Light-Induced Permanent Changes in Electric Resistance and Standing Potential Observed in Neonatal Chick Eyes

TADASI SATO

Department of Physiology I, School of Medicine, Iwate Medical University, Morioka

SATO, T. *Light-Induced Permanent Changes in Electric Resistance and Standing Potential Observed in Neonatal Chick Eyes.* Tohoku J. exp. Med., 1975, 115 (3), 277-284 — A group of neonatal chicks (subject) was kept in complete darkness before and after hatching, whereas another group (control) was raised under normal light conditions. The electric resistance and standing potential across the eyeball were measured between the cornea and occipital area on the 3-5 day after hatching. Under dark adaptation, the resistance of the subject group was much higher than that of the control. The standing potential of the subject group showed a large negativity of 4-15 mV on the cornea side, whereas that of the control group showed a slight positivity of 0.1-1.5 mV. The resistance and standing potential of the control eyeballs showed only a slight change during the light adaptation, whereas those of the subject group markedly decreased during the initial light adaptation. Ultimately, they became the same as the control group. These observed changes were irreversible, suggesting a certain structural change in the retina produced by the initial exposure to light. The results were discussed in relation to the morphological changes in photoreceptors observed after the initial exposure to light. ——— electric resistance of the retina; standing potential; morphological changes in the cones.

There are a few experiments which have measured the electric resistance of the adult retina. Brindley (1956) observed in the frog retina that a high resistance area is localized at a specific site in retinal layers and he named it the "R-membrane". Ogden and Ito (1971) also measured the electric resistance of the avian retina and described that the specific resistivity of the middle layer was 1.5 times greater than the inner or outer layer of the retina. There is, however, little information regarding the resistance change during the development of the retina after birth.

In a previous paper (Sato 1969b), the author observed that repetitive exposures of neonatal chick retina to light increased both the c-wave of the ERG and the light-induced peak of the standing potential. He also observed a certain morphological change in photoreceptors following the initial exposure to light. The present study is to find more basic factors underlying the changes in resistance and potential produced by the initial exposure to light and to correlate the factors further with the morphological evidence previously obtained.

Received for publication, December 16, 1974.

MATERIALS AND METHODS

Sixty white leghorn neonatal chicks aged 3 to 5 days (after hatching) were used in the experiments. They were divided into a control and subject groups. The control group consisted of 32 chicks commercially obtained which were kept under normal light conditions. The subject group included 28 chicks which were kept in complete darkness after hatching. In fact, the eggs were incubated in the dark for 7 days prior to hatching.

Each chick was anesthetized and immobilized by intramuscular injection of pentobarbital (0.1–1 mg) and d-tubocurarine chloride (0.15 mg) every 2 hr. Respiration was artificially maintained during the experiment. The entire preparation was made under dim red light. Body temperature was measured rectally and maintained at 39°C by a constant temperature device which was described in my earlier paper (Sato 1969a).

Two pairs of electrodes were placed between the cornea and occipital area of the head. One pair was used to feed a constant peak current (I) across the eyeball and the other pair (non-polarizable electrodes (Zn-ZnSO_4)) was used to record the IR drop, R being the resistance between the cornea and occipital area. Special precaution was made to reduce the direct current flow between the voltage recording electrodes by increasing the shunt resistance between the current and voltage electrodes.

The constant peak current had a triangular time course with a peak of $1\text{ }\mu\text{A}$ and duration of 0.5 sec. The resistance between the cornea and occipital area was continuously monitored by measuring the IR drop every 2 min. The resistance across the eyeball was actually calculated by subtracting the serial resistance between the sclera and occipital area which was normally 100–200 Ω . The standing potential was also recorded from the voltage electrodes placed between the cornea and occipital area through the high impedance input preamplifier (Sato et al. 1972 a, b) and DC-main amp. The recorder was a rectilinear polygraph; RJG-3024, Nihonkohden.

Photic stimulation was delivered by illuminating the entire visual field with a diffuse white light of 1,000 luxes measured at the surface of the cornea. All preparations were maintained in darkness at least 30 min before the measurement.

Fig. 1. Arrangement for measuring the electric resistance and the standing potential across the eyeball. PE: Potential electrodes; CE: Current electrodes.

