PATCH CLAMP RECORDINGS FROM THE EPITHELIUM OF THE LENS OBTAINED USING GLASSES SELECTED FOR LOW NOISE AND IMPROVED SEALING PROPERTIES

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We have measured currents from single ionic channels located in the apical membrane of lens epithelial cells from six species of animals. These cells require no enzyme treatment to prepare their surfaces for sealing. Because most of the channel types show fast flickering and some have small single channel conductances, we have spent considerable effort to lower electronic noise from the headstage and to investigate special glasses whose specifications predict low electrical noise when used as patch-clamp electrodes.

RESULTS

We have designed and constructed a headstage which utilizes a U430 dual JFET (Siliconix, Santa Clara, CA) as the input stage (1). The product of the input voltage noise and gate to source capacitance of the U430 (~2 $nV/Hz^{1/2}$ and 10 pF) is the lowest of commercially available FET suitable for patch-clamp applications. Thus headstages using a U430 common source input stage exhibit the lowest high-frequency noise levels that can be achieved at present. Selected units have gate currents below 0.5 pA for drain to gate voltages (V_{DG}) <4 or 5 volts. We operate the U430 at 6 mA per side and maintain V_{DG} at <3.5 volts. With a 50-GΩ chip resistor (National Micronetics, West Hurley, NY, kindly provided by F. Sigworth) in the feedback loop, the headstage has 0.10 pA rms noise for a 5 kHz bandwidth (−3 dB bandwidth of an 8-pole Bessel filter) with the input open-circuited. Its spectral density is shown in Fig. 1 *a*; at 5 kHz the headstage noise has reached a level approximately equal to the Johnson noise of a 6-GΩ resistor.

The interaction between glass and cell membrane resulting in a gigaseal is not well understood. Therefore, the selection of glasses for sealing properties is empirical. Also, because different cell membranes vary in their chemistry, morphology, and surface coatings, it is unlikely that any one glass will react in the same way with all cell membranes. We expected, however, that current noise associated with a glass used for a patch clamp electrode should be a direct function of its loss factor, and that a high volume resistivity and low dielectric constant are desirable for low noise. Our approach has been to select (10^6 Hz) glasses with loss factors at 10^6 Hz as low or lower than that of Corning #7740 (Pyrex) (Corning Glass, Corning, NY), and to compare their performance to Kimble #R-6 (soda lime) with respect to current noise and ability to seal to lens epithelial cells (Kimble Products Div., Owens-Illinois, Inc., Toledo, OH). All glasses were coated with #184 Sylgard (Dow Corning Corp., Midland, MI) to within 100 μm from the tip (2). #184 Sylgard was chosen following an extensive investigation of many other coatings; this particular Sylgard was found to provide the lowest noise over the frequency range of interest.

The noise comparison for three selected glasses gigasealed to Sylgard is shown in Fig. 1 *b–d*). As anticipated,

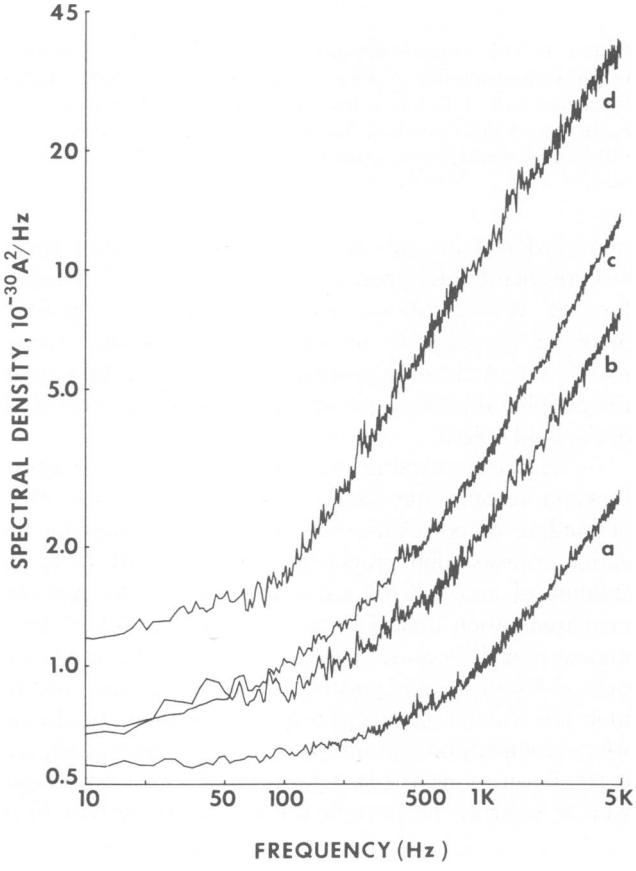

FIGURE 1 Power-spectral density of the noise of the headstage alone (*a*) and the total noise with patch electrodes made from three different electrode glasses, Sylgard-coated and sealed to Sylgard. Glasses are Corning #1723(*b*), Corning #7052(*c*), and Kimble #R-6(*d*). Spectral densities at 5 KHz in units of 10^{-30} A^2/Hz are: $a = 2.6$; $b = 7.3$; $c = 13.7$; $d = 36.4$. The curves were wild-point edited at multiples of 60 Hz. High-resistance seals to lens membranes with these glasses frequently give noise levels comparable to those in the figure when no field exists across the patch.

