Progress Report

The Stimulation of Crayfish Abdominal Nerves by Light and Dye.

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MASTER

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INTRODUCTION

An understanding of the mechanisms involved in the visual excitation process is difficult to achieve because of the complexity of most photo-receptor systems. One approach to the problem has been an attempt to produce a simple model system by sensitizing nerves or nerve-muscle preparations to light by staining with vital dyes (Chalazonitis, 1964; Rosenblum, 1960). Chalazonitis had experimented with vitally stained Sepia axons and the naturally pigmented neurons of Aplysia. Underlying these experiments is the hypothesis that the initiation of the nervous impulse is due to some chemical reaction, perhaps an oxidation-reduction, at the membrane surface. There are some indications that the dye molecules may be adsorbed on the membrane structure and be functionally operative in the photoactivation process. (Chalazonitis, 1964) It is also known that irradiated dyes sometimes react with oxygen of the air to form singlet oxygen.

We wished to see if these observations could be repeated on other more readily available nerves. The sciatic nerve of the frog and the abdominal nerve cord of the crayfish seemed to meet our requirements but, since the frog nerve is more complex and there seems to be less certainty of dye penetration, we soon switched to the study of the crayfish nerve cord exclusively.

If we were successful in achieving photoactivation of the dyed nerve, we could then test the hypothesis that singlet oxygen was involved, directly or indirectly, by generating it chemically and introducing it into our preparation to see if it would elicit a nervous impulse. If an oxidation-reduction reaction is indeed involved in the initiation of the impulse, then we might try a variety of oxidizing and reducing agents to see what effects they might have upon the nerve.

EXPERIMENTAL

Crayfish obtained locally were decapitated and the ventral nerve cord from each specimen was removed, tied at the anterior end with silk thread, and placed in cold Van Harreveld's solution prior to experimentation.

The nerve impulse was picked up with two Ag-AgCl wick electrodes, one of these being the ground electrode. The signals were led into a Textronix P-22 Pre-amplifier and thence into a cathode ray oscilloscope. Although in some of the earlier experiments we had used an electronic flash lamp or a high

intensity mercury vapor lamp, in the experiments described here the light source was a sealed beam headlamp controlled by a variable voltage power supply. The nerve was placed on a paraffin bed in the center of a metal stage through which cold water was circulated. A beam of light about 2 mm in diameter could be focused on the nerve, but there was considerable light scattering over the white paraffin background. All manipulations were carried out inside a wooden box shielded with metal screen.

The abdominal nerve cord of the crayfish shows spontaneous electrical activity. In some cases there are so many nerves firing and the frequency is so great, that it would be difficult to analyze any changes which might be induced in our experiments. Consequently, we had to take some measures to try to reduce the spontaneous activity. This is the reason for storing the nerve in cold saline solution and keeping it on a cold stage during experimentation. Another attempt to control activity was made by varying the potassium ion concentration of the saline solution. A solution for crayfish has been described by Van Harreveld and has been used by Kennedy and others. However, Kennedy (1960) reported using lower concentrations of potassium in an attempt to reduce activity of the nerve cord. There seems to be some variability in the effect of higher or lower concentrations of potassium ions (Prosser, 1943). Prosser has reported using higher concentrations to reduce spontaneous firing.

The dyes used in these experiments were riboflavin and crystal violet. The riboflavin was a 1:10 dilution of a saturated solution. A saturated solution of crystal violet was diluted until it would transmit light.

The sixth abdominal ganglion of the crayfish ventral nerve cord is known to be photosensitive (Prosser, 1934; Kennedy, 1957, 1958a, b.) In preliminary experiments we tested this response by illuminating the 6th ganglion and found that in most cases we could detect an increase in frequency of firing upon illumination. In cases where we were not successful the nerve was too active for us to analyze the discharges. After photoresponses had been obtained from the sensitized nerve, subsequent experiments were performed on nerve cords from which the sixth ganglion had been cut off in order to eliminate the possibility that this ganglion might be responding to scattered light on the paraffin stage.

