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Effects of Hydrogen Peroxide Oxidation and Calcium Channel Blockers on the Equatorial Potassium Current of the Frog Lens

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Hydrogen peroxide, in concentrations of 10–1000 μM, produces two major changes in the current–voltage relationships associated with the equatorial potassium current of the lens. First, the resting and reversal potentials become more negative than they were prior to treatment with hydrogen peroxide and second, the membrane resistance related to the equatorial current is decreased. The shift in the resting and reversal potentials is in the opposite direction from that produced by ouabain. Based on the Nernst equation, the shift in the reversal potential suggests that there is an increase in the concentration of potassium in the lens. The ^{86}Rb uptake and efflux are increased. These observations suggest that hydrogen peroxide stimulates the Na,K-pump. The decrease in membrane resistance is inhibited by 100 μM of quinine, a calcium-dependent potassium channel blocker, and does not decrease in a calcium-free medium. This suggests that the decrease in resistance may be secondary to an increase in lenticular calcium. These effects of hydrogen peroxide are similar to those of *p*-chloromercuriphenylsulfonate (*p*CMPS), a nearly impermeant sulfhydryl binding agent, and suggest that permeant hydrogen peroxide may increase calcium influx by acting on sulfhydryl groups on the outer surface of lens membranes. Verapamil, a calcium channel blocker, is reported to prevent cataract formation. D600, the methoxy analogue of verapamil, is a calcium channel blocker that increases the resistance associated with the equatorial current in the presence and absence of hydrogen peroxide. The gadolinium ion has a similar effect. Thus, D600 and Gd^{3+} partially counteract the reduction in membrane resistance produced by 50 μM hydrogen peroxide.

Key words: lens; lens equatorial current; hydrogen peroxide; oxidation; calcium channel blockers; D600; Gd^{3+}; cataractogenesis; vibrating probe.

1. Introduction

Hydrogen peroxide is an important factor in cataractogenesis (Bhuyan and Bhuyan, 1977, 1984; Spector and Garner, 1981; Bhuyan, Bhuyan and Podos, 1986). It is permeant and can oxidize SH groups on the aqueous or cytoplasmic side of lens membranes. The possible role of extracellular -SH groups in the pathogenesis of cataracts has been investigated with *p*-chloromercuriphenylsulfonate (*p*CMPS), a nearly impermeant sulfhydryl binding agent (Hightower, 1985, 1986; Marcantonio, Duncan and Rink, 1986; Hightower et al., 1989). Decreasing the available sulfhydryl groups, with *p*CMPS, is followed by an increase in the concentration of intracellular calcium and opacification of the lens. The possibility that an increase in intracellular calcium relates to cataract formation is suggested by reports that verapamil, a calcium blocking agent, can delay or prevent the development of diabetic cataracts (Fleckenstein, 1983; Pierce et al., 1989).

Changes, produced by pCMPS, in the current–voltage relationships of the equatorial potassium current of the frog lens may in part be due to an increase in the concentration of calcium within the lens (Walsh and Patterson, 1991). In addition, D600, the methoxy analogue of verapamil counteracts the change in resistance caused by *p*CMPS (Walsh and Patterson, 1992). It is the purpose of this study to determine whether hydrogen peroxide, a permeant oxidant and a physiological constituent of the lens, produces alterations in the current–voltage relationships of the equatorial potassium current of the lens by the same mechanisms that have been attributed to changes produced by *p*CMPS.

2. Materials and Methods

Lenses (20–30 mg) were obtained from adult frogs, *Rana pipiens*. Following decapitation the lenses were removed from the globe using a posterior approach. Lenses were supported on a platinum ring in a Petri dish and were bathed with a frog Ringers solution of the following composition (mM): 105 NaCl, 2·5 KCl, 2·0 $CaCl_2$, 1·2 $MgCl_2$, 5·0 glucose and 10 Hepes buffer at pH 7·4. Studies were conducted at room temperature (20–25°C). Details of the procedures have been published (Wind, Walsh and Patterson, 1988a, b). Lenses were oriented in a Petri dish so that the plane of the equator was horizontal and the anterior surface was down. Two microelectrodes with a tip resistance of 2–6 MΩ were inserted from above with the tips about 200 μm below the surface of the lens and about 1 mm apart. One electrode filled with

