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TEMPERATURE CHANGES ASSOCIATED WITH NERVE EXCITATION: DETECTION BY USING POLYVINYLIDENE FLUORIDE FILM

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SUMMARY: By using a pyroelectric film made of stretched and poled polyvinylidene fluoride, a small, fast rise in the temperature of the crustacean nerve associated with nerve excitation was detected.

INTRODUCTION

Polyvinylidene fluoride (PVDF) is a polymer material which acquires piezo- and pyroelectric properties when heated and stretched in the presence of a high electric field (1, 2, 3). Exploiting the pyroelectric property of PVDF, radiation detectors with a very high time-resolution have been constructed (4, 5). The present communication describes the results of our experiments demonstrating that rapid temperature changes associated with excitation of crustacean nerves can be detected by using PVDF film.

The existence of a small temperature rise associated with nerve excitation has been known for some time (6, 7, 8). However, the detectors employed in those previous temperature studies - thermocouples - had a rather limited time-resolution, the time required to reach half the maximum deflection being about 100 msec. The response time of PVDF thermal probe employed in the present studies was of the order of 1 msec, and our experiments gave definite and reliable information as to the thermal response of the nerve. A number of technical problems encountered in measuring small temperature changes in the nerve by the PVDF method are discussed.

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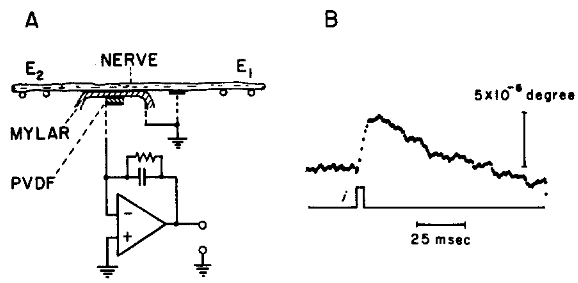

Fig. 1 A: Schematic diagram illustrating method of detecting temperature changes in nerve with polyvinylidene fluoride (PVDF) film. The size of the film used was roughly 5mm x 5mm. E's represent platinum electrodes. B: Response of PVDF thermal probe to Joule's heat generated by 1 msec long pulse of current of 0.72 mA that produced a potential drop of 1 v across 40 mg portion of a lobster nerve.

MATERIALS AND METHODS

Two types of PVDF film, 9 and 30 µm in thickness, were a generous gift of Kureha Chemical Co., Horidome-cho, Tokyo. The samples supplied had already been stretched and poled and had an about 30 nm thick aluminium layer deposited on each surface. The film has a pyroelectric coefficient of about 4×10^{-9} $coul.cm^{-2}.deg^{-1}$. It has been shown that a uniform, $1^{o}C$ change in temperature gives rise to a potential change of a few volts across the surface electrodes of the PVDF film.

The experimental setup employed is diagrammatically illustrated in Fig. 1, A. A claw nerve of the lobster, Homarus americanus, or a bundle of 3 claw nerves of the crab, Callinectes sapidus, was used. The excised nerve was immersed in artificial seawater containing 370 mM NaCl, 8 mM KCl, 50 mM $CaCl_2$ 20 mM $MgCl_2$ and 20 mM $MgSO_4$, its pH adjusted to 8 with Tris buffer. The blood vessel and connective tissue sheath were removed. The middle portion (roughly 10 mm long) of the nerve was divided further into smaller bundles of nerve fibers. This loosened portion was placed on a PVDF film attached to a 4 µm thick Mylar sheet fixed to a Teflon plate. The surface electrodes of the film were connected to an operational amplifier, AD515 (Analog Devices), located underneath the Teflon plate. The feedback capacitor in the circuit was 5×10^{-11} farad and the shunting resistance was 10^{10} ohm. The output of the amplifier was led to a signal averager (Nicolet Instrument, Model 1072) through an A.C.-coupled amplifier with a 60 db gain in the range between 250 and 0.2 Hz. Electric shocks, applied to the nerve through platinum electrodes, were repeated at intervals of 1 sec.

The electric resistance of the PVDF film is of the order of 10^{11} $ohm.cm^{-2}$. Because of this high resistance, large artefacts could be generated, when the nerve was excited, by virtue of electrostatic coupling between the nerve and the amplifier input. It was found that this type of artefacts could be eliminated by shielding the PVDF film with a thin aluminium layer deposited on a large and thin (4µm) Mylar sheet. When a very thin layer of Devcon epoxy resin was employed to glue the aluminium layer on the Mylar sheet to the PVDF film, secure electric contact could be established between the two metal layers.

