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CONTRACTILE RESPONSES OF DENDRITES IN THE BULLFROG RETINA

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Summary: Isolated retinae of the bullfrog were found to "contract" when excited either by electric stimulation of the optic nerve or by light stimulation of the photoreceptors. Involvement of the dendrites of the ganglion and amacrine cells in these contractile responses is suggested.

The frog retina is of interest to physiologists studying properties of neurons and synapses in the vertebrate central nervous system, since it is readily accessible to experimental manipulation. It is known that, in the inner plexiform layer in the frog retina, the dendritic arbors of the individual amacrine and ganglion cells spread over an expanse of 0.5 mm or more (1,2). In this paper, experimental findings are described suggesting that the long dendrites in the bullfrog retina "contract" when they are excited. These findings are in agreement with the view (3,4,5,6) that a change in the mechanical properties of the cell is a basic component of the excitation process.

MATERIALS AND METHODS

Isolated retinae of the bullfrog, Rana catesbeiana, were used. After dark-adapting the animals, the eyes were excised together with the connected optic nerve under illumination with dim red light. A piece of retina, that was in the shape of a 10 mm high equilateral triangle and was connected to the optic nerve in the middle of the about 8 mm wide base, was isolated. The lateral portions of the retina were clamped with plastic plates, and were fixed to the wall of a chamber filled with oxygenated Ringer's solution. The upper end of the retina was connected to a sensor by a thin thread. The optic nerve was passed through a hole in a plastic partition across which a pair of Ag-AgCl electrodes were placed. The separation between the optic disk and the partition was about 3 mm (Fig. 1, left).

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Contractile responses of the retina were detected with a piezoelectric sensor (purchased from Gulton Industries, Inc.) connected to the upper end of the thread (7). An initial tension of 300-400 mg was applied to the retina by displacing the sensor upwards; precaution was taken not to apply tension to the retina by way of the optic nerve. The piezoelectric sensor was 25 mm long, about 1.8 mm wide and weighed approximately 0.135g. The resonance frequency of the sensor was roughly 1.6 kHz. The electric charge generated by the sensor in response to a load applied near the tip was approximately 4.6×10^{-9} coulomb per gram weight. The charge generated was measured with an operational amplifier, AD515, in a current-voltage converting configuration. The feedback resistance of the amplifier was 2 or 5 times 10^9 ohms with a parallel capacitor of 1 pF. The output of the operational amplifier was led to a signal averager (Nicolet Model 1070) after 100-fold amplification with a capacity-coupled amplifier with a time-constant of 2 sec. Signals were recorded after averaging over 4-8 trials.

When a current is generated by the piezoelectric sensor in response to a force changing with time, a voltage is produced at the amplifier output that is proportional to the rate of change in the force multiplied by the feedback resistance of the operational amplifier. Most of the records presented below were obtained by this "differential method". The force-time curves were generated by integrating the signals obtained by the use of the integrating device of the signal averager.

Light pulses used for stimulating the retina were obtained from a quartz-iodine lamp used in conjunction with an electromagnetic shutter, a heat filter, an interference filter and a neutral-density filter. The intensity of the light employed was calibrated with a E. G. & G. Photometer (Model 550). The Ringer's solution used contained 110 mM NaCl, 1.5 mM KCl, 1.0 mM $CaCl_2$, 10 mM glucose and 5 mM Hepes (at pH 7.4). Precautions were taken to prevent mechanical disturbances from reaching the recording system. The room temperature was between 20 and 21°C.

RESULTS

Fig. 1 shows typical records of the mechanical changes in the bullfrog retina evoked by electric shocks applied to the optic nerve. In Record A, the top (smooth) trace represents the time course of the tension of the retina

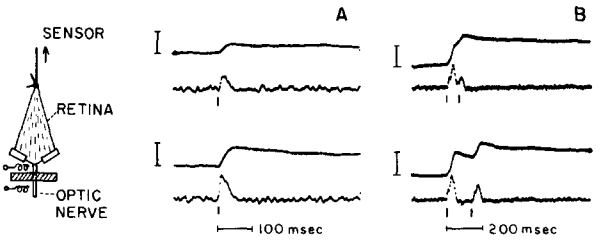

Fig. 1. Left: Schematic diagram of the experimental setup employed for recording contractile responses of the bullfrog retina (see Methods for details). A: Contractile responses evoked by electric stimulation of the optic nerve with a shock of 1 V in amplitude (top) and with a 12 V shock (bottom). The bars on the left represent a 0.01 mg rise in the tension. The time-derivatives of the responses are shown by the noisy traces. B: Summation of contractile responses evoked by two electric shocks at intervals of 60 (top) and 130 msec (bottom). The vertical bars represent 0.013 mg rise in the tension.

following the delivery of a weak shock. The noisy trace shown below represents the time derivative of the upper tension-time curve. The pair of lower traces in Fig. 1A were obtained using a much stronger shock. Since the majority of fibers in the optic nerve are non-myelinated (1,8), it was found necessary to raise the intensity of the 3 msec shock to about 10 V to obtain the maximal effect. No signs of shock artefacts were observed under the present experimental conditions. These records illustrate that the tension of the retina started to rise approximately 10 msec after the end of the shock. At high shock intensities, the tension reached at its peak (45-50 msec after the onset) was usually 8-20 μg weight. The falling phase of the tension-time curve was very long, the half-maximal time being roughly equal to or longer than 0.4 sec.

