Ba^{2+} release from soda glass modifies single maxi K^+ channel activity in patch clamp experiments

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ABSTRACT Glasses used to fabricate patch pipettes may release components which affect ion channels (Cota, G., and C.M. Armstrong. 1988. *Biophys. J.* 53:107–109; Furman, R.E., and J.C. Tanaka. 1988. *Biophys. J.* 53:287–292; Rojas, L., and C. Zuazaga. 1988. *Neurosci. Lett.* 88:39–44). The gating properties of maxi K^+ channels from *Necturus* gallbladder epithelium depend on whether borosilicate glass (BG) or blue tip hematocrit glass (SG) is used to construct the patch pipettes. The data are consistent with solubilization from SG of a component which exerts voltage-dependent, cytosolic-side specific block, closely resembling "slow block" by Ba^{2+} ions. Ringer's solution preincubated with SG, but not with BG, blocked inside-out maxi K^+ channels when used as bathing solution. Mass spectrometry revealed that Ba^{2+} is released by the glass from fast and slow-release compartments (SG contains 3% wt/wt BaO), and is the only ion found in the solution at concentrations consistent with the observed channel block. Additionally, SG released O^{2-}, Na^+, Ca^{2+}, and Mg^{2+}, all to micromolar concentrations. These elements do not interfere with maxi K^+ channels but they could in principle alter the properties of other ion channels. Thus, screening for channel-modifying substances released by the glass may be necessary for the adequate interpretation of patch-clamp results.

INTRODUCTION

Since the introduction of the patch-clamp technique a large number of glasses have been utilized for the construction of patch pipettes. Criteria for selection have included: (*a*) thermal and electrical properties of the glass, (*b*) tip shape after pulling and polishing, (*c*) sealing properties, and (*d*) ease of pipette construction (Corey and Stevens, 1984; Sakmann and Neher, 1984; Rae and Levis, 1984).

Recently, the release of K^+ channel blockers from pipette glass has been reported, as have glass-dependent alterations in the gating of acetylcholine-activated channels (Cota and Armstrong, 1988; Furman and Tanaka, 1988; Rojas and Zuazaga, 1988). It follows that an important consideration in the choice of glass is the possibility that substances released from the glass alter channel properties.

The apical membrane of the epithelial cells of *Necturus* gallbladder possesses a high conductance K^+ channel which is activated by membrane depolarization and by elevation of cytosolic Ca^{2+} (Segal and Reuss, 1990*a*,*b*; Copello et al., 1991). This channel shares properties with so-called maxi K^+ channels, widely distributed in different cells and species (Latorre et al., 1989; Rudy, 1988). Most patch pipettes used in our studies were constructed from either Blue Tip Coded hematocrit soft glass from Fisher Scientific (Pittsburgh, PA) (soda glass), or the hard glass TW150-6 from World Precision Instruments (New Haven, CT) (borosilicate glass).

Here, we present data on outside-out patches showing that soda-glass pipettes, but not borosilicate-glass pipettes, produce abnormal gating of maxi K^+ channels, suggesting a release (by soda glass) of substances that interact with the channels. In previous publications, the possible K^+-channel blockers in soda glasses were confined to the polyvalent cations, but the blockers were not identified (Cota and Armstrong, 1988; Furman and Tanaka, 1988). In this communication, we show that Ba^{2+} is released by the glass, reaches micromolar concentrations in the pipette solution, and accounts for the maxi K^+ channel block observed with soda glass.

MATERIALS AND METHODS

Patch-clamp methods

Patch-clamp techniques applied to dissociated epithelial cells from *Necturus* gallbladder have been described previously (Altenberg et al., 1990; Segal and Reuss, 1990*a*,*b*; Copello et al., 1991). Mudpuppies (*Necturus maculosus*) were obtained from Nasco Biological (Ft. Atkinson, WI) or Kon's Scientific (Germantown, WI), kept in tap water at 5°C, and anesthetized by immersion in a 1 g/l solution of tricaine methanesulphonate. The gallbladders were excised, sliced open, drained of bile, pinned mucosal side up in a Sylgard-coated Petri dish, and bathed with NaCl Ringer solution containing (in mM): 97.5 NaCl, 2.5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES/NaOH, pH 7.4, at room temperature (~ 22°C). Cell suspensions were prepared by a 7-min incubation of scraped epithelial sheets in 1 mg/ml hyaluronidase,

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followed by centrifugation and resuspension of the cells in hyaluronidase-free NaCl Ringer.

Maxi K^+ channels were also observed in basolateral membranes of scraped epithelial sheets. This preparation for patch clamp has also been described (Wehner et al., 1990).

Patch pipettes used in the present experiments were made of soda glass (blue-tip hematocrit capillary tubes; Fisher Scientific) or borosilicate glass (TW150-6; World Precision Instruments). The pipettes were pulled with a two-stage vertical puller (model PP-103; Narishige, Japan), and coated at the tip with Sylgard 184 (Dow Corning Co. Midland, MI). Borosilicate-glass pipettes were fire-polished under microscopic observation at 400×. Pipettes had electrical resistances of 8–10 Mohms when filled with standard solutions (see below).

Gigaohm seals were obtained by lightly touching the cells with the pipette under microscopic observation and applying gentle suction. Excision by tapping the microscope stage yielded inside-out patches one third of the time. In the other two thirds, vesicles formed, which could be disrupted by applying extreme holding voltages, yielding inside-out or outside-out patches (see Altenberg et al., 1990). Maxi K^+ channels were easily identified by their high conductance. To establish the orientation of the excised patch, we reduced the Ca^{2+} concentration in the bath from 10^{-3} to 10^{-7} M; in inside-out patches this maneuver changes the channel activity from near maximal to near zero, whereas in outside-out patches there is no effect. In addition, we confirmed the outside-out configuration by adding 1 mM tetraethylammonium (TEA^+) to the bath. At this concentration, the flicker block by TEA^+ reduces the current amplitude of outside-out patches by ~80%, but has only a slight effect on the current amplitudes of channels in inside-out patches (Segal and Reuss, 1990*b*).

Single-channel currents were measured with a List EPC-7 patch clamp (List Electronic, Darmstadt-Eberstadt, Germany) and stored on videotape using an Indec IR-2 digital instrument recorder (Indec Systems, Sunnyvale, CA). Patch recordings of 30–150 s duration were filtered at 2 kHz with an eight-pole Bessel filter (Frequency Devices, Haverhill, MA) and played onto a strip chart recorder (corner frequency 125 Hz) for analysis by hand, or digitized at 2 kHz using a Data Translation DT2801 analogue-to-digital conversion system (Data Translation, Inc., Marlborough, MA) for analysis by a microcomputer. Data acquisition and analysis were carried out with the program PAT V6.1 (generously supplied by J. Dempster, Department of Physiology and Pharmacology, University of Strathclyde, Glasgow, Scotland). Single-channel open probability (P_o) was estimated as

$$P_o = (1/N) \sum_{i=1}^{N} iP_i, \quad (1)$$

where i is a summation index, N is the number of channels in the patch, and P_i is the fraction of time during which i channels are open. Membrane voltages (V_m) are reported with respect to the extracellular side of the patch and correspond to $-V_p$ (pipette voltage) in inside-out patches or to V_p in outside-out patches. Currents depicted as positive (upward deflections) denote cation flux from the intra- to the extracellular compartment.