Another source of artefacts was derived from the piezoelectric property of the PVDF film. Because of this property, mechanical movements of the nerve during action potentials (9, 10) could produce signals that do not reflect temperature changes. It was found that these artefacts could be

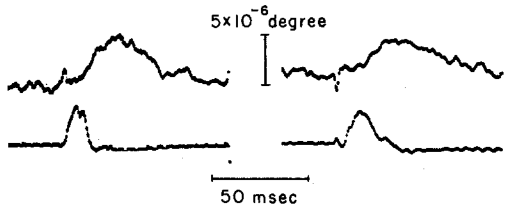

Fig. 2 Upper trace: Temperature changes in lobster nerves associated with propagated impulse. Lower trace: Externally recorded action potentials. 5^{o}C.

eliminated simply by keeping the nerve fibers on the PVDF film in a completely relaxed, more-or-less wavy form. [Note that the latency of the mechanical response is very short, whereas the thermal response of the nerve has a relatively slow rising phase (see later).]

The PVDF thermal detector was calibrated, at the end of each measurement, by using short pulses of electric current passing through the nerve. The voltage produced by the applied current was measured with separate platinum electrodes located near the PVDF film. The temperature rise at the end of an applied pulse, ΔT, was estimated simply by using the equation $\Delta T = (v.\ i.\ t)/(J.\ w)$, where v is the voltage, i the current intensity, t the duration, J the mechanical equivalent of heat and w the wet weight of the nerve between the two voltage-measuring electrodes. As expected, the magnitude of the observed deflection of the detector was independent of the direction of current passing through the nerve. The duration of the current pulse employed between 1 and 10 msec. An example of the temperature changes produced by current pulses is presented in Fig. 1, B. It is seen that the temperature rose rapidly during the period of current flow and fell gradually during the following period. Under the conditions of the present experiment, the thermal relaxation (half-maximal) time observed was usually between 30 and 60 msec.

The thermal detector described above had a high level of noise in its output. In addition to the Johnson and 1/f noises of the amplifier, we saw that room light, thermal disturbances and mechanical vibrations of the detector, as well as the humidity of the air around the input of the operational amplifier, could generate large, irregular potential variations in the output of the amplifier. To avoid thermal effects of the room light, both the probe and the operational amplifier were introduced into a metal box. In order to reduce mechanical disturbances, the box was placed on a "Servabench" (Barry Controls). To lower the humidity, dehumidified air was circulated gently in the metal box. Detection of temperature changes in the nerve was commenced only after the noise level of the detector became stationary. Most of the experiments were carried out at 5^{o}C. In most cases, the signals were averaged over 32 to 128 trials.

RESULTS

Two examples of the records obtained by the use of the PVDF thermal detector are shown by the upper trace in Fig. 2. The lower trace in the figure represents the action potential of the nerve. It can be seen that the rate of rise and fall of the upper trace is far smaller than that of the externally recorded action potential. The latency of the mechanical changes in the nerve associated with a propagated nerve impulse has been shown to be very similar to that of the action potential (9, 10). Therefore, the records taken with the PVDF film are quite distinct from the electric or mechanical responses of the nerve.

From the thermal conductivity ($1.3 \times 10^{-3} w.cm^{-1}.deg^{-1}$) and the heat diffusivity ($0.54 \times 10^{-3} cm^2/sec$) of the PVDF film used, it is expected that the response time of our 13 μm thick temperature probe is of the order of 1 msec. The rapidity of the response to a brief heat pulse (Fig. 1B) also indicates that the observed response of the nerve recorded with the PVDF film is actually a reflection of thermal processes involved. The claw nerve employed contain a large number of nerve fibers, large and small. Asynchronous arrival of the nerve impulses at the site of thermal detection is considered as the major factor contributing to the slowness of the temperature rise. With the experimental setup illustrated in Fig. 1, the time required to reach the peak of the thermal response at $5^{\circ}C$ was about 9 msec.

The magnitude of the temperature rise observed varied between 2 and 8 μdeg (measurements on about 25 nerves at temperatures between 2 - $20^{\circ}C$). There was no clear difference in the results between lobster nerves and crab nerves.

In most of the records obtained, the falling phase of the thermal responses associated with a propaged impulse was very similar to that of the response evoked by a brief heat pulse. Analyses of thermal responses were complicated by the temporal dispersion of propaged nerve impulses travelling along individual fibers. To examine the time course of the exo- and endothermic processes during the rising and falling phase of the action potential, the use of multi-fiber preparations presents serious difficulties.

The importance of measurements of the heat produced by the nerve has been emphasized by previous investigators (6-8). It is quite within the realm of possibility that the exothermic process of replacing calcium ions in the nerve with univalent cations (11) is at the base of the phenomenon of nerve swelling (10). It seems worthwhile, therefore, to pursue the relation between the phenomenon of swelling of nerve fibers during excitation and the temperature rise described in this communication.

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