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DEMONSTRATION OF HEAT PRODUCTION ASSOCIATED WITH SPREADING DEPRESSION IN THE AMPHIBIAN RETINA

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Summary Using thin film of synthetic pyroelectric material, polyvinylidene fluoride, sensitive heat-sensors were constructed for the purpose of detecting heat production associated with the phenomenon of spreading depression in isolated amphibian retinae. Measurements with these sensors revealed the existence of large heat production, which closely follows the electrical sign of spreading depression. Studies of the effects of chemical agents known to affect spreading depression have demonstrated the usefullness of heat measurements.

Spreading depression observed in the vertebrate central nervous system is a remarkable phenomenon which involves extremely slow spreading of reversible depression of electrical activities of nervous elements (1 - 5). Although this phenomenon has been studied extensively by recording both electrical and optical changes in the cerebral cortex and in the retina, the mechanism of production of the phenomenon has not yet been completely understood (6). The present report describes our experimental findings demonstrating the existence of large heat production associated with spreading depression in the amphibian retina.

In recent years, we have developed a technique of constructing a sensitive thermal detector using a thin film of synthetic pyroelectric material, polyvinylidene fluoride (7,8). The detectors we have constucted are well suited for detecting the heat generated by a flat tissue with a large area, because the heat-sensitive area of the detector can be adjusted to the area of the tissue under study. Using isolated retina of the bullfrog or toad in contact with a heat-sensor of this type, we found that a large amount of heat is produced at the onset of depression and that the time-course of heat production closely follows that of the potential difference across the retina. Taking heat production as an index, we have examined effects of various chemical agents known to affect the process of spreading depression. The results of these heat measurements throw fresh light on the physico-chemical nature of the process of initiation of the phenomenon.

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Materials and Methods

Isolated retinae of the bullfrog, *Rana catesbeiana*, and of the toad, *Bufo marinus*, were employed. In Ringer's solution bubbled with 95% oxygen and 5% carbon dioxide, the retina was detatched from the sclera of an eye-cup, usually free of the pigment layer. The Ringer's solution used had the following composition: 30 mM NaCl, 30 mM Na-isethionate, 2.5 mM KCl, 1.0 mM $CaCl_2$, 1.5 mM $MgSO_4$, 30 mM $NaHCO_3$, 1.0 mM NaH_2PO_4 and 20 mM D-glucose (pH 7.6-7.8). The retina was kept in Ringer's solution for 30 min or longer before use.

The design of the thermal detector was substantially the same as that employed previously (7,8). A 3x6 (or 2x4) mm^2 piece of pyroelectric film (purchased from Pennwalt Corp, Pennsylvania) was folded in the middle to form a square double-layer configuration. The film was glued to the metalized surface of a 4 μm thick Mylar sheet covering a 10x10 mm^2 hole in the middle of a plastic plate (see Fig.1, top). The upper surface of the Mylar sheet was coated with a thin layer of Urelane 8367 (Urane Products Co.). The two aluminum layers deposited on the surfaces of the pyroelectric film were connected, by way of an appendage, to the input of an operational amplifier (OPA 128) used as a current-to-voltage converter. The output of the amplifier was led to a digital recorder (Nicolet, model 1070) after a 100-fold DC amplification.

The thermal detector was provided with a pair of stimulating silver electrodes located approximately 1.5 mm away from the heat-sensitive area of the detector (see Fig.2, top). An isolated retina, roughly 11 mm in diameter, was placed in the detector in such a manner that both the heat-sensitive area and the stimulating electrodes were in direct contact with the external (receptor) surface of the retina. The detector carrying the retina was placed in a metal box kept at room temperature (20 - 23^{o}C). Thermal recording from each retina was repeated at intervals of about 30 min. During the resting period between successive recordings, the retina in the detector was kept submerged in Ringer's solution.

The output voltage of the operational amplifier is proportional to the rate of rise of the temperature of the surface of the retina in contact with the heat-sensor; its amplitude is determined by the pyroelectric coefficient of the film (about 25 $\mu coul.deg^{-1}.cm^{-2}$) and the feedback resistance of the amplifier (8 GΩ). The detector was calibrated using the procedure described previously (7,8).

Results

Fig. 1 shows two examples of thermal changes in the retina elicited by a short train of light pulses delivered through a glass plate covering the detector. In the record on the left, the first arrow indicates the delivery of five light pulses (550 nm in wavelength, about 60 $\mu W/cm^2$ in intensity and 2 ms in duration); only a heat signal associated with the radiant energy of the applied light stimuli was observed. The second arrow in this record represents the delivery of ten light pulses of the same intensity; it is seen that a burst of heat, which could be readily distinguished from the stimulus artefact, was evoked by the light pulses. The record on the right shows that a train of light pulses delivered to the retina shortly after the end of a heat burst was totally ineffective in eliciting a second heat burst. These findings strongly suggest that the observed heat bursts are, in fact, thermal manifestations of the phenomenon of spreading depression. It is well known

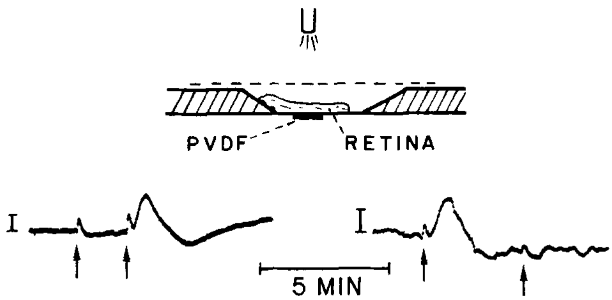

Fig. 1. Thermal changes in isolated retinae evoked by light pulses. The retinae used had been conditioned by immersion in modified Ringer's solution. PVDF represents a heat-sensor constructed with thin polyvinylidene fluoride film. Left: At the first arrow, the retina was stimulated with five light pulses repeated at 0.5 s intervals (subthreshold). At the second arrow, ten light pulses of the same intensity were delivered (suprathreshold). Right: both at the first and second arrow, ten light pulses were delivered.

that the generation of spreading depression is an all-or-none process which is followed by a long refractory period.