RESULTS

Resistance change before and after the single exposure to light

Sixty-six percent of the total eyeballs in the control group showed a resistance lower than 0.7 K Ω before they were illuminated, as shown in Fig. 2. In this group the resistance did not change appreciably after the exposure to light. 34% of the

Fig. 2. Relative incidence of the eye with different ranges of resistance. The data were obtained from the control group consisting of 32 chicks. The upper graph was obtained from eyes in the dark before illumination, whereas the lower graph was obtained from the same eyes but 3 hr after the onset of illumination.

control eyeballs, however, showed a resistance higher than $1.6 \text{ K}\Omega$ before illumination. Such eyeballs showed a marked decrease in resistance. During 3 hr of illumination, their values eventually became as low as those of the former group.

On the other hand, 93% of the eyeballs in the subject group which had never been exposed to light showed a resistance higher than $1.0 \text{ K}\Omega$ as shown in Fig. 3. Once exposed to the initial light, the resistance of these eyes decreased markedly to values similar to those of the control group. There were a small number of eyes whose resistances remained higher than $1.0 \text{ K}\Omega$ even 3 hr after the onset of illumination. The incidence of this tended to increase with the age of the chick.

Potential change before and after the single exposure to light

More than 80% of the low resistance eyeballs in the control group showed 0.1–1.5 mV in the dark, the cornea being slightly positive with respect to the occipital area of the head. This potential did not change markedly after a 3-hr exposure to light.

Fig. 3. Same as Fig. 2 but obtained from the subject group consisting of 28 chicks.

On the other hand, approximately 90% of the subject eyeballs which had never been exposed to light showed a large cornea-negativity of 4–15 mV. Once exposed to the initial light, this negativity markedly decreased and often reversed its polarity. For example, 65% of the eyeballs showed cornea positive potentials of 0.5–4.0 mV after a single exposure to 3-hr illumination. These cornea positive potentials were of the same order of magnitude as seen in the control group. It was noted that the light-induced polarity reversal of the standing potential was always associated with a marked decrease in electric resistance and that these changes were irreversible within 24 hr of the observation. Those eyeballs whose cornea negativity remained unchanged after exposure to light, usually demonstrated high resistance with little change at the end of 3-hr illumination.

Resistance and potential changes during the single exposure to light

As described above, 66% of the control eyeballs showed a low resistance which was not significantly altered by the exposure to light. A typical time course of the change in resistance is shown by the curve C in the upper half of Fig. 4. A simultaneous recording of the standing potential from the same eye is shown by the

Fig. 4. Time course of the change in the electric resistance (upper graph) and the standing potential (lower graph) after the onset of illumination. The curves A, a and B, b were obtained from the same eyeballs in the subject group whereas the curves C and c were obtained from an eyeball in the control group.

curve-c in the lower half. It is characterized by an initial slight positivity (0.2–0.5 mV) before illumination and gradual increase in positivity following a transient peak (lp) after the onset of illumination.

As described above there were two types of resistance changes in the subject group. Two samples were chosen to illustrate their time courses during a single exposure to light (see curves A and B on the upper half of Fig. 4). The standing potentials were recorded simultaneously from the same eyeballs as shown in the lower half (see curves a and b). It should be noted that both potentials were distinctly negative before the initial exposure to the light and that the negativity gradually decreased when the resistance fell during illumination. At the end of 3-hr illumination the curve-b ultimately reached the same positive level as that of the curve-c, whereas the curve-a remained negative after the initial decrease in negativity. Note the high resistance in curve A at the end of the illumination.

The eyeballs in the subject group which exhibited the characteristic time course shown by the curve B and b in Fig. 4 were kept in darkness for 24 hr after a series of illumination experiments. The resistance and potential of these eyeballs were found to be approximately the same as those values measured at the end of light-adaptation, suggesting that the changes due to the initial exposure to light were permanent and irreversible.

DISCUSSION

Change in electric resistance

The electric resistance of the eyeball which has never been exposed to light is significantly higher than that of the eye which has previously been exposed. Initial exposure to light prior to the 3rd-5th postnatal days seems to decrease the resistance to the value seen in the eye developed under a normal light environment. The exact time of light exposure and the intensity of illumination necessary to cause this effect remains to be studied. It should be emphasized that this effect of initial exposure to the light is permanent; the lowered resistance remains lower even after 24 hr of dark adaptation. This persistent effect suggests that the initial light exposure causes a certain structural alteration of the young retina.

