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D600 Increases the Resistance Associated with the Equatorial Potassium Current of the Lens

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The effects of D600, the methoxy analog of verapamil, on a pCMPS system were studied. A major effect of D600 is to increase the resistance associated with the equatorial potassium current of the lens. The increase in resistance is statistically significant at concentrations above 200 μM. At concentrations of 25–50 μM, D600 counteracts the decrease in resistance produced by binding sulfhydryl groups with 1 μM pCMPS. This effect is similar to that produced by quinine and by a calcium-free medium, and is attributed to the prevention of an increase in the calcium-dependent potassium conductance produced by pCMPS.

Key words: lens equatorial current; calcium-dependent potassium current; D600; pCMPS; lens.

1. Introduction

Verapamil, a calcium-blocking agent, prevents the development of cataracts in alloxan diabetic rats (Fleckenstein, 1983). In 13 rats receiving verapamil by stomach tube each day (25 mg kg^{-1} body weight), only one out of 26 lenses (3·9%) developed cataracts in 6·5 months compared with an incidence of 62% in the control group during the same period. The appearance of cataracts was attributed to an elevation of lens calcium. The addition of verapamil to a galactose diet (0·3%, > 150 mg kg^{-1} body weight) failed to delay cataracts in rats (unpubl. data). This suggests that the development of cataracts is a prolonged process and may involve seemingly minor effects acting over a long period of time. Therefore, it was felt that studies at an in vitro level might suggest a basis for the effects of a calcium-blocking agent.

Exposure of lenses to parachloromercuriphenylsulfonate (pCMPS), a nearly impermeant sulfhydryl-binding agent, increases calcium in the lens and causes the appearance of opacities (Hightower, 1985; Hightower, Reddan and Dziedzic, 1990). In frog lenses, pCMPS produces changes in the current–voltage relationships of the equatorial current of the lens that are attributed to an increase in the level of lens calcium (Walsh and Patterson, 1991). Therefore, D600, the methoxy derivative of verapamil and a more potent calcium-blocking agent, was tested to see if it would counter the results of exposing the lens to pCMPS.

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. The lens being studied was supported on a platinum ring in a Petri dish and was bathed with a frog Ringer 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; Jaffe and Nuccitelli, 1974). The lens was oriented in the Petri dish so that the plane of the equator was horizontal and the anterior surface was down. Microelectrodes were made with a Narashige puller and had a tip resistance of 2–6 MΩ. Two microelectrodes 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 3 mM KCl was used for measuring the potential difference (PD). It was connected to an amplifier WPI–S7100A and S7040A (WPI, New Haven, CT) 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). Positioning of the electrode within the lens was assured by using it to make the initial measurements of PD. 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). 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. Chemicals were obtained from Sigma (St Louis, MO). The following measurements were obtained directly from the recordings: (i) PD, the potential difference across the surface membranes; (ii) J, the current at the equator; (iii) R the input resistance. The ΔPD or the change in PD per μA of injected current, I. The average response with 1, 2 and 3 μA injected on each side of the PD is used; (iv) ΔJ, the change in J per μA of injected current; (v) $PD_{J=0}$,

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TABLE I

Effect of different concentrations of D600 on lens parameters associated with the equatorial current (mean ± S.E.; six or more lenses per concentration level)