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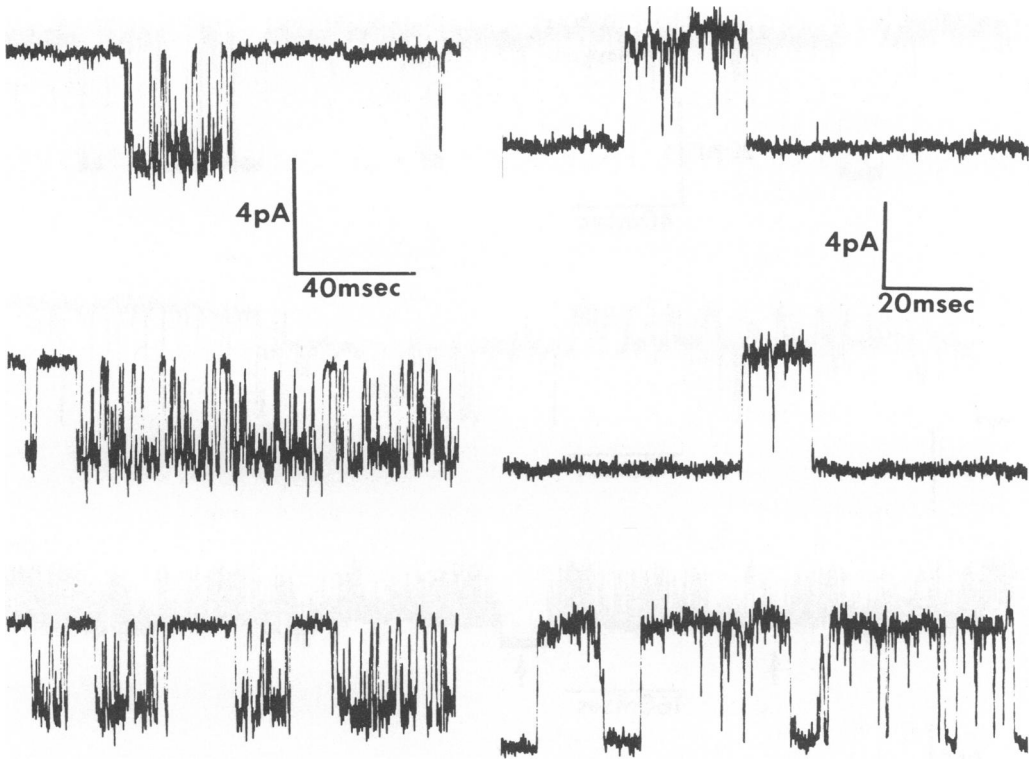

FIGURE 2 Representative time records of currents through the 25-pS channel (panel *A*) recorded with #7052 glass, and the 50-pS channel (panel *B*), recorded with #1723 glass. Both are on-cell patches recorded at a bandwidth of 3.5 KHz (−3 dB 8-pole Bessel).

the higher volume resistivity glasses have the best noise performance. Corning #1723 (aluminosilicate), with a loss factor of 1% at 10^6 Hz had the lowest noise of all glasses we tested, sealed easily to lens cells, and allowed fields of at least ±70 mV routinely across inside-out patches. This glass softens at high temperature (910°C), and thus rapidly degrades heater coils when it is pulled. In our hands, this is the best glass to use when low noise is of prime importance.

Corning #7052 (borosilicate Kovar sealing glass) softens at a temperature similar to that for soda lime glass (710°C) but has a loss factor of 1.3% at 10^6 Hz and thus has good noise properties. It was selected because of these specifications. It proved, however, to have exceptional sealing properties with our cells, producing 400 seals in 406 attempts during initial trials. In addition, multiple seals (up to seven) were often possible with the same electrode. It is the only glass with which we have obtained multiple seals. The seals were mechanically robust and allowed ±190 mV fields across inside-out patches. Although it is improbable that this glass will perform so laudably with all cells, it is likely to be a useful patch clamp glass for many preparations.

Kimble #R6 (soda lime), with a loss factor of 5.1% at 10^6 Hz and a softening temperature of 700°C, seals well and allows fields in excess of ±100 mV across inside-out patches. This glass is noisier than hard glasses we have tried, although its noise could be reduced by extending the Sylgard coating closer to the tip. Borolex (Rochester Scientific Co., Rochester, NY), a commonly used glass for patch clamping, has noise properties similar to Corning #7052 but seals to our cells infrequently.

