Some observations of the mode of action of ouabain upon the electrical activity of mammalian retinas

Yoshihito Honda

The effect of ouabain upon the electroretinogram (ERG) of mammals was studied in several specific conditions with the use of excised, perfused rabbit retinas. Retinas incubated in low-sodium media, in which sodium ions of the standard medium were replaced by lithium and choline ions, immediately lost their responsiveness to the luminous stimulus. However, the PII component of the ERG recovered when the sodium concentration of the medium was restored to normal. The PII component remained subnormal. The inhibitory effect of ouabain upon the ERG was not observed in low sodium media. This result was interpreted as providing supportive evidence that the mode of action of ouabain involves abolition of some of the sodium-dependent enzyme systems of retinal cells concerned with generation of the PII and PIII components of the ERG. The responses to the second flash of a pair of flashes survived longer than those to the first.

Key words: ouabain, PII component, PIII component, recovery of activity, sodium concentration and transport, paired two-flash stimuli.

It has been established that ouabain markedly inhibits the cation-activated ATPase activity of erythrocytes, resulting in abolition of transmembrane active ion transport. This explanation of the effect of ouabain has been suggested as applicable to all living cells. Several authors have reported evidence supporting this interpretation of the effect of ouabain on retinal receptors given normal extracellular ionic concentrations. The present work was intended to confirm the mode of action of ouabain upon the PII and PIII components of the electroretinogram (ERG) with the use of perfused mammalian retinas.

Methods

About 20 eyes of albino rabbits weighing approximately two kilograms each were utilized as study material. Excision and perfusion of the retinas were described in the preceding report, as were the stimulating and recording systems.

From the Department of Ophthalmology, Washington University School of Medicine, and the Oscar Johnson Institute, 660 South Euclid Ave. St. Louis, Mo. 63110.
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The sodium ions in the usual Ames solution come from 120 mM per liter sodium chloride and 23 mM per liter sodium bicarbonate. A low-sodium medium was used in this investigation and was made by substituting 120 mM per liter lithium chloride or choline chloride (Fisher Scientific Co., Fair Lawn, New Jersey) for the sodium chloride in the standard solution. The concentrations of the other ions and of glucose were constant throughout this investigation. Substitution of the incubating media was accomplished by completely draining the old medium and then refilling the chamber with the new medium. The substitution required about 15 seconds. The new medium was saturated with oxygen prior to substitution. The temperatures of the media were equal (36° C). Ouabain (Sigma Chemical Co., St. Louis, Mo.) was administered to the retinas by adding it to the standard or low-sodium media.

Results and discussion

Fig. 1 shows that $10^{-4}$M per liter ouabain suppressed the PII and, with a slight delay, the PHI components of the ERG when applied via the standard medium (Na+, 143 mM per liter). Suppression of both processes was complete within about four minutes after ouabain administration. Once poisoned, the retinas showed no recovery of activity even after they were reimmersed in the ouabain-free standard medium (compare time course study of the preceding report).

Figs. 2 and 3 show that the rabbit retina in vitro lost responsiveness to luminous stimulation in a low-sodium medium (Na+, 23 mM per liter). However, PHI recovered when the sodium concentration of the medium was restored to normal, although PII sometimes remained subnormal.

If the effect of ouabain on retinal activity is independent of extracellular sodium concentration, incubation with ouabain in the low-sodium medium would be expected to abolish PIII permanently, as it did in the standard medium. Thus, the effect of $10^{-4}$M per liter ouabain in the low-sodium medium (23 mM per liter) was studied. The retinas being incubated for four minutes under this condition, however, revealed prominent recovery of PIII upon return to the standard medium, as shown in Figs. 4 and 5. No recovery of PII was observed. The retina incubated in the low-sodium medium containing $10^{-5}$M per liter ouabain showed recovery of PII and PIII after being immersed in the medium of the standard sodium concentration. However, both processes were permanently abolished by $10^{-4}$M per liter ouabain contained in the succeeding medium (Fig. 6).

Fig. 7 shows the time course of loss of paired responses evoked by double-flash stimuli of 150 msec. interflash interval (stimulus frequency: one pair of flashes per second). The first response was more severely affected by $10^{-4}$M per liter ouabain.
Fig. 3. The effect of a low-sodium medium. Sodium chloride of the standard solution was replaced by lithium chloride. Here, the effect upon the PIII component was investigated, using a DC amplifier and stimuli of long durations (two and one-half seconds).

Fig. 4. The effect of 10^-M per liter ouabain in the low-sodium medium (replaced by choline chloride).

Fig. 5. The effect of 10^-M per liter ouabain in the low-sodium medium (replaced by lithium chloride). Spike waves on the responses of one and ten minutes are artifacts.

Fig. 6. The retina incubated in the low-sodium medium (containing 10^-M per liter ouabain) was immersed in the standard medium (containing 10^-M per liter ouabain).
than the second. The second response remained even after the first was no longer recordable, although the amplitude of the second also decreased with the lapse of time.

Based on these experiments with the use of perfused rabbit retinas, the following conclusions might be reached: First, choline and lithium ions could not substitute for sodium ions in the extracellular space for the generation of PII and PIII of mammalian ERGs. This supported the results of others for frog retinas.\textsuperscript{10-12} Choline and lithium ions probably had no toxic effect upon PIII, because PIII recovered immediately after the retina was reimmersed in the standard medium. Second, the effect of ouabain upon the mammalian retina appeared to be the abolition of some sodium-dependent enzyme system(s) of retinal cells concerned with the generation of PII and PIII. Third, the responses to the second flash of a pair of flashes survived longer than those to the first in this investigation. In the rabbit retina, two kinds of receptor cells, named Type I and II by Sjöstrand and Nilsson,\textsuperscript{13} have been described. The response to the second flash of a pair of flashes has been interpreted as more cone dependent than that to the first.\textsuperscript{14} It is not clear if Type I and II receptor cells in the rabbit do or do not correspond to the rod and cone cells of other species, respectively. If this proves to be the case, it might be possible that the two kinds of receptor cells and the cells of origin of PII were affected either on different time courses or in somewhat different manners. This possibility is interesting when considered in conjunction with the results of Gouras and Hoff which showed that in perfused cat eyes the rod component of the ERG was more sensitive to glucose or to oxygen deprivation than the cone component.\textsuperscript{15}

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