Single anion-selective channels in basolateral membrane of a mammalian tight epithelium

(patch clamp/chloride conductance/anion channel/epithelial transport)

J. W. HANRAHAN, W. P. ALLES, AND S. A. LEWIS

Department of Physiology, Yale University School of Medicine, New Haven, CT 06510

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ABSTRACT Basolateral membrane chloride permeability of surface cells from rabbit urinary bladder epithelium was studied using the patch-clamp technique. Two types of anion-selective channel were observed. One channel type showed inward rectification and had a conductance of 64 pS at -50 mV when bathed symmetrically by saline solution containing 150 mM chloride; the other resembled high-conductance voltage-dependent anion channels (VDACs). Both channels had the selectivity sequence Cl- ≈ Br- ≈ I- ≈ SCN- ≈ NO3- > F- > acetate > glucose > Na+ > K+ and were sensitive to the anion exchange inhibitor 4,4'-disothiocyanostilbene-2,2'-disulfonic acid. Basolateral chloride conductance in urinary bladder is apparently due to the 64 pS anion channel, which is active at physiological potentials. Imperfect selectivity of this channel against cations might also account for the low, but finite, sodium permeability of the basolateral membrane.

Membrane chloride conductance is an essential feature of cellular models for chloride transport across the cornea (1), trachea (2), and thick ascending limb of Henle's loop (3) and is thought to be an important regulatory site for fluid secretion and cell-volume regulation in some cells (4-7). However, unlike sodium and potassium channels, which have been characterized using fluctuation analysis (8, 9), little is known regarding anion permeability in most epithelia, and its constituent ion channels have not been identified (10). The rabbit urinary bladder is a model tight epithelium which has very high basolateral chloride permeability (11). We used the patch-clamp technique (12) to study this chloride conductance at the single-channel level.

MATERIALS AND METHODS

Epithelial cells were scraped from the luminal surface of rabbit urinary bladders and exposed to high-purity collagenase (100 units/ml; Worthington or Sigma) dissolved in a 1:1 mixture of medium 199 and Ham's nutrient mixture F-12 (GIBCO) at room temperature (22 ± 2°C). The epithelium of mammalian urinary bladder is transitional, consisting of large (∼70 µm diameter), flattened surface cells and two lower layers of much smaller (10 µm), cuboidal cells. Brief exposure to collagenase left a few intermediate cells attached to those from the surface layer, enabling us to identify the basolateral membrane during experiments. When obtaining seals, the patch pipette was placed against the surface cell's basolateral membrane next to the attached intermediate cells. The basolateral origin of patches was often confirmed by the presence of both anion- and potassium-selective channels because the apical membrane of this tissue does not exhibit potassium- or anion-selective conductances (13). Single-channel currents were recorded by use of pipettes made from borosilicate glass tubing (Boralex, Rochester Scientific, NY) that had been pulled and coated by standard methods (12). Seal resistances were typically 5-60 GΩ.

Currents were recorded on FM tape, low-pass filtered at 800 Hz during playback using an 8-pole Bessel filter (902 LPF, Frequency Devices, Haverhill, MA) and digitized at 2 or 4 KHz to give records containing 16,384 points. Each digitized record was displayed in blocks of 2048 points for inspection of events, and the mean baseline (leakage) current and its variance were calculated between two points that were set using a cursor. Data were normalized to the baseline, sometimes edited to remove transients and invalid events, and then analyzed to produce a histogram of current amplitudes. Open- and closed-time histograms were calculated using a threshold setting of one-half the open-channel current, as deduced from the amplitude histogram. Test pulses of various durations were used to determine the minimal duration that could be detected under experimental conditions. Events lasting <2 µsec but >400 µsec became distorted in shape but still crossed the 50% threshold and were counted. Nevertheless, the first bin in closed-time histograms (<500 µsec) was not used when fitting exponents, and time constants less than about 2 msec should be regarded as first estimates until a wider-pass band can be used. In both open- and closed-time histograms, the actual number of events in each bin was plotted and fitted rather than the cumulative distribution. To obtain power-density spectra, the data were low-pass filtered at 900 Hz with a 20-pole Cauer elliptic filter (LP120, Unicon, Mount Vernon, NY), digitized at 2 KHz, and read as 16 consecutive records of 2048 points each. Thirty-two spectra were obtained from overlapped data blocks by fast Fourier transform, averaged to yield final spectra, and fitted as the sum of linear and Lorentzian-type functions.

The pipette solution was 150 mM KCl (or NaCl)/10 mM Heps, pH 7.2/80 mM EGTA. The initial, extracellular solution was 140 mM NaCl/6 mM KCl/2 mM MgCl2/2 mM CaCl2/20 mM Heps pH 7.2. To determine selectivity among anions, patches were excised and the bath was perfused with a solution that was identical to that in the pipette except that chloride was replaced with a different anion. Liquid junctions were kept bionic, and those arising at the reference agar bridge were measured relative to a flowing 3 M KCl junction as follows. First, a 150 mM KCl solution was placed in the bath and a 150 mM KCl/4% agar bridge was connected to the bath reference electrode (Ag/AgCl). The tip of a patch pipette (containing another Ag/AgCl electrode) then was filled with 3 M KCl and immersed in the bath, and current flowing through the pipette was clamped at zero while the bath solution was replaced by other salts of potassium. The voltage deflections caused by substituting other anions for chloride agreed with those expected from their free-solution mobilities and were used to correct measured reversal poten-

Abbreviations: DIDS, 4,4'-disothiocyanostilbene-2,2'-disulfonic acid; VDAC, voltage-dependent anion channel.