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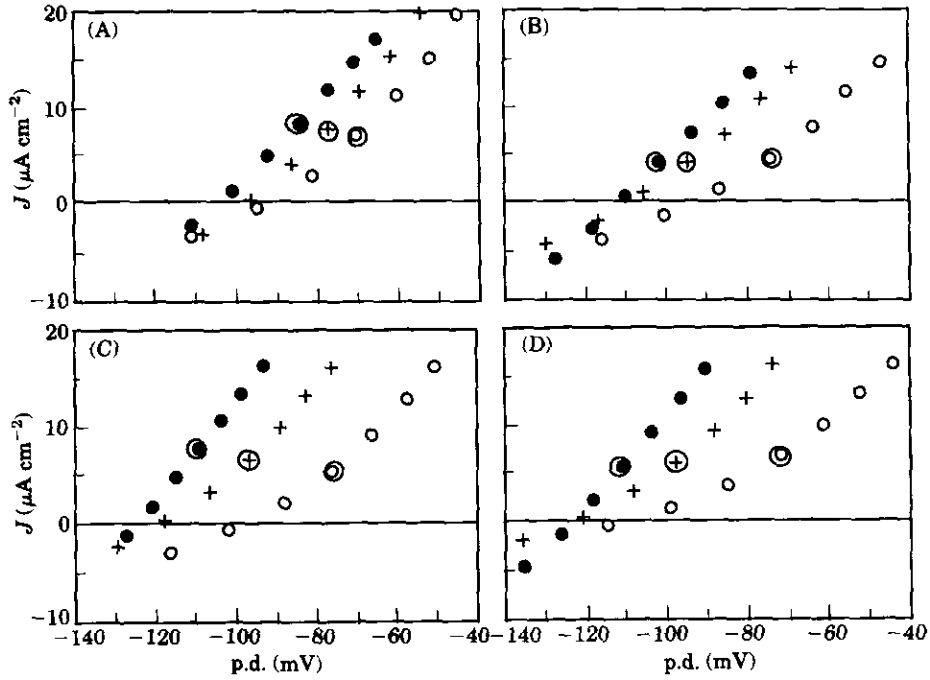

FIG. 1. Effect of different concentrations of hydrogen peroxide (A, 10 μM, $n = 3$; B, 50 μM, $n = 10$; C, 250 μM, $n = 8$; D, 1000 μM, $n = 4$) on the current–voltage relationships of the equatorial current of the frog lens. Hydrogen peroxide concentrations were maintained with an exchange pump. Note the shift to the left of the resting, p.d., and reversal, $p.d._{J=0}$, potentials and the change in the slope of the resistance, R_J. The resting values are encircled. (○) Initial, (+) 5 min, (●) 30 min. Data is shown in Table I.

TABLE I

Effect of different concentrations of hydrogen peroxide on the parameters associated with the equatorial potassium current of the frog lens [Mean ± S.E.; number of lenses in (parentheses)]

H_2O_2	p.d. (mV)	J (μA/cm^{-2})	R (kΩ)	ΔJ (μA/cm^{-2})	R_J (kΩ)	JR_J (mV)	$p.d._{J=0}$ (mV)
10 μM (3)							
Initial	−70 ± 3·7	7·1 ± 3·6	10·7 ± 0·2	3·9 ± 0·4	2·8 ± 0·4	18 ± 6·9	−88 ± 3·2
30 min	−85 ± 3·7	8·5 ± 3·4	7·5 ± 1·4	3·3 ± 0·4	2·4 ± 0·6	17 ± 2·8	−101 ± 3·5
50 μM (10)							
Initial	−74 ± 1·0	4·6 ± 0·6	11·4 ± 1·1	3·1 ± 0·2	3·9 ± 0·5	16 ± 1·9	−91 ± 2·4
30 min	−102 ± 3·1*	4·1 ± 0·7	8·1 ± 0·3	3·2 ± 0·3	2·7 ± 0·2	10 ± 2·0	−113 ± 3·0*
250 μM (8)							
Initial	−76 ± 1·1	5·5 ± 1·1	11·0 ± 1·1	3·3 ± 0·3	3·5 ± 0·4	17 ± 1·5	−93 ± 1·3
30 min	−109 ± 2·2*	7·8 ± 1·8	5·6 ± 0·5*	2·9 ± 0·4	2·0 ± 0·2*	15 ± 2·8	−124 ± 3·5*
1000 μM (4)							
Initial	−72 ± 0·8	6·8 ± 2·4	11·7 ± 2·0	2·9 ± 2·0	4·0 ± 0·5	24 ± 6·0	−97 ± 5·4
30 min	−111 ± 3·5*	5·8 ± 0·6	7·5 ± 0·6*	3·4 ± 0·4	2·2 ± 0·2*	13 ± 1·5	−124 ± 3·7*

* $P < 0.05$ with respect to initial value.

3 mM KCl was used for measuring p.d. It was connected to an amplifier WPI-S7100A and S7040A (WPI, New Haven, CT, U.S.A.) through a Ag/AgCl wire. The second electrode was used for the injection of current. It was filled with 1·5 mM potassium citrate and connected through a Ag/AgCl wire to a current source WPI-S7071A (WPI, New Haven, CT, U.S.A.). Positioning of the electrode within the lens was assured by using it to make the initial measurements of p.d. The vibrating probe was aligned along a radial line with the tip about 20 μm from the surface of the equator. The probe was attached to a lock-in analyser Model 5208, EG and G (Princeton Applied Research, Princeton, NJ, U.S.A.). After the probe was positioned, it was moved sufficiently far from the lens to establish a reference base and then returned to the lens to make the desired measurements. Output data were recorded on a chart recorder.