	PD (mV)	J (μA cm^{-2})	R (KΩ)	ΔJ (μA cm^{-2})	R_J (KΩ)	JR_J (mV)	$PD_{J=0}$ (mV)
Control							
10	−75 ± 1·06	6·0 ± 0·66	8·74 ± 0·59	3·76 ± 0·20	2·35 ± 0·18	13·9 ± 1·20	−89 ± 1·17
25	−73 ± 1·36	5·77 ± 0·94	9·99 ± 0·67	4·21 ± 0·24	2·39 ± 0·16	13·3 ± 1·93	−87 ± 2·57
50	−71 ± 2·54	5·63 ± 0·64	9·83 ± 1·04	3·85 ± 0·37	2·67 ± 0·36	14·1 ± 1·32	−85 ± 2·47
100	−74 ± 0·61	4·42 ± 0·53	10·97 ± 0·77	3·61 ± 0·35	3·12 ± 0·23	13·3 ± 1·07	−87 ± 0·82
200	−75 ± 0·89	4·53 ± 0·64	12·44 ± 0·59	2·92 ± 0·19	3·77 ± 0·30	15·9 ± 1·38	−91 ± 1·43
500	−73 ± 2·03	4·40 ± 0·77	12·73 ± 0·83	2·60 ± 0·14	4·92 ± 0·32	20·9 ± 2·87	−93 ± 2·71
D600							
10	−77 ± 1·31	6·38 ± 0·70	9·12 ± 0·48	3·81 ± 0·32	2·45 ± 0·20	15·3 ± 1·31	−92 ± 170
25	−72 ± 3·40	5·57 ± 0·91	10·79 ± 0·78	3·77 ± 0·22	2·90 ± 0·21	15·4 ± 1·65	−88 ± 3·12
50	−74 ± 4·67	−7·33 ± 1·18	9·77 ± 1·14	3·29 ± 0·30	3·09 ± 0·45	20·0 ± 0·98	−94 ± 4·52
100	−70 ± 1·67	3·84 ± 0·46	12·46 ± 0·59	3·36 ± 0·21	3·76 ± 0·19	13·9 ± 1·06	−86 ± 1·61
200	−71 ± 1·26	3·66 ± 0·26	11·24 ± 0·68	2·31 ± 0·20	5·02* ± 0·30	18·4 ± 1·86	−90 ± 1·41
400	−66 ± 3·70	3·37 ± 0·53	14·49 ± 1·13	2·29 ± 0·09	6·33* ± 0·44	20·5 ± 2·73	−86 ± 4·03

* $P < 0·05$ with respect to the control.

the reversal potential is obtained by injecting hyperpolarizing current until J equals zero.

The following are calculated from the above data and are given in the Tables. (i) R_J, the resistance of the segment of the K^+ loop that is being studied ($\Delta PD/\Delta J$) (ii) JR_J, the '*IR*' drop of Ohm's law or the driving force. It equals $PD - PD_{J=0}$ when the plot of J vs. PD is a straight line; (iii), $PD_{J=0}$, the reversal potential may be estimated from the relationship for the driving force ($PD_{J=0} = PD - JR_J$). This estimated value is used in the Tables.

3. Results

Effects of D600

Lenses suspended in a Petri dish were impaled with microelectrodes and a vibrating probe was positioned at the equator. After 10–15 min are the equatorial current, J, and the PD were stable. The responses of J and PD to the injection of 1, 2 and 3 μA of depolarizing and hyperpolarizing current, I, were then determined. Using the results obtained with several lenses, the average values for the resting potential, PD, the equatorial current, J, the input resistance, R, and the change in J per μA of injected current, ΔJ, were obtained. In addition, the resistance associated with the equatorial current, R_J the driving force, JR_J, and the reversal potential, $PD_{J=0}$, were calculated. These parameters are reflected in the current–voltage plots. The initial values for different parameters, obtained before the addition of D600, served as internal controls. Thereafter, D600 was added to the bathing medium at various concentrations and the current–voltage relationships were redetermined after 20 min. The results are shown in Table I. Biological and seasonal variations are evident in the control group so that experimental effects must be measured against the internal controls. If one considers the 10–50 μM range, D600 had little effect. With 200 or 400 μM of D600, only the levels of the resistance, R_J, were significantly higher ($P < 0·05$) than the control values. The percentage increases of R_J over control values at different concentrations of D600 are shown in Fig. 1. The connecting line is a computer-generated logarithmic curve. The values at 200 and 400 μM D600 in addition to being significantly higher than control values are also significantly higher than those obtained with 10 μM D600.