The electron microscopic study of the neonatal chick's retina and some morphological changes due to initial light exposure have been published previously (Sato 1969b). According to Penn and Hagins (1969), the inner segment layer has a higher electric resistance than the outer segment layer of the receptor cells. The electron microscopic analysis of the inner segment revealed certain structural changes presumably as the result of the initial exposure to light. The cone of the retina which has not been exposed to light usually includes an oil droplet in the inner segment which normally stains as an electron dense substance as illustrated in Fig. 5. This electron dense material, however, disappears after repetitive exposure to light and seems to be replaced by an electron lucent substance (Sato 1969b). It is conceivable that the electric resistance of the electron dense substance is higher

Fig. 5. Schematic illustration of the electronmicrograph, showing the change in receptor cells before and after initial illumination.

OS: outer segment BB: basal body of connecting cilium M: mitochondria
IS: inner segment DPM: double plasmic membrane. Note the pore on the DPM of the cone on the right, indicated by P.
O₁ and O₃: oil droplets

than that of the electron lucent substance, thus contribution to the higher resistance of the eyeball which has never been exposed to the light.

Another morphological finding characteristic to the retina which was once exposed to light, is partial discontinuity of the double plasmic membrane of the cones (Fig. 5). This has never been seen in retinae kept in the dark after hatching. If the electrical resistance of the double plasmic membrane is significantly high, the light induced disruption of this membrane could also contribute to the lower resistance of the illuminated retina. The presence of cytoplasmic continuity between the inner and outer segments other than the connecting cilium has also been demonstrated in the mammalian retina by Richardson (1969).

Change in standing potential

The standing potential recorded from the eye which has never been exposed to light demonstrated a large negativity on the corneal side with respect to the occipital side. If the electromotive force is due to the charge separation at the boundary between the outer and inner segment of the receptor layers as suggested by Penn and Hagins (1969), the inner segment should be negatively charged with respect to the outer segment to give the large negativity recorded on the corneal side. Then, the metabolic current in the dark should be flowing from the outer to the inner segment. It should be noted that the direction of the dark current measured from the adult rat retina by Penn and Hagins (1969) is just opposite to that described above.

On the other hand, the standing potential recorded from eyes after the initial exposure to light usually shows a slight positivity on the corneal side. This corneal positivity remains unchanged even after 24 hr of dark adaptation. The dark current should flow from the inner to the outer segment in this situation, the direction of the flow now being consistent with that reported by Penn and Hagins (1969). Accordingly, it is suggested that the polarity of the charge separation between the inner and the outer segment of the receptor cell in the neonatal retina which has never been exposed to light is opposite to that usually seen in the adult dark-adapted retina.

As described in the results, there were a certain number of eyes in the subject group which showed neither the marked decrease in electric resistance nor the irreversible change in polarity of the standing potential. The relative incidence of such eyes seemed to increase with the age of postnatal life at the time of the first exposure to light. However, this needs further investigation.

Acknowledgment

I am grateful to Prof. T. Mita and Associate Prof. M. Sato for their valuable advice and criticism during the preparation of this manuscript and Dr. T. Nikara for his helpful discussion.

References

- 1) Brindley, G.S. (1956) The passive electrical properties of the frog's retina. *J. Physiol. (Lond.)*, **134**, 339-352.
 - 2) Ogden, T.E. & Ito, H. (1971) Avian retina. II. An evaluation of retinal electrical anisotropy. *J. Neurophysiol.*, **34**, 367-373.
 - 3) Penn, R.D. & Hagins, W.A. (1969) Signal transmission along retinal rods and the origin of the electroretinographic a-wave. *Nature (Lond.)*, **223**, 201-205.
 - 4) Richardson, T.M. (1969) Cytoplasmic and ciliary connections between the inner and outer segments of mammalian visual receptors. *Vision Res.*, **9**, 727-731.
 - 5) Sato, T. (1969a) A convenient device for maintaining a constant body temperature of the experimental animal. *J. Iwate med. Ass. (Jap.)*, **21**, 175-177.
 - 6) Sato, T. (1969b) The relation between the development of the electrical responses to the light and the electron micrographic structure of the visual cell in the chick's eye. *J. Iwate med. Ass. (Jap.)*, **21**, 513-520.
 - 7) Sato, T., Mita, T., Sugawara, Y. & Nunokawa, S. (1972a) A method of continuous recording of the change in biological slow potentials. *J. Physiol. Soc. Jap.* **34**, 471. (abstract in Japanese)
 - 8) Sato, T., Ishizuka, T. & Nunokawa, S. (1972b) High input impedance preamplifier for physiological examination. pp. 445-446, Proceedings of the 11th Conference, Japan Society of Medical Electronics and Biological Engineering, April 21-22, Kurume-shi, Japan. (abstract in Japanese)
-