The following measurements were obtained directly from the recordings: (1) p.d., the potential difference across the surface membranes. (2) J, the current at the equator. (3) R, the input resistance. The change in

p.d., or Δp.d. per μA of injected current, I. The average response with 1, 2 and 3 μA injected on each side of the p.d. was used. (4) ΔJ, the change in J per μA of injected current. (5) $\text{p.d.}_{J=0}$, the reversal potential was obtained by injecting hyper-polarizing current until J equals zero. The following are calculated from the above data and were used in the tables: (1) R_J, the resistance of the segment of the K^+ loop that was being studied ($\Delta\text{p.d.}/\Delta J$). (2) JR_J, the 'IR' drop of Ohm's law or the driving force. It equals $\text{p.d.} - \text{p.d.}_{J=0}$ when the plot of J vs. p.d. is a straight line. (3) $\text{p.d.}_{J=0}$, the reversal potential was estimated from the relationship for the driving force ($\text{p.d.}_{J=0} = \text{p.d.} - JR_J$).

Chemicals were obtained from Sigma (St Louis, MO, U.S.A.). Solutions containing hydrogen peroxide were administered with the aid of an exchange pump so that constant concentrations could be maintained.

^{86}Rb (New England Nuclear, Boston, MA, U.S.A.) uptake was determined by incubating a lens in 1 ml of medium containing ^{86}Rb (10^6 cpm ml^{-1}). After incubation for 30 min the lens was rinsed in 'cold' medium and placed in a counting flask containing 1 M hyamine hydroxide (New England Nuclear, Boston, MA, U.S.A.). After sitting for 60 min, 6 ml of aqueous counting scintillant (Amersham, Arlington Heights, IL, U.S.A.) was added to the flask. Two 5-μl aliquots of the medium were placed in separate counting flasks and treated the same way to obtain counts for the medium. The ratio of the cpm ml^{-1} of lens water (60% of wet weight was used) to cpm ml^{-1} of medium (C_L/C_M) was used to indicate uptake. To measure ^{86}Rb efflux, lenses were loaded by incubating lenses in 5 ml of medium containing ^{86}Rb (10^6 cpm ml^{-1}). The lenses were then given a quick rinse and transferred to individual flasks containing media without ^{86}Rb. After incubating for 2 hr, counts were made on each lens and on aliquots of medium as described for ^{86}Rb uptake. The efflux was reported as the percent efflux or the total counts in the medium times 100 divided by the counts in the lens plus medium after 2 hr of incubation.

3. Results

After a lens was placed in the bathing medium in a Petri dish and the electrodes were in place, a steady state was attained in about 10 min. Current, I, of 1, 2 and 3 μA was then injected on each side of the resting potential. This provided data for the initial current–voltage plot that served as an internal control. In addition to J and p.d. which were recorded on the chart the slope of the plot indicated the resistance, R_J, and the point at which the plot intersected the zero line indicated the reversal potential, $\text{p.d.}_{J=0}$. An exchange pump that controlled the inflow and outflow of medium was used to introduce and maintain a constant concentration of hydrogen peroxide around the lens. At different intervals additional J–p.d. plots were determined by injecting current. The responses to different concentrations of hydrogen peroxide are shown in Fig. 1 with the data in Table I. When the results obtained after 30 min exposure to hydrogen peroxide were compared with the control values there were two findings of significance. First, the resting and reversal potentials became more negative. This shifted the whole plot to the left and was usually apparent in 5 min. This shift is the opposite of that produced by

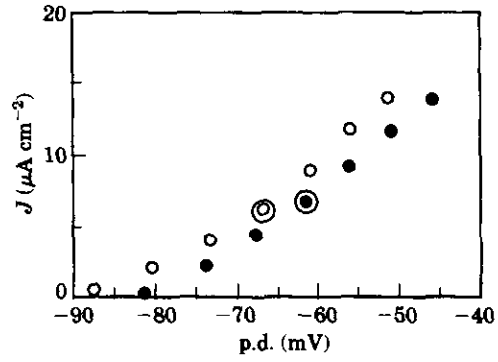

FIG. 2. The effect of ouabain on the current–voltage relationship on the resting and reversal potentials is the opposite of that produced by hydrogen peroxide. (○) Control, (●) Ouabain 20 min; $n = 7$.

TABLE II

Effect of H_2O_2 on the efflux (% efflux in 2 hr) and uptake of ^{86}Rb (C_L/C_M) with contralateral lenses as paired controls (Mean ± S.E.)

^{86}Rb uptake 30 min 2·5 mM K^+	n	Control	Experimental	% Diff.	P
H_2O_2; 50 μM	9	1·43 ± 1·00	1·70 ± 1·30	+18	< 0·01
250 μM	3	1·27 ± 0·06	1·28 ± 0·10	—	—
1000 μM	4	1·38 ± 0·04	0·95 ± 0·12	−31	< 0·05
^{86}Rb efflux*					
H_2O_2; 250 μM	4	16·3 ± 2·1	24·9 ± 1·8	+53	< 0·01
500 μM	4	15·3 ± 0·9	27·5 ± 0·9	+80	< 0·01
1000 μM	4	18·3 ± 2·2	25·0 ± 0·4	+37	< 0·01

* All media contained 2·5 mM K^+ and 10^{-3} M ouabain.