Mass spectrometry methods

An estimate of the number of ion released from the glass to the pipette solution was obtained by incubating 12 hematocrit- or borosilicate-glass pipettes (see dimensions below) with 4 ml of KCl-Ringer solution containing (in mM): 100 KCl, 10 NaCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 Hepes/KOH (pH 7.4). The volume of incubation solution chosen gave sufficient resolution to determine the $[Ba^{2+}]$ over time (limit of resolution 0.01 μM Ba^{2+}). For a cylindrical pipette 7 cm long, with an internal diameter of 0.12 cm, the ratio of glass surface to volume of Ringer within the pipette (S/V) is 33 cm^{-1}. For the extraction experiment, in which the solution also bathed the external surface of the pipette (external diam 1.5 mm), the S/V is 18 cm^{-1}.

The pulling procedure increases the pipette S/V, with the ratio varying along the pipette length. Measuring transverse diameters of a patch pipette by observation under microscope (600×), and considering the pipette as made of interlocked conical sections, each 10 μm long, the average S/V of the first 10 μm from the tip is ~5,000 cm^{-1}; for a length of 100 μm it is 1,100 cm^{-1}. To take into account the S/V in assessing the rate of ion release, pipettes were pulverized with an agate mortar. Particles had variable shapes with diameters ranging from 10 to 250 μm. From these dimensions, the minimal increase in glass S/V (assuming a cubic shape for all particles) ranges from 240 to 6,000 cm^{-1}. Batches of 12 pulverized pipettes (borosilicate glass and soda glass) were incubated in test tubes with 4 ml of KCl-Ringer for 2.5–120 min. Then, the solution was separated by centrifugation for 20 s at 1,000 *g* followed by filtration of the supernatant with a 0.2 μm-pore filter (Acrodisc, Gelman Sciences, Ann Arbor, MI).

To obtain additional information on the mechanism of ion release from the glass, some patch-clamp experiments were performed after prolonged washout of the pipette solution (see Results). The number of washes necessary to eliminate Ba^{2+} from pulverized soda glass was also assessed. Batches of 60 pulverized soda-glass pipettes were extracted for 10 min, twelve times, with 20 ml KCl-Ringer.

Inductively coupled plasma (ICP)-mass spectrometry was used to quantitate trace metals present in the KCl-Ringer solutions. The technique is based on the generation by argon ICP of an ion source for quadrupole mass spectrometry. The instrument used (VG Plasmaquad-1, V.G. Elemental Inc., Cheshire, England) allows for multielement (up to 75) analysis with sub ng/ml detection limits and rapid quantitative analysis for the entire mass spectrum of elements (Date and Gray, 1989). Analysis of the samples was performed using indium (50 ng/ml) as internal standard. $[Mg^{2+}]$ and $[Ca^{2+}]$ were estimated in a KCl-Ringer solution devoid of the ion to be analyzed.

To assess their release from the glass, $[Na^+]$ and $[K^+]$ were determined by flame photometry, replacing them in the Ringer solution with Li^+ and Rb^+, respectively.

Statistics and curve fitting

Unless otherwise noted, experimental values are expressed as mean ± standard error. Curve fitting was done by nonlinear least-squares analysis of pooled data. Fitting routines were based on the Marquardt-Levenberg algorithm and are commercially available (Sigmaplot 4.02; Jandel Scientific, Corte Madera, CA; NFIT; Island Products, Galveston, TX).

RESULTS

Maxi K^+ channels in outside-out patches: evidence for channel block with soda glass pipettes

The maxi K^+ channel of the *Necturus* gallbladder epithelial cells is activated by internal Ca^{2+} and by membrane depolarization. The channel is blocked by Ba^{2+}, which acts selectively from the cytosolic side (Segal and Reuss, 1990*a*,*b*). In inside-out patches obtained with either borosilicate- or soda-glass pipettes, when the Ca^{2+} concentration on the cytosolic surface ($[Ca^{2+}]_i$) is 1 mM, the Ca^{2+} gating sites saturate and the channel is fully

activated ($P_o \sim 0.95$) over a wide range of voltages (Segal and Reuss, 1990*a*,*b*; Copello et al., 1991). In outside-out patches obtained with borosilicate glass, P_o values averaged 0.94 at $[Ca^{2+}]_i = 1$ mM over the voltage range of −50 to 70 mV (see Fig. 1). Note that P_o values do not differ when either NaCl-Ringer or KCl-Ringer is used as extracellular (bath) solution.

Channels in outside-out patches obtained with soda glass pipettes, also with a saturating $[Ca^{2+}]_i$ (1 mM), had strikingly different gating properties. P_o was dependent on both V_m and whether the bath solution contained Na^+ or K^+. With NaCl- or KCl-Ringer in the pipette ("cytosolic" solution) and KCl-Ringer in the bath ("extracellular" solution), P_o was ~0.95 at $V_m = -40$ or −30 mV. With further depolarization, long-lasting quiescent periods were observed, which increased in frequency at more positive V_m values (Fig. 2*A*). P_o gradually fell to near 0 at 60 mV (Fig. 2*B*). In patches exposed to KCl-Ringer in the pipette and NaCl-Ringer in the bath, the P_o–V_m curve was shifted ~75 mV to more negative voltages (Fig. 2*B*). At constant V_m, channel activity could be rapidly increased by replacing extracellular NaCl- with KCl-Ringer (Fig. 2*C*). This effect resembles the release of intracellular Ba^{2+} block by increasing extracellular K^+, as observed by Vergara and Latorre (1983).