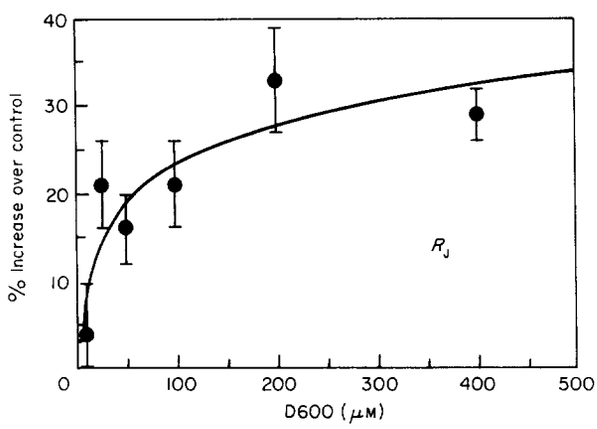

FIG. 1. Effect of D600 concentration of the % increase, above the internal control lens, of the resistance, R_J, associated with the equatorial current of the lens. (Mean ± S.E.; six or more lenses per point.)

Effect of D600 on pCMPS Treatment

Lenses were placed in the measuring apparatus in a standard Ringer solution and the current–voltage relationships were determined on all lenses after they reached a steady state. D600 was added to half of the lenses at a final concentration of 25 or 50 μM. The

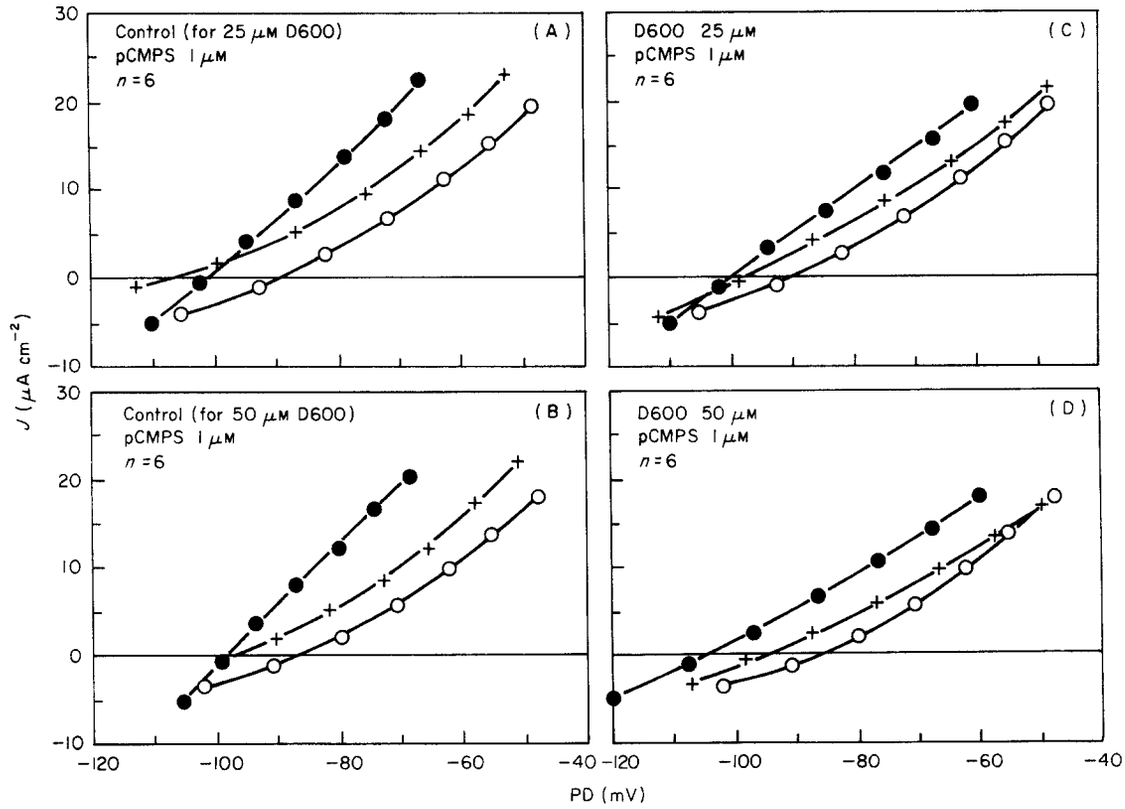

FIG. 2. Effects of pCMPS on the current–voltage relationships of the equatorial current of the lens are shown on the left. Note the shift to the left at 5 min and the change in slope of resistance, R_J, at 20 min. The contralateral lenses on the right were treated with D600 before the addition of pCMPS. Note a shift to the left without a change in R_J (Table I). The middle point in each set shows the resting values. (A), (B), (○) comb initial; (+) control 5 min; (●) control 20 min: (C), (D), (○) comb initial; (+) experimental 5 *n*; (●) experimental 20 min.