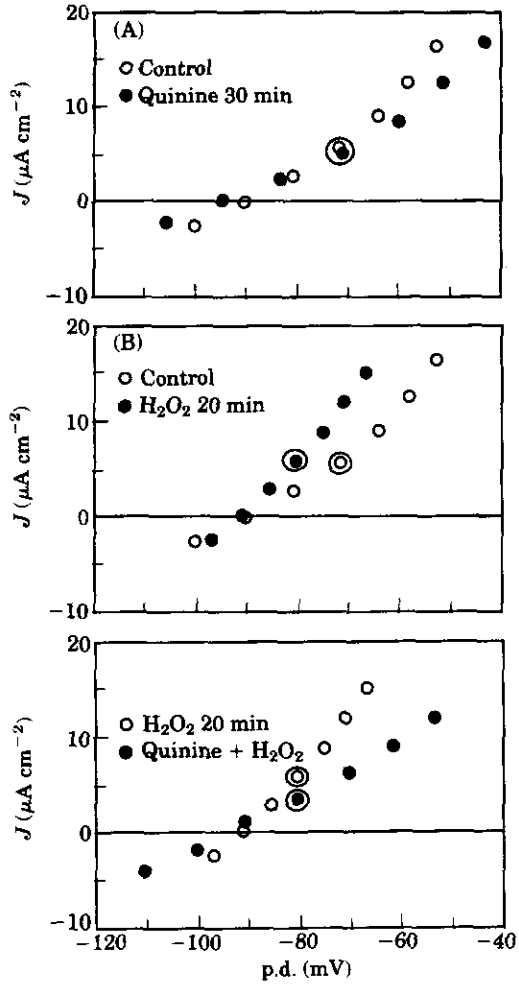

FIG. 3. Current–voltage relationships were determined on (A) control lenses (○) and lenses treated with 100 μM quinine in the medium for 30 min (●), (B) control lenses (○) and lenses with 250 μM hydrogen peroxide in the medium for 20 min (●) and (C) lenses with 250 μM hydrogen peroxide added to the medium for 20 min alone (○) and following 30 min of treatment with 100 mM quinine (●). Exposure to H_2O_2 decreased the resistance, R_J and hyperpolarized the lens. Quinine blocked the effect on R_J, but had no effect on the hyperpolarization. Resting values are encircled. Data is shown in Table III.

ouabain (Fig. 2). Second, there was an increase in the slope of the plot indicating a decrease in the resistance, R_J.

Hydrogen peroxide, at low concentration (50 μM), stimulated ^{86}Rb uptake (Table II). At a high concentration (1000 μM) the net uptake was inhibited. The ^{86}Rb uptake data (Table II) were consistent with previous observations (Delamere, Paterson and Cotton, 1983; Giblin et al., 1987; Spector et al., 1987). The efflux was determined in the presence of 10^{-3} M ouabain so that it would not be complicated by the recycling of ^{86}Rb that enters the medium. The ^{86}Rb efflux, with concentrations of 250–1000 μM hydrogen peroxide, increased on average by 57%. The percentage changes at the different concentrations of hydrogen peroxide were not significant. The ^{86}Rb uptake measurements are net determinations since simultaneous efflux can not be eliminated. In order to decrease the effects of efflux, ^{86}Rb uptake measurements were limited to 30 min.

Quinine has been recognized as an inhibitor of calcium activated potassium channels. Therefore, its effects on the changes produced by hydrogen peroxide were tested. The concentration of hydrogen peroxide was, in all instances, maintained at a constant level with an exchange pump. Current–voltage relationships were determined under four different conditions: (1) control conditions with a standard medium, (2) with 100 μM quinine in the medium for 30 min, (3) with 250 μM hydrogen peroxide in the medium for 20 min and (4) for 20 min with 250 μM hydrogen peroxide added to the medium following 30 min of treatment with 100 mM quinine. The results are shown in Fig. 3 with the data in Table III. The presence of quinine alone produced little effect. The resting points were unchanged. There was some increase in the resistance, R_J, that was significant ($P < 0.05$) when compared with six contralateral paired lenses in the control group. The presence of hydrogen peroxide alone decreased the resistances, R and R_J, and made the resting and reversal potentials more negative than the control values. The effects produced by hydrogen peroxide on the resting and reversal potentials were not affected by the presence of quinine. In the presence of quinine, however, the resistances, R and R_J, were not decreased by hydrogen peroxide and were similar to those produced by quinine alone. In the presence of quinine and hydrogen peroxide the

TABLE III

Effect of 250 μM hydrogen peroxide on the parameters associated with the equatorial potassium current in the absence and presence of 100 μM of quinine (Mean ± S.E.)

	p.d. (mV)	J (μA/cm^{-2})	R (kΩ)	ΔJ (μA/cm^{-2})	R_j (kΩ)	JR_j (mV)	p.d.$_{j=0}$ (mV)
Control (12)	−71·7±0·70	5·7±0·42	8·0±0·37	3·1±0·18	2·64±0·19	14·4±0·94	−86·1±0·88
Quinine (6)	−71·1±0·97	5·2±0·67	10·8±1·36	3·1±0·25	3·45±0·39	16·9±1·19	−88·0±1·09
H_2O_2 alone (12)	−80·6±0·71*	6·0±0·61	5·1±0·31*	2·9±0·18	1·77±0·13*	10·1±0·83	−90·6±0·91*
With quinine (6)	−80·6±0·62*	3·6±0·58*	9·7±0·36*	2·7±0·20	3·72±0·24*	13·3±2·17	−93·8±1·96*

*$P < 0.05$ with respect to control.