The records presented in Fig. 2 were obtained by using electric stimuli (10 pulses of about 15 V in amplitude and 2 ms in duration). By placing a pair of non-polarizable (Ag-AgCl-agar) electrodes across the retina (see the diagram in Fig. 2, left), it was possible to record changes in the potential difference simultaneously with changes in the output voltage of the heat-sensor. With the present experimental arrangement, the heat-sensor was located between the stimulating and recording electrodes. Under these conditions, the thermal changes invariably preceded the onset of the potential change. The polarity and the magnitude of the observed potential change were close to what is expected on the basis of previous studies (4).

Fig. 2, right, shows an example of the records obtained by using a thermal detector with two small heat-sensors (each 2x2 mm^2 in size) separated

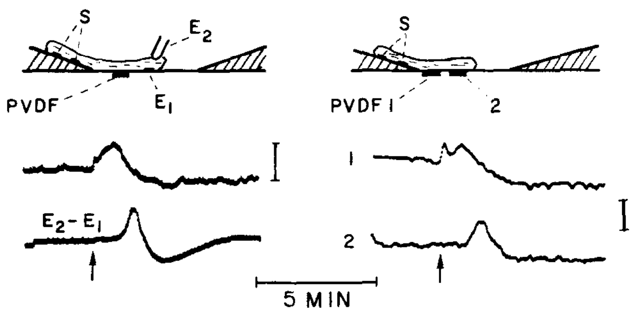

Fig. 2. Left: Thermal changes in a bullfrog retina generated in response to strong electric stimulation (top trace), recorded simultaneously with the potential difference across the retina (bottom trace). S represents a pair of stimulating electrodes (2 mm apart). E_1 represents a wet filter paper connected to an agar electrode. The potential variation (between E_1 and E_2) was about 1.2 mV. Right: Thermal changes in a bullfrog retina recorded simultaneously with two heat-sensors (PVDF 1 and 2).

by a space of about 1 mm. It is seen that the peak of the upper trace (i.e. the record taken with the sensor close to the stimulating electrodes) preceded the peak of the lower trace by about 0.8 s. These measurements indicate the site of enhanced heat production travels along the retina at roughly 4 mm/min, namely at the speed at which spreading depression is known to travel (5,9). These findings prove that the production of spreading depression is associated with a readily detectable increase in the rate of the temperature rise of the retina.

In most preparations studied, the duration of the positive phase of the thermal change associated with spreading depression was between 1 and 2 min. The maximum value of the rate of temperature rise varied between 5 and 15 mdeg/min. The wet weight of the retinal preparation used, including remaining vitreous humor and Ringer's solution, was usually between 25 and 35 mg. Assuming that the specific heat of the retina to be 0.9 $cal.g^{-1}.deg^{-1}$, the heat generated by the retina during the positive phase of the thermal change is estimated to be roughly 0.3 mcal/min. The positive phase of the thermal change was followed by a negative phase during which the temperature of the retina fell, sometimes below the steady temperature level before the onset of spreading depression.

By taking the generation of a heat burst as an index, effects of several chemical agents known to affect spreading depression in the retina were examined. The results obtained were, in general, in good agreement with those of previous studies performed by Martins-Ferreira (9) and others. Among the agents examined, the effects of elimination of Ca-ions in the medium attracted our special attention. Ca-free Ringer's solution was prepared either by simply eliminating the Ca-salt or substituting the Ca-salt with an equivalent amount of Mg-salt. Retinae immersed in such a medium were incapable, as Hanawa et al (11) have already shown, of generating spreading depression. This effect was reversible. A point of great interest is that the heat bursts generated in retinae pre-treated with Ca-free Ringer's solution were followed frequently by a large negative heat production. An important problem for future investigation is to elucidate the origin of this positive and negative heat production associated with spreading depression.

Discussion

The rate of oxygen consumption by the frog retina at rest reported by Sickel (10) is 0.235 µmol/h. By taking the value of 5 cal per cm^3 of oxygen as the heat produced by oxidation of glucose, it is found that the frog retina, in its resting state, produces heat at the rate of 0.44 mcal/min, which has the same order of maginitude as the maximum rate of heat production associated with spreading depression. We thus find that the energy expenditure of the retina is roughly doubled when a wave of spreading depression is evoked in the retina. The present heat measurements provide new information concerning the energy metabolism during spreading depression.

Quite recently. the behavior of diffusion-dependent traveling chemical waves in a variety of solutions has been investigated extensively by a number of chemists and mathematicians (12). These chemical waves in inanimate systems represent transient changes in macroscopic variables, such as concentrations, temperature, etc. At the wave front, there is a transition from one (non-equilibrium) steady state of the system to another. Undoubtedly, there is a close analogy between these waves in inanimate systems and the phenomenon of spreading depression in the nervous system. It is probable that many aspects of the theories proposed to explain the behavior of traveling chemical waves are applicable to the processes taking place in the nervous system.

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