TABLE II

Effect of 1 μM pCMPS on frog lenses in the absence (control) and presence (Exper.) of D600 (mean ± SEM; number of lenses in parentheses)

	PD (mV)	J (μA cm^{-2})	R (KΩ)	ΔJ (μA cm^{-2})	R_J (KΩ)	JR_J (mV)	$PD_{J=0}$ (mV)
50 μM D600							
Initial (12)	−71 ± 1·54	5·6 ± 0·37	8·97 ± 0·69	3·7 ± 0·26	2·52 ± 0·22	13·4 ± 0·77	−84 ± 1·61
pCMPS (20 min)							
Alone (6)	−87* ± 1·36	8·0 ± 0·94	6·28* ± 0·67	4·3 ± 0·24	1·58 ± 0·16	11·7 ± 1·93	−99* ± 2·57
With D600 (6)	−86* ± 3·84	6·7 ± 1·70	10·01 ± 1·60	3·8 ± 0·70	2·91 ± 0·48	15·7 ± 1·45	−102* ± 4·62
25 μM D600							
Initial (12)	−72 ± 1·21	6·8 ± 0·84	9·82 ± 0·98	4·1 ± 0·32	2·37 ± 0·16	15·3 ± 2·16	−89 ± 2·98
pCMPS (20 min)							
Alone D600 (6)	−87* ± 2·19	8·9 ± 1·80	7·51 ± 0·82	4·6 ± 0·62	1·68* ± 0·14	14·3 ± 2·68	−101 ± 3·43
With D600 (6)	−84 ± 5·01	−7·4 ± 1·76	8·59 ± 0·54	4·2 ± 0·26	2·12 ± 0·22	17·2 ± 4·77	−102 ± 2·53

* $P < 0·05$ with respect to the control.

contralateral lenses were used as controls. Both groups of lenses were treated with 1 μM pCMPS and current–voltage plots were determined at 5 and 20 min. The results are shown in Fig. 2 and Table II. The control lenses reflected the effects of the first two phases that have been described for pCMPS (Duncan, Emptage and Hightower, 1989; Walsh and Patterson, 1991). The first phase was noted in the control group at 5 min with a shift of the plot to the left. This was associated with the PD and reversal potential becoming more negative than they were prior to the addition of pCMPS. The second phase was noted at 20 min with the reversal potential staying the same as is was at 5 min while the slope became steeper. The curvature of the initial plot was lost, the resistances R and R_J were reduced and the current, J, was elevated.

In the presence of D600, the results were more pronounced with 50 μM D600 than they were with 25 μM D600 (Table II). The PD and reversal potential were shifted to the left at 5 min, as expected for the