TABLE IV

Effect of 50 μM hydrogen peroxide on the parameters associated with the equatorial potassium current in calcium free medium (Mean ± S.E.; n = 6)

	p.d. (mV)	J ($\mu A/cm^{-2}$)	R (kΩ)	ΔJ ($\mu A/cm^{-2}$)	R_j (kΩ)	JR_j (mV)	$p.d._{j=0}$ (mV)
Combined initial	−65·2 ± 1·0	8·6 ± 0·9	3·9 ± 0·2	1·8 ± 0·1	2·22 ± 0·13	18·3 ± 1·3	−83·6 ± 1·4
No H_2O_2							
5 min	−63·0 ± 0·8	7·5 ± 1·1	3·7 ± 0·2	1·6 ± 0·2	2·36 ± 0·11	17·3 ± 2·4	−80·3 ± 1·8
30 min	−53·7 ± 4·3*	10·1 ± 1·8	3·2 ± 0·3	1·4 ± 0·1	2·25 ± 0·13	22·3 ± 3·8	−76·0 ± 2·9
With H_2O_2							
5 min	−68·3 ± 2·4	9·9 ± 1·7	3·4 ± 0·2	1·7 ± 0·1	2·07 ± 0·24	19·7 ± 2·8	−87·9 ± 3·0
30 min	−76·0 ± 2·8*	10·1 ± 1·0	3·0 ± 0·2	1·5 ± 0·1	2·08 ± 0·24	21·1 ± 3·7	−97·1 ± 4·7*

* $P < 0.05$ with respect to combined initial.

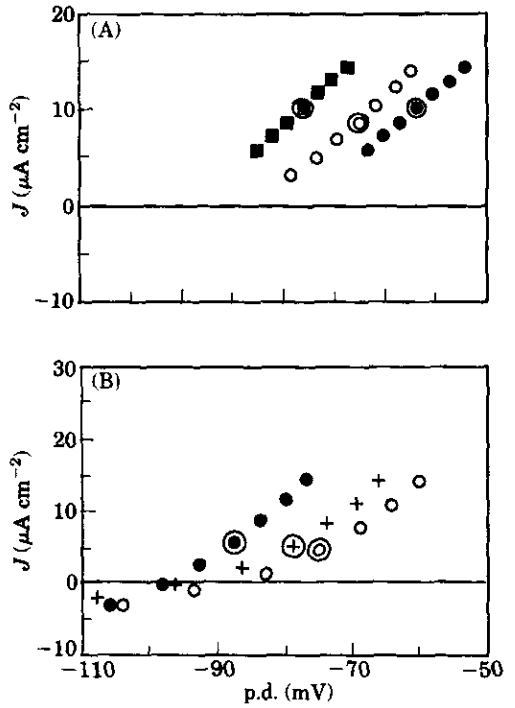

FIG. 4. (A) Current–voltage relationships were determined on 12 lenses in a calcium-free medium (○). This accounts for some depolarization and some decrease in the resistance. Subsequently six of the lenses were followed for an additional 30 min (control, ●). The other six lenses were immediately treated with 200 μM hydrogen peroxide and followed for 30 min (■). With the exchange pump in operation the control group depolarized with time. In the presence of hydrogen peroxide the lenses hyperpolarized with time. There was no significant change in R_j (Table IV). (B) Lenses in a standard medium, containing calcium, that were treated with 200 μM of hydrogen peroxide showed changes that were comparable to those in Fig. 1, (○) initial, (+) 5 min, (●) 30 min. In the presence of calcium, R_j decreased 30% ($P < 0.05$) after 30 min of exposure to hydrogen peroxide. Resting values are encircled.

