315-351.
3. Grunfest,H., and Bennett,M.V.L. (1961) In Bioelectrogenesis. (C.Chagas and A.P.deCarvalho. Ed) pp.57-95. Elsevier Publishing Co., Amsterdam.
4. Abbott,B.C., Hill,A.V.,and Howarth,J.V.(1958) Proc.Roy.Soc.B 148.149-187.
5. Tasaki,I., Kusano,K., and Byrne,P.M. (1989) Biophys.J. 55.1033-1040.
6. Abbott,B.C., Aubert,X.,and Fessard,A.(1958) J.Physiol.(Paris) 50.99-102.
7. Aubert,X.,and Keynes,R.D. (1968) Proc.Roy.Soc.B. 169. 241-263.
8. Albe-Fessard,D., and Chagas,C. (1951) C.R.Soc.Biol.Paris. 145.248-251.
9. Tasaki,I., Byrne,P.M. (1992) Biopolymers. 32.1019-1023.