Using all of these glasses, we have been able to record single-channel currents from lens epithelial cells. To date, we have seen six different kinds of channels based on single-channel conductance alone. The three most common channels are shown in Figs. 2 and 3. The 25 pS channel (Fig. 2 *A*) has properties similar to the nonselective cation channel reported from other cells (3–5). This channel flickers at a particularly high rate and shows Ca^{++} dependence. The 50 pS channel (Fig. 2 *B*) is also quite nonselective, but in preliminary selectivity studies shows about a 1.4:1 selectivity for Na^{++} over K^{++}. The channel shown in Fig. 3 varies in conductance from ~400 pS to 1.9 nS in different patches and usually requires a voltage field in excess of ±70 mV for many seconds to activate. It shows no selectivity for small anions or cations.

We speculate that the "channel" results from a cooperative gating of gap junction protochannels. Our speculation arises both from the observation that lens membranes contain a high density of "gap junction" particles and from the notion that it is unlikely for a nonselective channel with such a large conductance to connect the cell's interior with its extracellular space, because opening of the channel would rapidly dissipate the cell's ionic gradients. Therefore, our evidence that the "channel" is related to gap

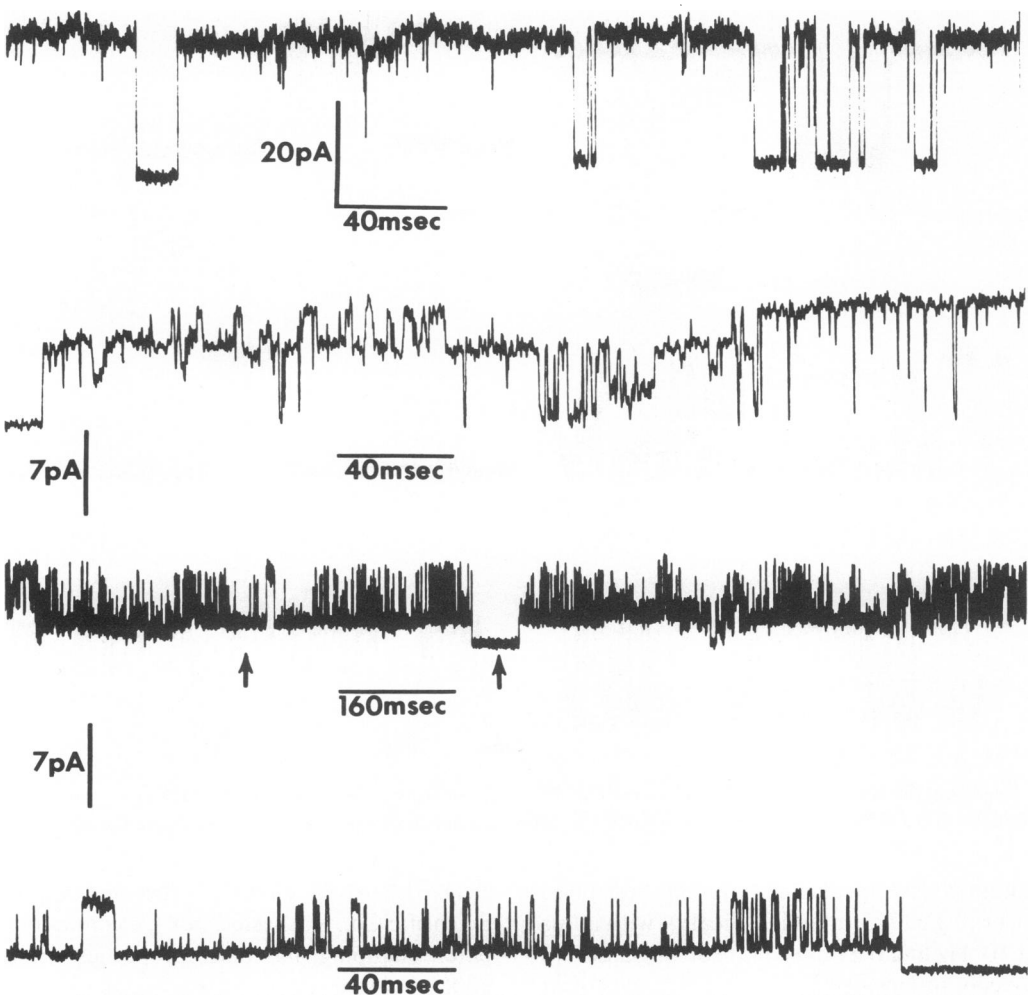

FIGURE 3 Time records of currents through the 400 pS-1.9 nS channel of the lens epithelium. The top trace show one of the relatively rare instances when the "channel" is well behaved, showing numerous full openings and closures of reasonable duration. Typical behavior is shown in the bottom 3 traces where the "channel" flickers rapidly between apparent subconductance levels which might alternatively be parallel channels of ~100 pS conductance operating with a high degree of cooperativity. The bottom trace is an expanded plot of the region between the arrows from trace 3.

junctions is simply that the "channel" does not seem to be anything else.

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