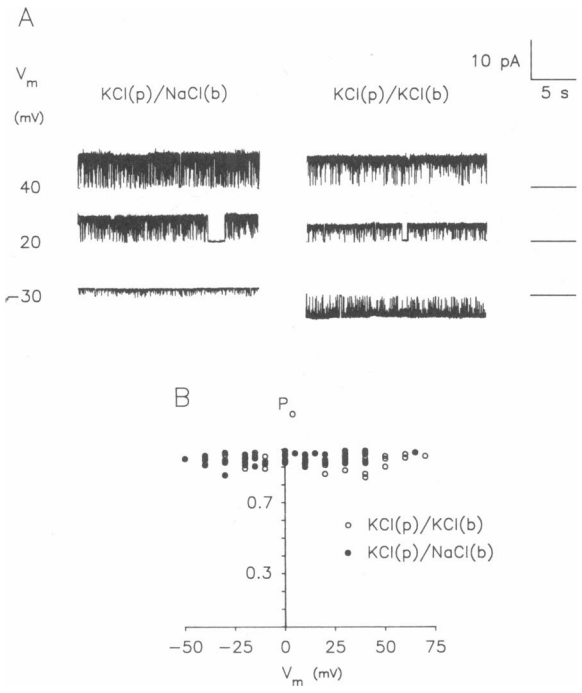

FIGURE 1 Effect of membrane voltage (V_m) on maxi K^+ channel activity in outside-out patches obtained with borosilicate glass. $[Ca^{2+}]_i$ was 1 mM in all cases. (*A*) Maxi K^+ channel currents at 40, 20, and −30 mV. Pipette (cytosolic solution) was KCl-Ringer containing (in mM) 100 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 Hepes/KOH, pH 7.4. The bath solution was NaCl-Ringer (*left*) or KCl-Ringer (*right*). Pipette and bath solution are denoted by (*p*) and (*b*), respectively. (*B*) Single-channel P_o as a function of V_m in patches exposed to KCl Ringer in the pipette and KCl or NaCl Ringer in the bath solution. Data shown were obtained in 14 patches. P_o (on average 0.94) was independent of V_m.

In contrast with the marked differences in channel gating between patches obtained with soda glass or borosilicate glass, we found that the channel current amplitudes at comparable voltages were the same (Figs. 1*A* and 2*A*). The slope conductances in NaCl / KCl or KCl / KCl solutions fell in the range of previously reported values, i.e., 110–150 pS and 150–220 pS, respectively (Segal and Reuss, 1990*a*,*b*; Copello et al., 1991).

When $[Ca^{2+}]_i$ is below saturation levels for the channel gating site(s), the voltage dependence of the channel is the same for inside-out patches in soda or borosilicate glass (Segal and Reuss, 1990*a*; Copello et al., 1991). Although in those studies channels exhibited a wide variability in their Ca^{2+} and voltage sensitivities, at a fixed $[Ca^{2+}]_i$ of 0.3 μM P_o increased continuously when the membrane was clamped to progressively more positive voltages until it reached plateau values of 0.90–0.99 at V_m ranging from −20 to 40 mV. Maxi K^+ channels in outside-out patches obtained with borosilicate glass had the same gating characteristics (Copello et al., 1991). This is illustrated in Fig. 3*A*. At 0.3 μM $[Ca^{2+}]_i$, P_o increased with depolarization and reached plateau values near 0.95 at $V_m > 10$ mV.

In contrast, in outside-out patches obtained with soda glass pipettes, in symmetrical KCl solutions and $[Ca^{2+}]_i = 0.3$ μM, the plateau was not observed. The shape of the P_o–V_m curves varied among individual experiments ($n = 5$). Fig. 4 shows one of these experiments, in which P_o increased as V_m was changed from −40 to 20 mV, but either did not change or decreased thereafter. With KCl(*p*)/ NaCl(*b*) and $V_m > 10$ mV P_o continuously decreased as V_m increased (in all five experiments, P_o at 40 mV was ≤ 0.2). Single-channel current amplitudes observed in membrane patches obtained with borosilicate or soda glass were not different when the membrane surfaces were exposed to comparable solutions.

The different gating properties observed in outside-out patches obtained with soda glass both at high and low $[Ca^{2+}]_i$ suggest the possibility that soda glass pipettes release interfering agents which block the channel at a cytosolic site, in a voltage-dependent manner. To test this hypothesis, we used KCl-Ringer preincubated with soda glass as a bath solution in inside-out patches. As illustrated in Fig. 5, voltage-dependent block was clearly observed. As expected, there was no effect on channel properties when the bath solution was KCl-Ringer preincubated with pulverized borosilicate glass (Fig. 5).

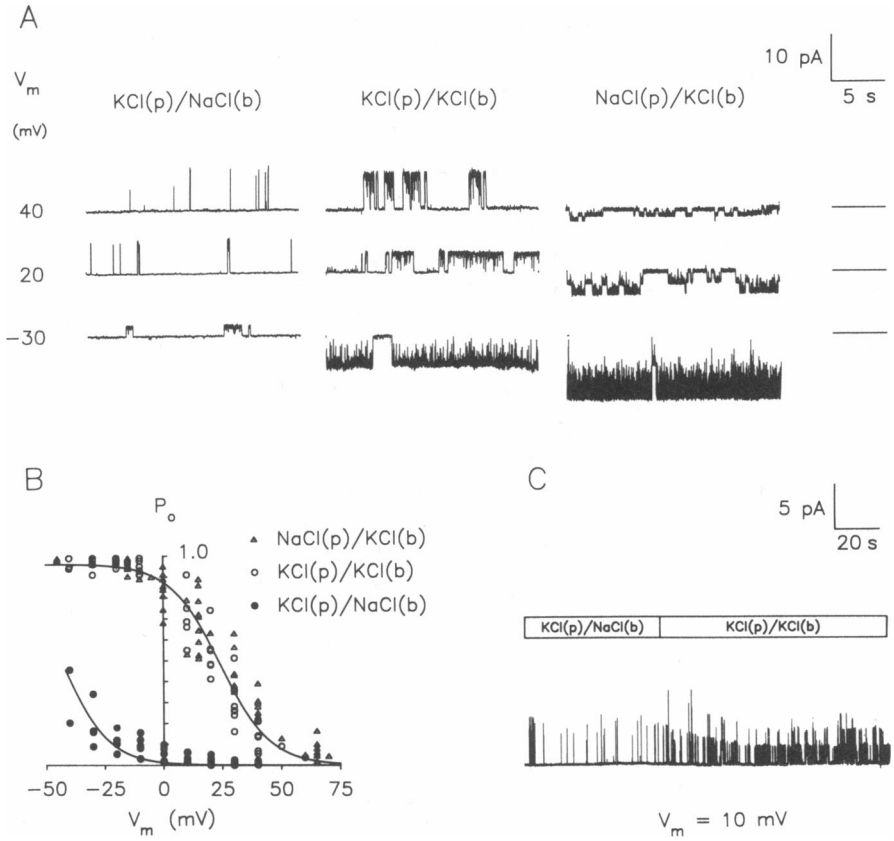

FIGURE 2 Effect of membrane voltage (V_m) on maxi K^+ channels in outside-out patches obtained with soda glass. $[Ca^{2+}]_i$ was 1 mM. (*A*) Maxi K^+ channel currents at 40, 20, and −30 mV. Pipette and bath solutions were KCl/NaCl (*left*); KCl/KCl (*center*), and NaCl/KCl (*right*); the patches contained 1, 1, and 2 channels, respectively. Single channel current amplitudes were similar to those in borosilicate glass under comparable conditions (compare Fig. 1 *A*). (*B*) Single-channel $P_o - V_m$ relationship. Curves represent nonlinear least squares fits of Eq. 2 to the data; for details see text. P_o decreased with depolarization, in contrast with the experiments with borosilicate pipettes (compare Fig. 1 *B*). Note the lowest P_o values were obtained in KCl(*p*)/NaCl(*b*). (*C*) Effect of replacing NaCl with KCl (bath solution) on single channel currents (pipette solution KCl-Ringer; V_m = 10 mV). Note the increase in channel openings when NaCl-Ringer is replaced with KCl-Ringer.

