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RAPID MECHANICAL CHANGES IN THE AMPHIBIAN RETINA EVOKED BY BRIEF LIGHT PULSES

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SUMMARY: Dark-adapted retinae of the toad and bullfrog were found to respond to brief light stimuli with a succession of rapid mechanical changes. The latencies of the mechanical responses, as well as the effects of chemicals known to block the synapses on photoreceptor cells, indicate that the first mechanical response represents swelling of the photoreceptor cells. The first response is followed by mechanical changes in the postsynaptic elements. It is suggested that the observed response of the photoreceptor cells is a mechanical expression of the process underlying heat production by the cells.

A brief light pulse applied to the dark-adapted vertebrate eye is known to produce a hyperpolarizing response of the photoreceptor cells, followed by the electric responses of various postsynaptic elements in the retina (see e.g. 1, 2). Quite recently, we have shown that the electrical response of the amphibian photoreceptor cell is preceded by rapid production of heat (3). Since the electrical response of the squid retina to a light pulse is accompanied by both thermal and mechanical changes in the photoreceptor cells (4, 5), it seemed reasonable to assume that the amphibian photoreceptor cells also respond to light stimuli with mechanical changes. The present communication describes our method of detecting mechanical changes in the isolated retina and the results obtained by this new method.

MATERIALS AND METHODS

Dark-adapted retinae of the toad, *Bufo marinus*, or the bullfrog, *Rana catesbeiana*, were separated from the sclera and the pigment epithelial layer under dim red light. A rectangular piece of the isolated retina, approximately $7x9\ mm^2$ in size, was employed for measurements (Fig. 1). The lower side of the

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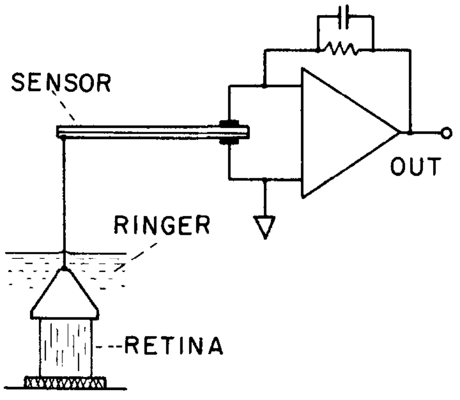

Fig. 1. Schematic diagram of the experimental setup employed for measuring changes in the tension developed by the amphibian retina. "Sensor" represents a piezoceramic bender. Calf serum (10% by volume) was added to Ringer's solution which contained 110 mM NaCl, 1.5 mM KCl, 1 mM $CaCl_2$ and 5 mM HEPES (pH 7.3 - 7.6). The solution was oxygenated by O_2 bubbling (except during measurements). The operational amplifier had a feedback resistor of 1.4×10^9 ohms with a small (approximately 1 pF) parallel capacitor.

retina was fixed to the bottom of a plastic chamber filled with oxygenated Ringer's solution. Its upper side was held with 3M (Scotchmate) hook-and-loop fastening sheets (roughly 40 mg). A thin thread was used to connect the top of the fastening sheets to a piezoelectric sensor. Initially, the retina was stretched so that its length was increased by 15 - 25%.

As in our previous studies of mechanical changes in various excitable tissues (4, 6), a piezoceramic bender (G1195) purchased from Gulton Industries, Inc., New Jersey, was used for measurements. The resonant frequency of the bender was about 1.6 kHz and the electric charge generated by the force applied suddenly at the tip was approximately 4.6×10^{-9} coulomb per gram weight. An operational amplifier (AD515 or OP111) was employed to convert the current generated by the sensor into voltage. The output of the operational amplifier was led to a signal averager (Nicolet Model 1070) via a capacity-coupled amplifier. Brief pulses of light from a light-emitting diode, usually 490 nm in wavelength, 10 msec in duration and 8×10^{-6} W/cm^2 in intensity, were delivered to the inner (vitreal) surface of the retina at intervals of 11 - 14 sec. Records of mechanical responses were obtained after averaging over 8 - 32 trials.