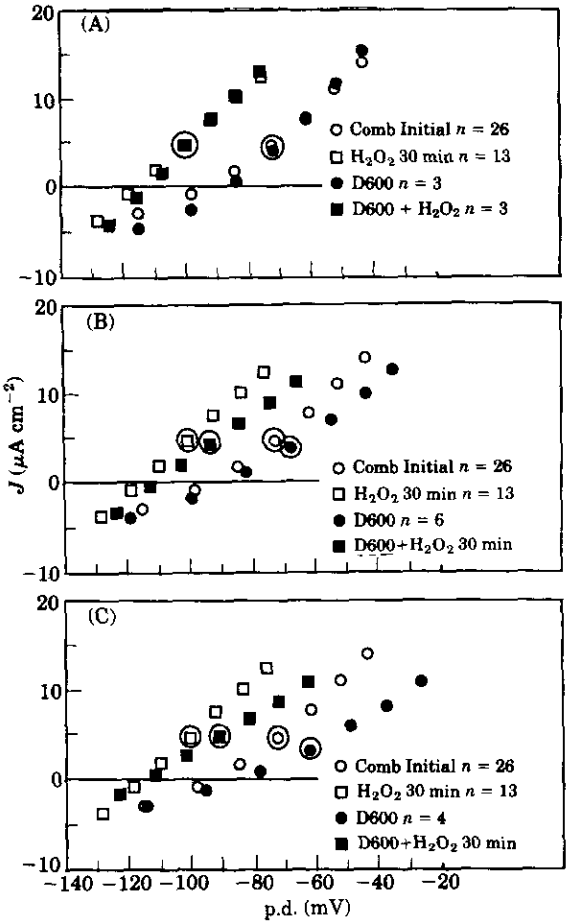

FIG. 5. After obtaining current–voltage curves on 26 lenses half the lenses were treated with (A) 100 ($n = 3$), (B) 200 ($n = 6$), or (C) 400 ($n = 4$) μM of D600 (●). The changes in the J–p.d. plots were noted. All 26 lenses were then treated for 30 min, using an exchange pump to maintain concentration, with 50 μM of hydrogen peroxide [(□) control, (■) +D600]. D600 increased R_j. Depending on the concentration of D600, the slope of the J-p.d. plot was changed about the same amount in the presence as it was in the absence of hydrogen peroxide. Resting values are encircled. Data in Table V.

J-p.d. plot became a straight line indicating a lack of a voltage-dependent change in resistance.

The effects of hydrogen peroxide on the resistance, R_j, in a calcium free medium were determined. Initial current–voltage relationships were determined on 12 lenses 10 min after they were placed in a calcium free medium. One set of six lenses was treated with 200 μM hydrogen peroxide for 30 min with current–voltage

TABLE V

Effect of D600 on the parameters associated with the equatorial potassium current in the absence and presence of 50 μM hydrogen peroxide [Mean ± S.E.; number of lenses in (parentheses)]

	p.d. (mV)	J ($\mu A/cm^{-2}$)	R (kΩ)	ΔJ ($\mu A/cm^{-2}$)	R_j (kΩ)	JR_j (mV)	p.d.$_{J=0}$ (mV)
Before H_2O_2							
Combined initial (26)	−72·5±0·5	4·8±0·3	11·7±0·5	2·7±0·2	4·22±0·21	19·3±1·1	−91·8±1·2
D600							
100 μM (3)	−72·1±2·5	4·2±0·7	11·7±0·5	3·4±0·4	3·49±0·32	14·1±1·4	−86·2±2·5
200 μM (6)	−67·8±2·6	3·9±0·5	14·0±0·8	2·8±0·2	5·02±0·32	18·9±1·9	−86·7±1·2
400 μM (4)	−62·4±4·8	3·3±0·7	14·5±1·6	2·3±0·1	6·21±0·54*	19·8±4·1	−82·2±4·6
H_2O_2 30 min							
H_2O_2 alone (13)	−100·7±2·0	4·7±2·8	8·8±0·4	2·7±0·2	3·30±0·16	15·5±1·3	−118·3±2·0
D600 + H_2O_2							
100 μM (3)	−99·9±3·6	4·8±2·8	7·9±0·9	2·9±0·3	2·79±0·35	11·5±6·6	−111·4±4·7
200 μM (6)	−93·5±5·6	4·4±1·3	9·6±0·3	2·4±0·2	4·24±0·42	19·1±6·2	−112·6±3·1
400 μM (4)	−91·2±7·8	4·9±1·1	10·0±0·9	2·1±0·2	4·81±0·19*	22·9±4·9	−114·0±1·1

*$P < 0·05$ with respect to values without D600.

relationships being determined at 5 and 30 min. The other set of six contralateral lenses (control) was followed in the same manner without being treated with hydrogen peroxide. The results obtained in calcium-free media, in the absence and presence of hydrogen peroxide are shown in Fig. 4 (A) with the data in Table IV. For comparative purposes a graph showing the effects of hydrogen peroxide on lenses in a standard medium with calcium is included in Fig. 4 (B). Switching lenses from a standard to a calcium-free medium shifted the current–voltage plot to the right. A decrease in the resistances, R and R_J, a depolarization of the lens and an increase in the equatorial current were consistent with earlier findings on frog lenses (Patterson, Walsh and Wind, 1989). When control lenses were maintained in a calcium-free medium for 30 min, in the absence of hydrogen peroxide, there was a further shift to the right. The current–voltage relationships of lenses treated with hydrogen peroxide in a calcium free medium were shifted to the left with the resting and reversal potentials, p.d. and p.d.$_{J=0}$, becoming more negative. The resistance, R_J, was not significantly lower than it was in a calcium-free medium.