Mass spectrometry confirms that the maxi K^+ channel blocker Ba^{2+} is released by soda glass

Block of maxi K^+ channels in outside-out patches obtained with soda glass pipettes resembles the "slow block" by Ba^{2+} described in gallbladder epithelium (Segal and Reuss, 1990*a*,*b*) as well as in other preparations (Vergara and Latorre, 1983; Hille, 1984; Guggino et al., 1987; Sheppard et al., 1988). The soda glass has a bulk [BaO] of 490 mM, while borosilicate glass contains no Ba^{2+} (Table 1), suggesting that Ba^{2+} could be the channel blocker released by soda glass.

Quantitative determinations of $[Ba^{2+}]$ by mass spectrometry were carried out in KCl-Ringer after a 1.5-min to 2-h incubation with soda-glass or borosilicate-glass tubes, pulverized to increase the average surface to volume ratio to values near those estimated at the pipette tip (see Methods). The time course of Ba^{2+} release from soda glass is shown in Fig. 6. Most of the release occurs during the first 10 min. Because the time elapsed in a typical experiment between pipette filling and patch excision is 10–20 min, $[Ba^{2+}]$ in the pipette solution is approximately constant during patch clamp experiments. After a 60-min incubation in KCl-Ringer, $[Ba^{2+}]$ was 9.1 ± 0.7 μM (range 6.2–15.1 μM, n = 6). In contrast, the $[Ba^{2+}]$ after a 60-min incubation of pulverized borosilicate glass was 0.04 ± 0.02 μM (n = 4).

Ba^{2+} release from soda glass can account quantitatively for the blocking effect

An estimate of $[Ba^{2+}]$ at the tip of the soda glass pipette can be obtained by comparing the P_o–V_m relationship in

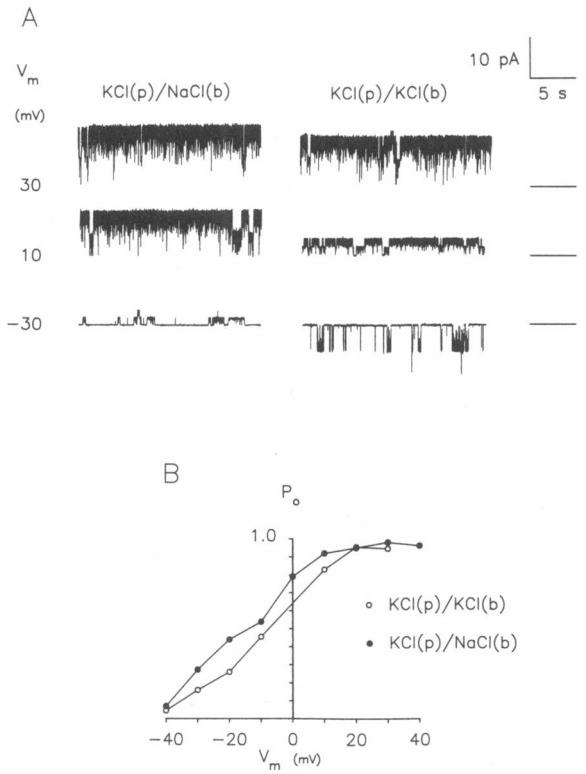

FIGURE 3 Effect of V_m on maxi K^+ channel activity in outside-out patches obtained with borosilicate glass. The pipette solution was KCl-Ringer containing 4.18 mM $CaCl_2$; the free [Ca^{2+}] was buffered to 0.3 μM with 5 mM ethyleneglycol-*bis*(beta-aminoethylether)-*N*,*N*'-tetraacetic acid (EGTA). Bath solution was either NaCl-Ringer or KCl-Ringer. (*A*) Single channel currents at 30, 10, and −30 mV. Pipette and bath solution are denoted by (*p*) and (*b*), respectively. (*B*) Single-channel P_o vs. V_m. Note that P_o increases with more positive V_m values reaching in both cases a maximum of ~0.95.

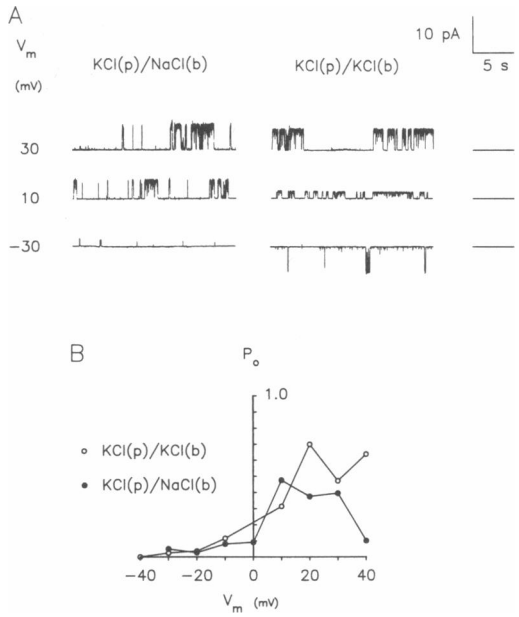

FIGURE 4 Effect of V_m on maxi K^+ channel activity in outside-out patches obtained with soda glass. Pipette solution was KCl-Ringer; [Ca^{2+}] was buffered to 0.3 μM with 5 mM EGTA. (*A*) Example of maxi K^+ channel currents. Single channel current amplitudes were similar to those in borosilicate glass under comparable conditions (compare Fig. 3 *A*). Pipette and bath solution are indicated at the top. (*B*) Single-channel P_o vs. V_m. In KCl(*p*)/KCl(*b*) P_o did not increase for V_m > 20 mV. With NaCl-Ringer as the bath solution and V_m > 10 mV, P_o continuously decreased.