RESULTS

Fig. 2A shows a typical record obtained. The upward deflection of the middle (smooth) trace in the record represents a decrease in the tension of the retina (associated with a small upward movement of the bender). This trace was obtained by integration of the top trace which represents the time-derivative of the tension. It is seen that the retina responded to a light pulse with a succession of rapid mechanical changes. The initial upward deflection, representing a fall of 0.5 - 3 µg in tension, was followed by a large, long-lasting downward deflection. The top trace indicates further that the downward deflection was

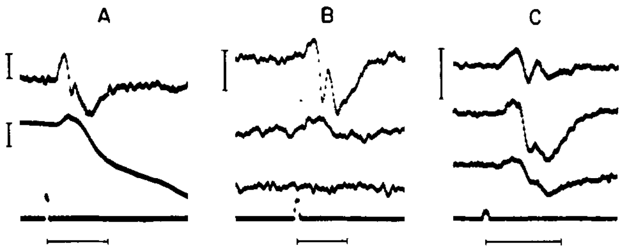

Fig. 2. Mechanical responses of toad (A and B) and bullfrog (C) retinae evoked by brief light pulses (490 nm in wavelength, 8 $\mu W/cm^2$ in intensity, and 10 ms in duration). A fall in the tension of the retina, which tends to move the sensor upwards, generates an upward deflection in the record. A: Both the change in the tension developed by the retina (middle trace) and its time-derivative (top trace) are shown. The vertical bars represent 10 μg/sec and 2 μg. B: The top trace was taken from a retina immersed in normal Ringer; the middle trace, from the retina immersed in Ringer containing 4 mM Na-glutamate; the bottom trace, after exposure of the same retina to room light. The bar, 50 μg/sec. C: Top and bottom traces were taken from a retina immersed in normal Ringer; middle trace, from the same retina in Ringer containing 0.1 mM d-tubocurarine; the vertical bar, 50 μg/sec. The time-markers, 0.2 sec. Temperature, 21°C.

interrupted by a second upward deflection. The magnitude of these early mechanical changes was found to be affected, to a considerable extent, by the initial tension and the chemical environment of the retina.

It is known that both electrical and thermal responses of the photoreceptor cells can be isolated by blocking the synapses at the proximal end of the photoreceptor cells with Na-aspartate or glutamate added to the medium (3, 7). Record B shows the effect of Na-glutamate. [Only the time-derivative of the change in tension is shown in the figure.] It is seen that the downward deflection of the top trace was eliminated by the amino acid. Hence, the initial, upward deflections of the top traces in Fig. 2 are interpreted as representing the mechanical response of the photoreceptor cells and the downward deflections as reflecting physiological activities of the postsynaptic elements. Addition of Na-aspartate to the medium produced a similar effect. The initial phase of the mechanical response represents a slight elongation of the isolated retina in the direction of initial stretching. A small increase in the diameter (i.e. swelling) of individual photoreceptor cells is expected to produce the observed phenomenon. The peak of this response was reached usually between

60 and 80 ms after the end of the brief light pulse. The response lasted slightly more than 100 ms.

The bottom trace in Record B was taken after 2 min exposure of the retina to room light. As in the case of thermal responses (3), mechanical responses of the amphibian photoreceptor cells were readily suppressed by light-adaptation and anoxia, as well as by inhibitors of oxygen utilization, such as cyanide and azide. A preliminary experiment using an incandescent light source and interference filters suggested that the mechanical responses are induced, as the thermal responses are (3), by light absorbed by red rods. The time-course of the mechanical response of the photoreceptor cells is comparable to that of the thermal response. Therefore, we suggest that the swelling of the photoreceptor cells is a mechanical counterpart of the thermal response.

Next, properties of the mechanical responses of the postsynaptic elements are briefly discussed. According to Dowling (8), the latency of the electric responses of the horizontal and bipolar cells (35 ms in mudpuppy) are far shorter than that of the amacrine cells (110 ms). It seems reasonable therefore to interpret the downward deflections of the top traces in Fig. 2A, B and C as representing shrinkage of the cell-bodies of the horizontal (and probably bipolar) cells and the second upward deflections as reflecting swelling of the amacrine cells. This interpretation is consistent with the previous findings that, upon diffuse illumination, the horizontal (and many bipolar) cells hyperpolarize and the amacrine cells depolarize (1,2,8). [Note that neurons are expected to shrink when they are hyperpolarized.] Record C in the figure shows that the downward deflection was enhanced reversibly by 0.1 - 1.0 mM d-tubocurarine, a competitive blocker of acetylcholine involved probably in an inhibitory circuit in the retina (9). A similar enhancement was observed with picrotoxin and bicuculline, antagonists of gamma-aminobutyric acid (10).

We have shown in the communication that measurements of mechanical changes in the retina provide new information about the process of phototransduction, as well as about the sequence of events that take place in various postsynaptic elements in the eye.

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