The effects of D600 on the changes produced by hydrogen peroxide were determined (Fig. 5 and Table V). An initial current–voltage curve was determined on 26 lenses and the lenses were divided into two groups. The first group of 13 lenses was exposed to 50 μM hydrogen peroxide for 30 min, and then another current–voltage plot was determined. The second group of thirteen contralateral lenses was treated with D600 before being exposed to 50 μM hydrogen peroxide for 30 min. Current–voltage relationships were determined on the second group of lenses before and after treatment with D600. The combined group of 26 lenses served as a control for the determination of the effects of D600 in the absence of hydrogen peroxide. As the concentration of D600 was increased from 100 to 400 μM, in the absence of hydrogen peroxide, the slope of the current–voltage plot decreased, indicating a graded increase in the resistance, R_J. The first group of 13 lenses that was not exposed to D600 served as the control for determining the effects of hydrogen peroxide in the presence of 100, 200 or 400 μM of D600. After the 30 min exposure of this control group to hydrogen peroxide there was a significant ($P < 0·01$) hyperpolarization with the p.d. and p.d.$_{J=0}$ becoming more negative than they were before the lenses were exposed to hydrogen peroxide. In addition the resistance was significantly reduced. After treatment with hydrogen peroxide, in the presence of D600, there was a graded increase in the resistance, R_J, as the concentration of D600 was increased from 100 to 400 μM. The current–voltage plots were the same with and without 100 μM D600 and became significantly different with 400 μM D600. The decrease in the resistance, R_J, that occurs after the treatment of fresh lenses with hydrogen peroxide was counteracted by 200 and 400 μM D600. However, the increase in resistance produced by D600 is about the same in the absence and presence of hydrogen peroxide so that the decrease in resistance following treatment with hydrogen peroxide is, on average, the same in the absence and presence of D600.

4. Discussion

Hydrogen peroxide is well established as a factor in cataractogenesis (Bhuyan and Bhuyan, 1984). The level of hydrogen peroxide in the aqueous humor of humans, using the dichlorophenol-indophenol procedure, is reported to be about 26 μM in normal eyes

with a mean of 69 μM in 17 cataractous eyes (Spector and Garner, 1981). A hydrogen peroxide concentration of 50 μM has been suggested as the maximum level that can be tolerated by intact rabbit lenses without apparent damage (Giblin et al., 1987). Lens epithelial cells from young rabbits tolerate 30 μM of hydrogen peroxide in tissue culture, whereas, at this concentration the growth of cells from older rabbits is diminished and the level of glutathione and glutathione reductase activity is decreased (Reddan et al., 1988; Ikebe et al., 1989). A recent report indicates that assays of hydrogen peroxide concentrations made with the dichlorophenol–indophenol method are lower than previously reported and that concentrations of hydrogen peroxide in normal aqueous humor may be in the range of 1–5 μM (Garcia-Castineiras et al., 1992). This reservation, however, does not apply to estimates of hydrogen peroxide toxicity that are based on the addition of known concentrations of hydrogen peroxide to experimental preparations. A concentration in the range of 30–50 μM of hydrogen peroxide in the bathing medium may well be appropriate for defining a limit below which damage is not detected.

After 30 min of exposure of frog lenses to 10–1000 μM hydrogen peroxide the current–voltage relationships are altered in two ways. First, the resting and reversal potentials become more negative and second, the resistance, R_J, is decreased.

The changes in the resting and reversal potentials produced by hydrogen peroxide are the opposite of those produced by ouabain. Based on the Nernst equation, the observed increase in the negative value of the reversal potential indicates that hydrogen peroxide raises the intralenticular concentration of potassium and stimulates the Na, K-pump. This conclusion is supported by the demonstration that hydrogen peroxide increases the ^{86}Rb uptake of the lens (Table II, and Delamere, Paterson and Cotton, 1983; Giblin et al., 1987; Spector et al., 1987). This effect of hydrogen peroxide is clearly evident with hydrogen peroxide concentrations as high as 250 μM. At higher concentrations the effect may decrease or be masked.

The decrease in the resistance, R_J, produced by hydrogen peroxide is inhibited by quinine and the $J-$p.d. plot changes from a curve to a straight line. The latter is consistent with a loss of voltage-dependent resistance. Quinine is recognized as a drug that has an inhibitory effect on calcium-dependent potassium channels (Armondo-Hardy et al., 1975). The change in resistance produced by hydrogen peroxide is not observed if calcium is removed from the medium. This is consistent with the decrease in resistance being dependent on the presence of calcium but inconclusive since a calcium-free medium itself may have exhausted the possibility of any additional decrease. In other tissues increasing the concentration of intracellular calcium by injection (Krnjevic and Lisiewicz, 1972), by exposure to an ionophore (Oliveira-Castro and Dos Reis, 1981) or by inhibiting the calcium pump with vanadate (Varecka and Carafoli, 1982) is followed by an activation of calcium-dependent potassium channels. In rabbit lens epithelial cells the free calcium concentration has been measured with Indo-1, a fluorescent calcium indicator (J. R. Reddan, Oakland Univ., pers. commun.). The basal level was 10–200 nM. Following exposure to a single dose of 50 μM H_2O_2 there was a gradual increase in calcium concentration. These findings suggest that the decrease in resistance following exposure to hydrogen peroxide may be related to an increase in the concentration of intracellular calcium. The increase in the resting and reversal potentials produced by exposure of lenses to hydrogen peroxide is not affected by quinine or by the removal of calcium from the medium bathing the lens.