experiments with soda glass pipettes (Fig. 3) with the effect of "cytosolic" [Ba^{2+}] on the P_o–V_m relationship (Fig. 7). We analyzed the voltage dependence of the cytosolic Ba^{2+} block using a modified version of the equation derived by Woodhull (1973) for block of a one-site, two-barriers channel model which has been shown to be applicable to maxi K^+ channels (Segal and Reuss, 1990*b*):

$$P_o = P_o^{max}/[1 + ([Ba^{2+}]/K_{Ba}) \exp(z'V_mF/RT)], \quad (2)$$

where F, R, and T have their usual meanings, P_o^{max} is maximal P_o measured in the absence of Ba^{2+}, K_{Ba} is the Ba^{2+} concentration at which block is half-maximal at 0 mV, and z' is the "equivalent valence of the block" (Hille, 1984), a parameter which is influenced by blocker valence, binding site location within the membrane field, K^+ – Ba^{2+} interactions in the pore, and the possibility of multi-site Ba^{2+} block. The fits of the data shown in Fig. 7 give K_{Ba} = 90.2 ± 2.8 μM; z', allowed to vary depending on [Ba^{2+}] concentration, ranged between 2.5 and 2.7. Mean values of [Ba^{2+}] in soda-glass pipette tips filled with either KCl or NaCl (with KCl in the bath) were obtained by fitting Eq. 2 to all data points in Fig. 2 *A*. From the calculated K_{Ba}, and with z' = 2.6, [Ba^{2+}] was estimated to be 9.3 ± 0.8 μM (symmetrical KCl, n = 7), 6.2 ± 0.6 μM (NaCl/KCl, n = 11), or 8.0 ± 0.6 μM (pooled data, n = 18 patches). From the average [Ba^{2+}] = 8.0 μM, and z' = 2.6, we estimated K_{Ba} = 0.09 μM in KCl(*p*)/NaCl(*b*), i.e., a 10^3-fold increase in channel sensitivity to Ba^{2+} with respect to KCl-Ringer solution. This value was used in Fig. 2 to fit Eq. 2 to the KCl(*p*)/NaCl(*b*) data.

We also estimated free [Ba^{2+}] when EGTA is present in the pipette solution buffering [Ca^{2+}]$_i$ to 0.3 μM (Fig. 4). From the apparent affinity constant of EGTA for Ba^{2+} (4.8×10^4 M^{-1} at 20°C, pH 7.4, and ionic strength 0.1; Martell and Smith, 1974), the estimated total [Ba^{2+}] upon release from soda glass pipettes (5–28 μM), and the computed free [EGTA] of 0.73 mM, the calculated free [Ba^{2+}] ranges from 0.1 to 0.8 μM. These values could account for the block of maxi K^+ channels ob-

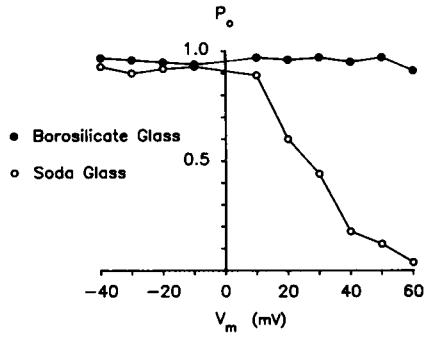

FIGURE 5 Effects of KCl-Ringer incubated with pulverized borosilicate or soda glass on channel P_o. A maxi K^+ channel in an inside-out patch obtained with borosilicate pipettes was first exposed to KCl-Ringer (1 mM [Ca^{2+}]) on both sides; then, the bath solution was changed to KCl-Ringer that had been preincubated with pulverized pipettes of either borosilicate or soda glass (4 ml of Ringer/12 pulverized pipettes, 60 min, see Methods). Note the blocking effect obtained with soda glass (compare with Fig. 2 *b*).

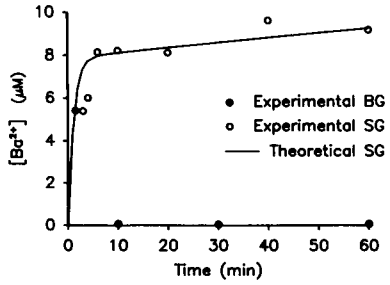

FIGURE 6 Ba^{2+} release from soda glass (SG) or borosilicate glass (BG). Batches of 12 pulverized pipettes were incubated with KCl-Ringer solution for 1.5 to 60 min. The time course of Ba^{2+} release was studied by measuring [Ba^{2+}] in the solution with mass spectrometry. The theoretical curve represents the best visual fit of the solution of Eqs. 4–8 to the data in SG. Parameter values were: k_1 = 0.6 min^{-1}, l_1 = 0.02 min^{-1} μM^{-1}, k = 0.004 min^{-1}, St = 10 μM. For details see Appendix.

served when pipette [Ca^{2+}] is buffered at 0.3 μM with EGTA (Fig. 4).

Mechanism of Ba^{2+} release

The above results indicate that Ba^{2+} is the blocker of maxi K^+ channels released by soda glass, but do not establish the process of Ba^{2+} release. This process could be restricted to the surface or could also involve release from the bulk glass. The pulling procedure elevates the glass surface area relative to the volume of the pipette to an extent similar to that obtained by pulverization of the glass. Pulling is also likely to change the structure of the glass and therefore Ba^{2+} release could be facilitated by devitrification, i.e., the transformation, at the surface of the glass, of ions in an insoluble "glass structure" to a soluble "crystalline structure" (Morey, 1954). This process is kinetically favored by the combination of high temperature and increase in the glass surface area which occurs upon pulling.

If Ba^{2+} release is exclusively a surface phenomenon, it should be reduced progressively with repeated washes of the pipettes with Ringer after pulling. Thus, repeated washing should prevent the block of maxi K^+ channels in outside-out patches. To test this possibility, patch pipettes were backfilled with KCl-Ringer and the filling solution was replaced ten times, at 10-min intervals, by aspiration and backfilling. Then, the pipettes were filled with KCl and kept at room temperature for 12–24 h, immersed in a large volume of KCl-Ringer. After that, the pipette solution was again changed ten times. Finally, a stream of solution was passed through the tip by applying positive pressure. These manipulations reduced the yield of gigaohm seals from ~25% to <5%, lowered the mean electrical seal resistance from 15 to 2 Gigaohms, and reduced the duration of the seals. Regardless of these experimental limitations, the results were clear: in outside-out patches (n = 5) bathed with symmetrical KCl-Ringer there was voltage-dependent slow block of maxi K^+ channels, although at all voltages studied, the decrease in P_o was less than that expected with 1 μM Ba^{2+} (see Fig. 7). When NaCl-Ringer was the bath solution, the blocking effect increased and P_o was ≤ 0.5 at $V_m \geq 0$ mV. This indicates that Ba^{2+} is continuously released into the pipette solution from the bulk glass.

Multiple washes of soda glass pipettes decreased but did not eliminate Ba^{2+} release. After 12 10-min extractions, Ba^{2+} released from the glass resulted in concentrations of 0.3–0.4 μM in 10 min (Fig. 8). The amount of Ba^{2+} released in the first 30 min represents <0.01% of the total Ba^{2+} present in the glass. From four different batches of glass, using the same protocol of Fig. 8, an

TABLE 1 **Composition* of soda glass (SG) and borosilicate glass (BG) (values are % wt/wt)**

	SG	BG
SiO_2	70	80
B_2O_3	1	13
Al_2O_3	3	2.25
Na_2O	14	3.5
K_2O	2	1.0
BaO	3	—
CaO	5	—
MgO	2	—
Fe_2O_3	—	<0.05

*Approximate, stated by manufacturers.