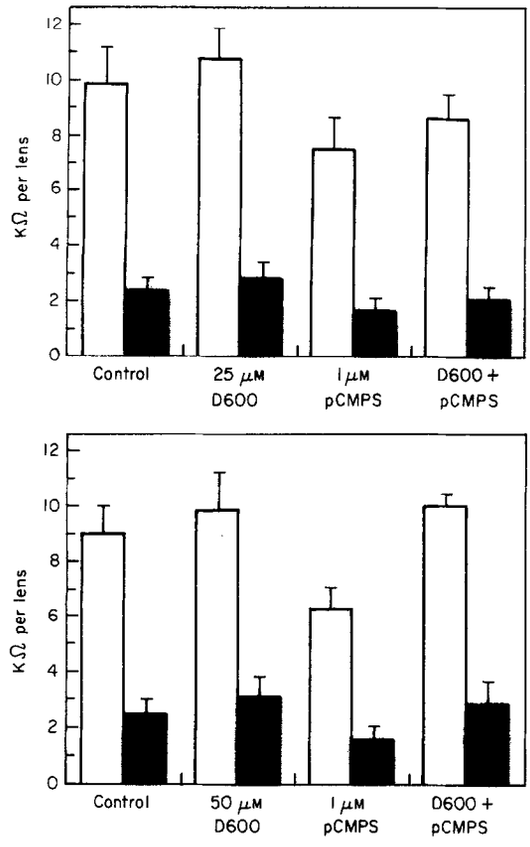

FIG. 3. Values of the resistances R (□) and R_J (■) in control lenses without D600 or pCMPS; in lenses with only D600 added; in lenses with only pCMPS added; and in lenses with both D600 and pCMPS. (Mean ± S.E. for six lenses.)

first phase of the pCMPS effect. However, an increase in resistance was also evident so that the plot does not parallel the initial J–PD relationship. The important difference, as a result of the presence of D600, was that the resistances, R and R_J, which were reduced as part of the second-phase effect of pCMPS, were not reduced when compared with the initial levels. The effects of D600 and pCMPS on the resistances, R and R_J, are illustrated in Fig. 3. The resistances, R and R_J, are reduced about 30% by the addition of pCMPS. This is prevented if D600 is also present.

4. Discussion

Verapamil produces its effect by preventing an increase in the level of lens calcium. Fleckenstein (1983) found that cataracts were first observed when the calcium level was over four times the normal value. Hightower (1985), while studying the effects of pCMPS on calcium levels in rabbit lenses in vitro, found that opacities could be produced in 20 hr and that the lens calcium threshold (0·91 mM) was four to five times the level in fresh lenses (0·21 mM). Thus, the increase in lens calcium required to produce an opaque lens appears to be the same whether the change is the result of diabetes or the treatment of the lens in vitro with pCMPS.

The changes that occur following the exposure of frog lenses to low concentrations of pCMPS are three-fold (Duncan, Emptage and Hightower, 1989; Walsh and Patterson, 1991) (Fig. 2, control group). The first phase is a hyperpolarization with a shift in the current–voltage relationship to the left. It is characterized by the PD and reversal potential becoming more negative than the initial values and is attributed to a stimulation of the Na, K pump. This is based on the following observations: (i) the shift to the left of the current–voltage plot is the opposite of that produced by ouabain; (ii) the shift to the left is prevented by ouabain; (iii) the ^{86}Rb uptake is increased. The second phase is an increase in conductance with an increase in the slope of the current–voltage relationship. It is characterized by a decrease in the resistance, R_J, and is attributed to an increase in calcium-dependent potassium conductance. This is based on the following findings: (i) the change does not occur in a calcium-free medium; (ii) the change is blocked by quinine; (iii) lens calcium concentration can be elevated by pCMPS. The third phase is a rapid depolarization that is attributed to a continued increase in calcium concentration.

D600 prevents the decrease in resistance associated with phase 2 when frog lenses are treated with pCMPS. This is accomplished with 25 and 50 μM D600 which have little effect in the absence of pCMPS. These results are similar to those found with quinine (Armando-Hardy et al., 1975; Duncan, Emptage and Hightower, 1989; Walsh and Patterson, 1991) and are consistent with, but not proof of, an inactivation of calcium-dependent potassium channels by D600. This might occur by either of two possible mechanisms. (1) Verapamil and D600 act as blocking agents on open calcium channels (Lee and Tsien, 1983). Although voltage-gated calcium channels have not been demonstrated in the lens, the prevention, by verapamil, of an increase in the lens calcium concentration of diabetic rats (Fleckenstein, 1983) indicates that verapamil can counteract an influx of calcium. Consequently, any increase in lens calcium due to pCMPS would be reversed as the calcium level is lowered by an active calcium pump. (2) D600 may act directly to block calcium-dependent potassium channels (Gola and Ducreux, 1985). In the absence of pCMPS, when the concentrations of D600 are greater than 200 μM (Table I) the resistance, R_J, is increased. This may be the result of a direct action of D600 on calcium-dependent potassium channels. It is concluded that D600 prevents the increase in calcium-dependent potassium conductance produced by pCMPS.

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