The changes in the $J-$p.d. plots that occur following treatment with hydrogen peroxide are qualitatively similar to those produced by the impermeant sulfhydryl binding agent, *p*CMPS (Walsh and Patterson, 1991), and have been attributed to a loss of membrane sulfhydryl groups either by binding to *p*CMPS or by oxidation after exposure to hydrogen peroxide. The decrease in the resistance, R_J, but not the changes in resting and reversal potentials, were ascribed to an increase in free calcium.

The oxidation of sulfhydryl groups is thought to initiate the opacification process of cataractogenesis and appears to begin at the lens fibre membrane (Garner and Spector, 1980). Opacification is associated with an increase in lens calcium (Hightower and Harrison, 1982; Hightower, 1985, 1986; Marcantonio et al., 1986; Duncan, Emptage and Hightower, 1988; Clark, Danford-Kaplan and Delaye, 1988; Hightower et al., 1989; Shearer et al., 1992). The calcium pumps of calf lens fibre cells (Galvan and Louis, 1988) and of human red cells (Varecka and Carafoli, 1982) are located on the external aspect of the membranes. Therefore, the sulfhydryl groups of the calcium pump are readily accessible to *p*CMPS and hydrogen peroxide (Hightower, 1986; Galvan and Louis, 1988) and an imbalance between passive calcium influx and active calcium efflux could account for an increase in intralenticular calcium and the opacification of the lens.

Opacification associated with the appearance of diabetic and galactosemic cataracts in rats is a relatively sudden occurrence that is accompanied by a loss of the semipermeable properties of lens membranes (Patterson and Bunting, 1964; 1965). The smaller lens proteins are lost and small molecules enter and leave the lens in accord with the concentration gradient. On the basis of organ culture studies of bovine lenses it appears that protein loss is preceded by an increase in lens calcium and followed by a sudden increase in high molecular weight proteins (Marcantonio et al., 1986). Calcium is involved in binding lens proteins to fibre membranes (Broekhuyse

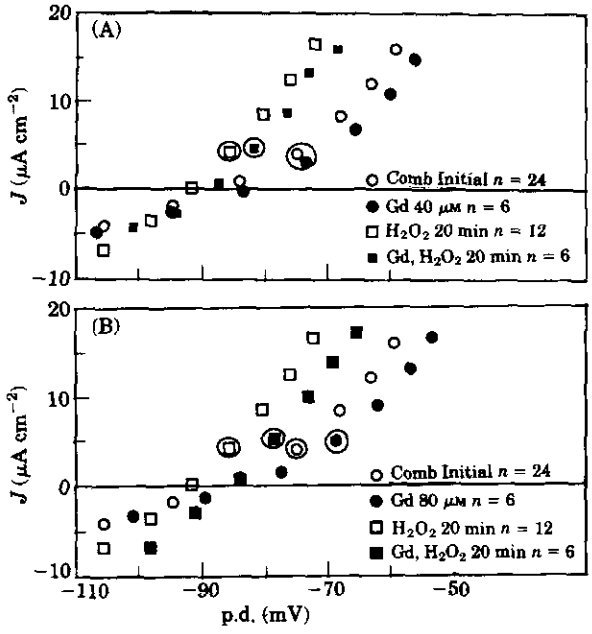

FIG. 6. Gd^{3+}, 40 (A) or 80 (B) μM, alters the slope of the current–voltage relationships about the same amount in the absence and presence of 200 μM hydrogen peroxide. Resting values are encircled.

and Kuhlmann, 1978; van den Eijnden-van Raaij, de Leeuw and Broekhuyse, 1985) and calcium induced opacity is accompanied by an increase in high molecular weight proteins with subsequent light scattering (Jedziniak et al., 1972; Spector, Adams and Kruul, 1974). Thus the oxidative properties of hydrogen peroxide may be followed by calcium induced changes that produce opacities in the lens.

The prevention of diabetic cataract formation in rats by verapamil (Fleckenstein, 1983; Pierce et al., 1989) is supportive of the proposed role of calcium in cataractogenesis. In human red blood cells calcium influx occurs through a verapamil sensitive carrier-mediated system and calcium efflux is dependent on a Ca ATPase pump (Varecka and Carafoli, 1982). A comparable electrically neutral system may be present in the lens in as much as calcium channels have not been found in lens membranes. It was suggested earlier that the ease of calcium influx can be gauged by changes in the resistance of the equatorial current of the lens, R_J. In the absence of hydrogen peroxide, D600, a verapamil analogue, increases the resistance associated with the equatorial current of the lens (Walsh and Patterson, 1992). The gadolinium ion, a non-specific inhibitor of calcium influx (Hambly and dos Remedios, 1977; Bourne and Trifaro, 1976; Overall and Jaffe, 1985), produces a similar effect (Fig. 6). Each of these substances increases the resistance, R_J. Hydrogen peroxide has the opposite effect and decreases R_J. In the presence of hydrogen peroxide, D600 and Gd^{3+} increases R_J approximately the same amount that they do in the absence of hydrogen peroxide (Figs 5 and 6). Therefore, it is suggested that D600 and the gadolinium ion partially block carrier-mediated calcium influx so that the effect of hydrogen peroxide on calcium influx and on cataractogenesis is diminished.

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