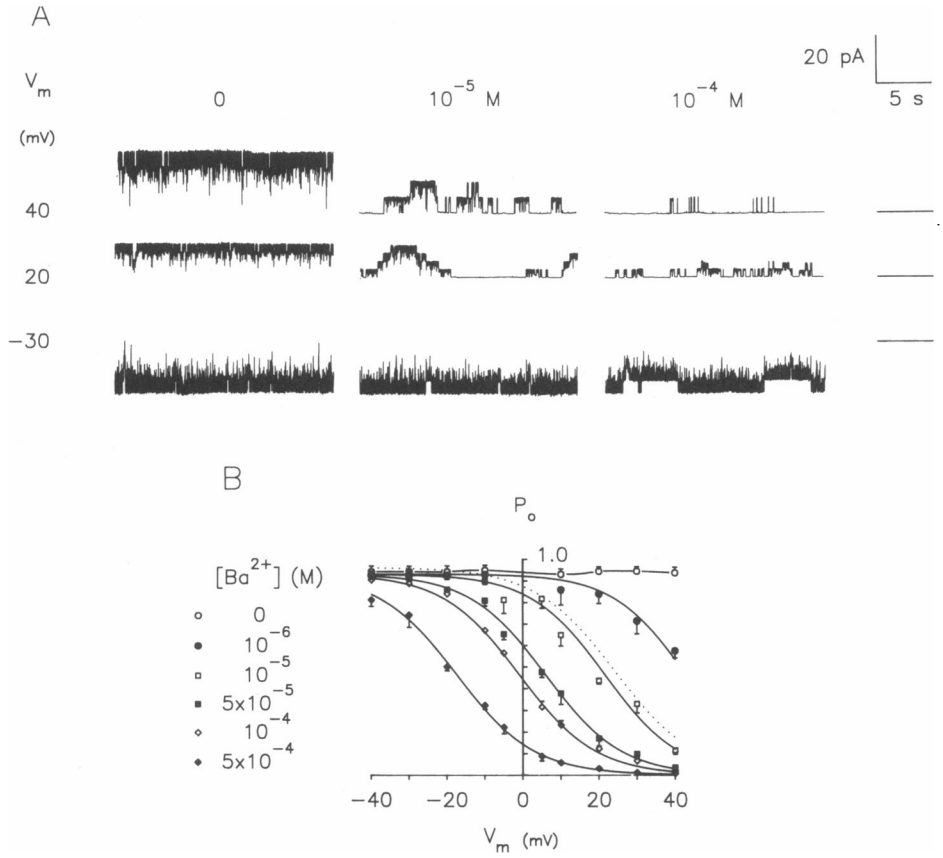

FIGURE 7 Cytoplasmic-side Ba^{2+} block of maxi K^+ channels. Inside-out patches were exposed to symmetrical KCl-Ringer with $[Ca^{2+}]_i$ = 1 mM, varying bath ("cytosolic") $[Ba^{2+}]$. (*A*) Effects of Ba^{2+} on single-channel currents at different V_m values at the concentrations indicated on the top. (*B*) P_o vs. V_m, for channels exposed to 0–5 · 10^{-4} M $[Ba^{2+}]$. Note that Ba^{2+} reduced P_o, but had no significant effect on single-channel current amplitudes. Data shown are means ± s.e.m. of four paired experiments. The solid lines depict fits of Eq. 2 to the data. The dotted line is the P_o vs. V_m relationship from Fig. 2 *b* [soda glass, KCl(*p*)/KCl(*b*) or NaCl(*p*)/KCl(*b*)]. $[Ba^{2+}]$ in the soda glass pipette solution was estimated to be 8.0 ± 0.6 μM.

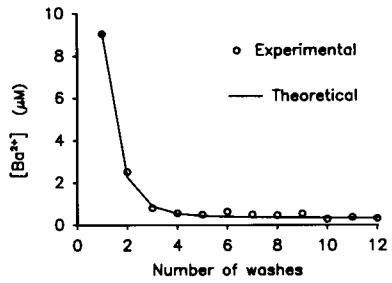

FIGURE 8 Ba^{2+} release from soda glass (SG) by consecutive washes with KCl-Ringer. Batches of 50 pulverized soda glass pipettes were exposed for 10 min to fresh KCl-Ringer (20 ml); this procedure was repeated 12 times. The $[Ba^{2+}]$ in Ringer solution after each 10-min release period was determined using mass spectrometry. The theoretical curve represents the best visual fit of the solution of Eqs. 4–8 to the data. Parameter values were: k_1 = 0.6 min^{-1}, l_1 = 0.02 min^{-1} μM^{-1}, k = 0.004 min^{-1}, St = 11.5 μM. For details see Appendix.

additional extraction was carried out for periods of 40, 60, 90, or 120 min. The $[Ba^{2+}]$ in the solution increased with the duration of the extraction period.

Thus, it appears that release of barium from either micropipettes or crushed glass occurs in two phases, a rapid release from the surface followed by a maintained slower efflux from the bulk glass which continues despite repeated washings. To estimate the binding affinity, the amount of barium bound to the surface, and the rate of diffusion of barium from the interior of the glass to the solution, a model was developed and fit to the data from the experiments described above (see Appendix). The parameters obtained from fitting the model to the data were then used to estimate the $[Ba^{2+}]$ inside a pipette. If the pipette was simply filled with solution then the predicted $[Ba^{2+}]$ after 20 min was 10–30 μM; if the pipette was washed repeatedly to remove the barium on the surface, then the predicted $[Ba^{2+}]$ was 0.1–1 μM.

Both of these values are consistent with the Ba^{2+} concentrations under these conditions, estimated from the degree of barium block.

Other ions released from the glasses do not block maxi K^+ channels

Mass spectrometry of KCl-Ringer solutions pre-exposed to pulverized SG and BG for 60 min showed that the level of all elements, with the exception of Ba^{2+}, were not significantly different from those already present in KCl-Ringer solution. Determinations of $[Mg^{2+}]$ and $[Ca^{2+}]$ in solutions devoid of these cations show that they are released to final concentrations of 54 ± 20 μM ($n = 4$) and 30 ± 10 μM ($n = 6$), respectively, from pulverized soda glass but not from borosilicate glass (our limit of detection for Ca^{2+} is ~10 μM, i.e., near the concentration measured in nominally Ca^{2+}-free Ringer). Mg^{2+} is a positive allosteric modulator of maxi K^+ channels in gallbladder epithelium (Copello et al., 1991) and in other tissues (Golowasch et al., 1986; Squire and Petersen, 1987). It has been argued that Ca^{2+} can block maxi K^+ channels in a similar way as Ba^{2+}, but at concentrations of 1–20 mM (Latorre et al., 1983; Vergara and Latorre, 1983; Laver, 1990). Our experiments appear to rule out a Ca^{2+} block for the following reasons: (*a*) there was no voltage-dependent block when the cytosolic side of the channel was bathed with KCl-Ringer plus 1 mM Ca^{2+} (Fig. 1), (*b*) $[Ca^{2+}]$ is expected to increase by only 5% from Ca^{2+} release from the glass, and (*c*) the experiment in Fig. 4 *B* showed that, although block is observed at $V_m > 20$ mV, P_o is reduced to low values (0–0.20) with hyperpolarization, which implies that free $[Ca^{2+}]$ is not high enough to saturate maxi K^+ channel Ca^{2+} gating sites.