Records in Fig. 1B demonstrate that, when two electric stimuli are delivered to the optic nerve with short intervals, the contractile responses "summate." A similar summation of contraction has been reported in crab nerve fibers (7). It was found that non-myelinated fibers swell (i.e., expand laterally) during the period of enhanced membrane conductance following stimulation, and that the swelling is associated with a long-lasting lengthwise shortening of the fibers. The duration of the rising phase of the shortening response was comparable to the duration of the action potential. We believe that the summation phenomenon observed in the stretched retina is analogous to that observed in crab nerve fibers (7).

It is known that electric stimulation of the optic nerve evokes antidromic action potentials in the ganglion cells (9). The antidromically evoked contractile response of the retina was not affected by light-adaptation. In addition, application of a 10 mM Na-aspartate, which is known to interrupt synaptic transmission between the photoreceptors and the bipolar cells (10), had no effect on these responses. Similarly, a 2 mM Na-cyanide failed to influence these responses. All these findings are consistent with the interpretation that these contractile responses derive from excitation of the dendrites of the ganglion cells. The mechanical responses of the cell

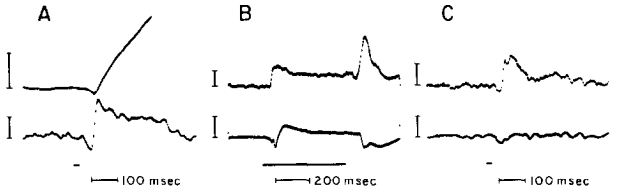

Fig. 2. A: Contractile response of dark-adapted retina evoked by a 10 msec light pulse (indicated by the short horizontal bars). The vertical bars represent 0.01 mg for the tension-time curve (top) and 0.1 mg/sec for the time-derivative (bottom). B: Contractile response (top) and action potential led from the optic nerve (bottom) evoked by a 500 msec light pulse. The bars represent 0.1 mg/sec for the time-derivative of the tension-time curve (top) and 0.1 mV for the action potential trace (bottom). C: Contractile responses (time-derivatives) recorded from a dark-adapted retina before (top) and after (bottom) application of a 2 mM Na-aspartate Ringer. The bars represent 0.1 mg/sec.

bodies are known to represent an increase in the volume (11,12) which is expected to make a negative contribution to the observed rise in the tension. The contribution of the optic nerve fibers in the retina cannot be completely excluded; but, it is probably very small because the sum of the lengths of all the dendrites is expected to be far greater than that of the nerve fibers.

Fig. 2 shows the records of contractile responses of the dark-adapted retina to light pulses. The light stimuli employed were 500 nm in wavelength and about $6 \times 10^{-6} W/cm^2$ in intensity. The contractile responses observed under these conditions lasted much longer than those evoked by antidromic stimulation. Records in A were taken by using a brief light pulse, the top trace representing a tension-time curve and the bottom trace being its time derivative. Records in B were obtained by using relatively long light pulses. It was found that d-tubocurarine, which is known to enhance the electric off-respnses (13), also enhanced the mechanical off-response (not shown). Record C in Fig. 2 shows that a 2 mM Na-aspartate solution applied to the retina suppressed the contractile response, indicating that the response derived from the postsynaptic elements in the retina. Light-adaptation was found to suppress these responses completely.

For the following reasons, we postulate that the major portion of the light-evoked contractile response of the retina derives from the dendrites of the amacrine cells: (i) these dendrites are likely to be the longest processes

of neurons (2,14); (ii) depolarization of the amacrine cells lasts much longer than that of the ganglion cells (14); (iii) off-depolarization is typical of the amacrine cells, while bipolar cells respond to uniform illumination of the retina with hyperpolarization (2,14); (iv) similar contractile responses could be observed in a piece of retina sliced and stretched in the direction roughly perpendicular to the pathway of the optic nerve fibers; and (v) a contractile response evoked by optic nerve stimulation could be superposed on the light-evoked contraction (without occlusion).

Finally, the origin of the small downward deflection of the mechanical traces in Fig. 2A and C is considered. The latency of this small response is close to that of the electric response of the photoreceptor (10). It is suppressed by light-adaptation but not by application of Na-aspartate. It is suggested therefore that this small deflection represents a mechanical response (probably a sign of swelling) of the photoreceptor cells associated with the production of the receptor potential.

DISCUSSION

The experimental evidence presented here indicates that excitation of long neuronal processes in the retina, most probably impulse propagation along the dendrites of the ganglion and amacrine cells, is associated with contraction of the retina. It is not clear at present what physiological role these contractile responses play in the function of the retina. However, the experimental findings indicate that, at a microscopic scale, the central nervous system is an assembly of cells with highly mobile processes.

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