RESULTS AND DISCUSSION

The results reported here should be regarded as preliminary because of the few cases studied. The time of our study ran out just as we began to obtain some interesting data. Photoresponses were obtained from nerves stained with riboflavin and with crystal violet. In addition, a response was obtained when hydrogen peroxide was added directly to the nerve.

1. Riboflavin. High concentrations of riboflavin are apparently lethal. Two nerves which had soaked in saturated solutions ceased all spontaneous activity. However, nerves soaked in a dilute riboflavin solution and illuminated in the region of the 3rd or 4th ganglion show a marked increase in the frequency of spontaneous firing. After the light is turned on, there is a few seconds latency before the speed up. After the light is turned off, there is a continued discharge for about 40-50 seconds before the firing returns to the "resting" frequency. This effect was seen in at least three different nerves. However, the sixth ganglion was still intact in these preparations and we had not eliminated the possibility that the photoeffect could be due to the receptors in that ganglion. Subsequent experiments, however, would seem to indicate that the sixth ganglion was not implicated in the effects that were observed.

2. Crystal violet. Two different types of responses were obtained from nerves stained with a dilute solution of crystal violet. The sixth ganglion had been removed from each of these nerves.

a. An increase in frequency of pulses. After illumination began, there was a latency of from 2-5 seconds before there was a sudden burst of pulses which lasted for about 15-20 seconds. If illumination continued beyond this time, the nerve still returned to its former firing rate, apparently becoming light adapted. This signal could be repeated many times provided there was a sufficient dark interval between successive illuminations. The increase in pulses seemed to be due to the firing of new units.

b. An inhibition of spontaneous firing. In another nerve which was firing at a countable rate, the number of impulses decreased markedly a few seconds after the onset of illumination. After some 30 seconds of illumination, the nerve returned to its former firing frequency, apparently becoming light adapted. After being returned to darkness, the nerve would respond to a new burst of light in the same way: In fact, after this nerve was removed

from the stage, and stored for several hours in cold Van Harreveld's solution, then tested again, it still gave the same response to light.

The studies described seemed to indicate that the crayfish abdominal nerve cord could be sensitized to light by means of dye. If the interaction of the light and dye somehow involves an oxidation-reduction reaction which in some way is responsible for the initiation of the neural impulse, then perhaps the same effect could be achieved by the direct application of an oxidizing or a reducing agent. It was decided to test the effects of a hydrogen peroxide solution on an unstained nerve. When an approximately one percent solution of H_2O_2 was dropped on the nerve there was an immediate increase in the number of spikes. After the peroxide was removed, it was replaced with Van Harreveld's solution for a few minutes. The excess solution was taken off and the nerve tested again. Spiking frequency had returned to near normal. This procedure was repeated several times and each time spiking would increase upon the addition of the peroxide. Although the "resting" frequency would decrease after the nerve was bathed in VH, it seemed to be somewhat higher after a number of treatments than at the beginning of experimentation. At the end of the testing period, the nerve was observed under the stereomicroscope and bubbles were present under the nerve sheath, being especially abundant in the region of the 2nd and 3rd ganglia.

The meaning of the results with H_2O_2 is not clear since there are probably a number of things that could be happening. The peroxide could be acting as a reducing agent for some molecule in the membrane, thus producing some chemical change which results in a depolarization of the membrane and a consequent initiation of a nerve impulse. Oxygen could be released in sufficient quantity to collect beneath the nerve covering. However, H_2O_2 might also be acted upon by catalase which may be present in the nerve tissue. This reaction would also result in the production of oxygen. Since an oxidation-reduction reaction is also involved in this case, such a reaction could also affect the cell membrane. Perhaps the peroxide could react with some intermediate substance which in turn initiates a reaction in the membrane. Our present results do not yet provide sufficient support for any hypothesis.

The procedures outlined in this paper seem promising, since we have apparently elicited two types of response which are known to occur in visual systems. However, the experimental conditions need to be standardized in

order to insure reproducibility of results. For example, we should control the light intensity, temperature, ion concentration of solutions and dye concentrations. We should also try the effects of some other dyes.

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