The release of Na^+ and K^+ from soda and borosilicate glasses was assessed by flame photometry replacing Na^+ with Li^+ and K^+ with Rb^+ in the Ringer solution. After a 60-min incubation with pulverized soda glass, $[Na^+]$ was 880 ± 140 μM and $[K^+]$ was 50 ± 10 μM ($n = 5$). Pulverized borosilicate glass yielded 160 ± 40 μM Na^+ and 13 ± 3 μM K^+ ($n = 4$). In some maxi K^+ channels Na^+ can produce a flickery block, but only in the absence of extracellular K^+ (Yellen, 1984*a*,*b*).

We also measured pH changes in KCl-Ringer solutions after preincubation with both glasses. After a 60-min incubation with soda glass, the pH of KCl-Ringer changed from 7.40 to 7.44 ($n = 6$) in solutions buffered with 10 mM Hepes, and from pH 6.02 to 9.95 ($n = 2$) when KCl-Ringer was nominally buffer free. After incubation with borosilicate glass, the pH changed slightly from 7.40 to 7.39 ($p < 0.005$, $n = 5$) in KCl-Ringer with 10 mM Hepes. In unbuffered Ringer, the pH rose from 6.02 to 6.8 ($n = 2$).

DISCUSSION

Ba^{2+} is the maxi K^+ channel blocker

Our results demonstrate that maxi K^+ channels in outside-out patches obtained using soda glass exhibit altered gating properties attributable to Ba^{2+} release from the glass. The characteristics of the Ba^{2+} block of maxi K^+ channels (Hille, 1984; Wolff et al., 1986; Guggino et al., 1987; Sheppard et al., 1988; Segal and Reuss, 1990*a*,*b*) are the same as those observed in experiments with soda glass pipettes: (*a*) Ba^{2+} blocks selectively from the cytoplasmic side of the channel. (*b*) Blocking and unblocking events last much longer than gating events. (*c*) The blocking effect is favored by positive voltage on the blocker (cytoplasmic) side of the channel; therefore, the block could be solely expressed as a fall of P_o at positive V_m values. (*d*) A partial release of the blocking effect at constant V_m can be obtained by increasing K^+ on the extracellular side of the channel; hence, the observed difference in P_o with Na^+ or K^+ in the pipette solution would erroneously suggest Na^+ block. In agreement with the patch-clamp observations, mass spectrometry studies showed that pulverized soda glass, when incubated with KCl-Ringer, releases Ba^{2+} to "steady-state" concentrations of 5–15 μM. Similar elevations of $[Ba^{2+}]$ in the pipette tip could fully account for the observed block.

In addition, Ca^{2+}, Mg^{2+}, Na^+, and K^+ are also released by soda glass but in small amounts, insufficient to alter significantly the concentrations of these ions in our pipette-filling solutions.

Furman and Tanaka (1988) have suggested the possibility of a role of Na_2O release from soda glass, which could cause solution alkalinization and have effect on channel function. In our preparation changing pH_i from 7 to 9 did not block, but activated maxi K^+ channels when $[Ca^{2+}]_i$ was below saturation for the Ca^{2+} gating site(s) (Copello et al., 1991). In KCl-Ringer incubated with pulverized soda glass pH increased only from 7.40 to 7.46; however, those solutions were able to block maxi K^+ channels (Fig. 5) similarly to the block observed with soda glass (Fig. 2 *b*).

Ba^{2+} interference is difficult to avoid

Soda-glass Ba^{2+} is released from two compartments: a fast-release compartment (probably on the surface) and a slow-release compartment still manifest after 24 h. Clearly, repeated replacement of the pipette solution is not a practical way to avoid Ba^{2+} interference. We also tested pipette-filling solutions containing 1–50 mM SO_4^{2-}, aiming to precipitate Ba^{2+} as $BaSO_4$. The solubility product for $BaSO_4$ in distilled water is 10^{-10} M^2 (Goode-

nough and Stenger, 1973). Fixing $[SO_4^{-2}] \geq 10^{-3}$, a $[Ba^{2+}] \leq 10^{-7}$ M should be obtained. The maxi K^+ channel block observed in outside-out patches with soda glass was significantly less when this solution was used but it was not abolished. In outside-out patches bathed with 50 mM KCl and 25 mM K_2SO_4 in the pipette, and KCl-Ringer in the bath, when V_m increased from 0 to 40 mV P_o decreased from ~0.95 to 0.80 ($n = 2$). When NaCl-Ringer was the bath solution P_o was $\leq$ 0.60 at $V_m \geq 10$ mV ($n = 2$).

Cota and Armstrong (1988) and Furman and Tanaka (1988) prevented pipette-glass related blocks by using 5–20 mM EGTA and nominally 0 Ca^{2+} in the pipette solution (free $[Ca^{2+}] < 10^{-9}$ M). However, maxi K^+, as well as other channels, require $[Ca^{2+}] > 0.1$ μM to be active. To reduce soda-glass free $[Ba^{2+}]$ to <0.1 μM when free $[Ca^{2+}]$ is 0.3 μM, one must increase the total [EGTA] used from 5 to 50 mM (see Figs. 3 and 4). However, in the experiments shown in Fig. 2 (1 mM Ca^{2+}), the calculated K_{Ba} for KCl(p)/NaCl(b) was ~10^{-7} M (see Results), a value similar to that reported for maxi K^+ channels in cultured medullary thick ascending limb cells (Guggino et al., 1987). Consequently, the reduction of free $[Ba^{2+}]$ promoted by 50 mM EGTA would be insufficient to prevent Ba^{2+} block of maxi K^+ channels.

Although in principle the effect of Ba^{2+} can be reduced by different procedures, there is no available method which adequately prevents the interference of this ion, unless the pipette solution is continuously replaced. Hence, soda glass pipettes should not be used to study channels with a high sensitivity to Ba^{2+}.

Channel block can also occur with glasses other than soda glass

Concentrations of BaO ranging from 0.5 to 5% wt/wt are commonly present in technical glasses because BaO decreases the viscosity of the glass at all temperatures, increases glass density and improves the chemical durability of glass (Morey, 1954; Volf, 1961). Thus, the soda glass tested in our studies is not the only glass expected to cause artifactual Ba^{2+} block of K^+ channels. Furman and Tanaka (1988) reported great variation in the I-V relations of cGMP-activated ion channels of frog rod photoreceptors (in inside-out patches) when six different glasses were compared. Four glasses contained 0.3–0.5 M BaO. When we incubated 4 ml of KCl-Ringer for 60 min with one of those glasses (12 pulverized Kovar 7052 sealing glass tubes), the final $[Ba^{2+}]$ was 0.8–3 μM.

Ba^{2+} is not the only component of glasses that could interfere with channel gating. Furman and Tanaka (1988) observed differences between the I-V relations obtained with two Ba^{2+}-free soda lead potash glasses (KG-12 and 0010). A borosilicate glass (Kwik-Fil Standard) alters the gating properties of acetylcholine-activated channels, relative to a Ba^{2+}-containing soft glass (Rojas and Zuazaga, 1988; Zuazaga and Steinacker, 1990).

Na^+ and K^+ are the major components of technical glasses. Other ions found in different kinds of glasses include Mg^{2+}, Al^{3+}, Ca^{2+}, Mn^{2+}, Zn^{2+}, As^{3+}, Sb^{3+}, and Pb^{2+} (Morey, 1954; Volf, 1961). Most of these ions can exert positive or negative modulatory effects on channels (Begenisich and Lynch, 1974; Hille, 1984; Audesirk, 1987; Matsuda, 1988; Latorre et al., 1989; Oortgiesen et al., 1990; Zhang and Colombini, 1990; Ravindran et al., 1991). For example, Pb^{2+} blocks ionic currents in central neurons of *Lymnaea stagnalis* (Audesirk, 1987), but is a channel activator in mouse neuroblastoma cells (Oortgiesen et al., 1990).

Conclusions

Our studies demonstrate that soda glass releases Ba^{2+} at rates that produce concentrations in the pipette solution sufficiently high to block maxi K^+ channels in the outside-out patch configuration. Hence, soda glass should be avoided when studying maxi K^+ channels. Inasmuch as other glasses also release ions, the possibility of artifactual results should be considered in all experiments involving glass micropipettes.

APPENDIX

The release of Ba^{2+} from soda glass was simulated with the following model, which includes Ba^{2+} diffusion within the glass and binding and unbinding of Ba^{2+} from the surface of the glass. Ba^{2+} is assumed to be distributed uniformly throughout the glass at a concentration of 0.49 M (corresponding to the density of BaO of 3% wt/wt), which yields a 1.5-nm distance between ions. Movement of Ba^{2+} within the glass occurs by simple diffusion defined by a rate constant k. At the surface, Ba^{2+} can dissociate from its binding site and go into solution with forward and reverse rate constants k_1 and l_1, respectively. The first eight layers of Ba^{2+} and the surface layer can be represented by the following kinetic scheme:

$$S_8 \underset{k}{\overset{k}{\rightleftharpoons}} S_7 \underset{k}{\overset{k}{\rightleftharpoons}} S_6 \cdots\cdots S_1 \underset{k}{\overset{k}{\rightleftharpoons}} S_0 \underset{l_1}{\overset{k_1}{\rightleftharpoons}} S + Ba^{2+}, \qquad (3)$$

where S_i is the $[Ba^{2+}]$ in the ith layer from the surface and S is the concentration of surface sites which do not have Ba^{2+} bound to them. The following differential equations describing the movement of Ba^{2+} were then solved simultaneously with the appropriate boundary conditions:

$$dS_i/dt = k(S_{i+1} + S_{i-1}) - 2kS_i \qquad i = 1, 7 \qquad (4)$$

$$dS_8 = k(S_7 - S_8) \qquad (5)$$

$$dS_0/dt = k(S_1 - S_0) + l_1[Ba^{2+}](S_t - S_0) - k_1 S_0 \qquad (6)$$

$$d[Ba^{2+}]/dt = k_1 S_0 - l_1[Ba^{2+}](S_t - S_0) \qquad (7)$$

$$S_t = S + S_0, \qquad (8)$$

where $S_i(0) = S_i$ the initial $[Ba^{2+}]$ in the glass, $S_0(0) = 0$ and $[Ba^{2+}](0) = 0$. k, k_1, l_1, and S_i were then varied to give the best visual fit to the data for the experiments described below. Limiting the model to only 8 states was quite sufficient because over the time course of the longest simulation S_8 decreased by $<0.000001\%$.

Fig. 6 shows the model fit to an experiment in which 1.7 g (0.68 cm^3) of broken glass were added to 4 ml of distilled water and the $[Ba^{2+}]$ measured over time (see legend for parameter values). The early rapid release phase corresponds to the relatively fast unbinding of Ba^{2+} from the surface and the slow phase corresponds to the much slower diffusion of Ba^{2+} from within the glass to the surface, where it is released. From an estimate of the surface area one can calculate the $[Ba^{2+}]$ that would be reached if all the Ba^{2+} from the surface (at a density of 0.44 ion $\cdot$ nm^{-2}) went into solution. The crushed glass particles were approximately cubic with sides ranging from 10 to 250 μm. The average surface to volume ratio of these particles is 583 cm^{-1} which gives a total surface area of 583 $cm^{-1} \times 0.68\ cm^3 = 396\ cm^2$. The total number of Ba^{2+} ions on the surface is then 1.8×10^{16}. If these are all released into 4 ml of water the resulting $[Ba^{2+}] = 7.3$ μM, remarkably close to the measured value in this experiment, namely 8 μM.

Fig. 8 shows a fit with the same kinetic parameter values to data from an experiment in which broken glass was washed repeatedly, by soaking for 10 min, after which solution was removed and fresh solution added. This experiment was simulated by solving the differential equations over a 10-min period and then using the final occupancies of each state as the initial occupancies for the next 10-min interval, with $[Ba^{2+}]$ set to zero. After the first few washings, which remove the Ba^{2+} bound to the surface, there is a fairly constant, small efflux corresponding to 4.2×10^{-15} mol $\cdot$ $cm^{-2} \cdot s^{-1}$. At the end of 2 h, the $[Ba^{2+}]$ in the first layer is diminished by 32%, in the second layer by 6%, and in the remaining layers by $<1\%$. Thus, Ba^{2+} is significantly depleted only within 3 nm of the surface of the glass. The magnitude of the small remaining Ba^{2+} efflux compares well with the value of 5.3×10^{-15} mol $\cdot$ $cm^{-2} \cdot s^{-1}$ for Cs^+ uptake into NAS glass after an initial rapid uptake into the surface layer (Eisenman, 1969). The rapid uptake phase of Cs^+ corresponded to 3.9×10^{-13} mol $\cdot$ $cm^{-2} \cdot s^{-1}$ (average over the first 15 min) compared with a value of $\sim 1.2 \times 10^{-13}$ mol $\cdot$ $cm^{-2} \cdot s^{-1}$ from our fitting. These numbers are remarkably close given the differences in valence and glass type and considering that the density of Cs^+ in NAS is ~ 2.5 times that of Ba^{2+} in soda glass. Thus, our model can accurately describe the time course and degree of Ba^{2+} release from soda glass with reasonable parameter values.

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