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tials. Experiments were performed at room temperature (~22°C).

RESULTS

Conductance and Selectivity

Low-Conductance Anion Channel (g64). Fig. 1A shows the current recorded from an inside-out patch excised from the basolateral membrane of a surface cell and held at −50 mV, approximately the normal potential in situ (11). This particular recording was obtained when the patch was bathed symmetrically by 150 mM KCl, but similar results were obtained when the solution on either (or both) sides of the membrane contained NaCl. Single-channel currents displayed inward rectification (Fig. 1B), and their open-channel current/voltage (I/V) relationship yielded a mean slope conductance in six patches of 64.0 ± 4.7 pS between −40 and −60 mV. Fig. 1B also shows the I/V relationship after substitution of fluoride for chloride. With a 5:1 KCl gradient, single-channel currents changed sign at approximately −20 to −35 mV, from which we calculated a value for $P_{K}/P_{Cl}$ (permeability ratio) of 0.043 ± 0.016 (mean ± SEM), n = 6 patches, using the constant field equation (14, 15). We measured similar reversal potentials under bicionic conditions and measured nearly identical slope conductances between −40 and −60 mV when currents were carried by anions from the bath. These observations indicated that this channel is equally permeable to Cl−, Br−, I−, NO3−, and SCN−, within measurement error (P > 0.2, n = 3). However, we calculated $P_{F}/P_{Cl}$ = 0.479 (n = 1), $P_{acetate}/P_{Cl}$ = 0.310 ± 0.036 (n = 3), and $P_{glutamate}/P_{Cl}$ = 0.07 ± 0.040 (n = 3). Bicarbonate ions also permeate, but less well than chloride ions.

Discrimination between fluoride and other halides having lower free energies of hydration suggests that permeating anions interact with a site having low field strength, whereas acetate and gluconate are presumably distinguished by size. Importantly, g64 was often seen when we recorded in the cell-attached mode, indicating that it normally contributes to membrane conductance. The number of anion channels per patch ranged from zero to six. The overall probability of observing an anion channel after obtaining a gigahm seal was about 0.3, however, there was day-to-day variability in the number of channels obtained even when care was taken to keep all experimental conditions constant. The cause of this variability has not been determined.

High-Conductance Anion Channel (g62). A much larger unit conductance (362.3 ± 11.1 pS; mean ± SEM, 9 patches), referred to here as the high-conductance channel or g62, was observed in excised patches after a variable delay of 30 sec to several minutes (Fig. 2A). This channel usually did not appear until large (ca. ±50 mV) voltage steps were applied, but once evoked, it would remain spontaneously active at potentials between +20 and −20 mV. We assessed the selectivity of g62 under the same bicionic conditions used to study g64 (Fig. 1B). As before, the reversal potential with a 5:1 KCl gradient present was approximately −35 mV, yielding $P_{K}/P_{Cl}$ = 0.59 ± 0.01 (n = 10 patches). Moreover, replacing bath Cl− with NO3−, SCN−, I−, or Br− had no significant effect on the reversal potential (P > 0.2). We estimated $P_{F}/P_{Cl}$ = 0.57 ± 0.05 (n = 3), $P_{acetate}/P_{Cl}$ = 0.30 ± 0.03 (n = 3), and $P_{glutamate}/P_{Cl}$ = 0.07 ± 0.01 (n = 4). In summary, the selectivities of g64 and g62 were identical for all ions tested, providing evidence that the sites (i.e., filters) responsible for selectivity in both channels might have structural similarities despite their very different conductances.

Kinetics

The low-conductance anion channel (g64) remained active indefinitely at normal potentials (ca. ±50 mV) but became quiescent after several seconds when the membrane potential ($V_m$) was made more negative than −80 mV (Fig. 1C). This deactivation occurred in <1 sec at more hyperpolarized potentials, but channel activity was restored when the membrane potential was returned to a value more positive than about −80 mV. In contrast, g62 inactivated at the normal membrane potential of −50 mV. A stimulus, such as stepping $V_m$ to the opposite polarity, was required to cause g62 to reopen, although it would rapidly inactivate again. We noticed that g62 usually inactivated outside the range ±20 mV and that this occurred much more rapidly at large positive potentials than at large negative ones, which sometimes required >4 min. Such voltage-dependent behavior is of uncertain physiological significance (see below) but suggests that this channel would not normally be active in situ. Finally, in contrast to the single conductance level of g64, the high-conductance channel displayed numerous subconductance states. These transitions were rectangular, clearly resolved, and often lasted for >10 msec. One substate flickered to the fully open state and is shown with low time resolution in Fig. 2C.
weakening the presence of channel open states was not reported. Except for somewhat higher selectivity, the high-conductance anion channel \( g_{362} \) is strikingly similar to voltage-dependent anion channels (VDACs) from mitochondrial outer membrane (19, 20) and to anion channels recently described in primary cultures of rat muscle, rabbit urinary bladder epithelium, and rabbit glial cells and in several continuous cell lines (embryonic carcinoma, A6, and MDCK cells) (21–27). Nearly all plasmalemma VDACs have sometimes been used as evidence for anion exchange; however, this is apparently not a useful criterion in distinguishing exchange from conductive mechanisms in rabbit bladder because both anion channels in this study were sensitive to DIDS (0.1 mM) when applied to the internal membrane surface, and \( g_{362} \) was irreversibly inhibited by external DIDS at the same concentration after 2–3 sec.

**DISCUSSION**

To our knowledge, the low-conductance anion channel \( g_{364} \) described here has not been detected by use of the patch-clamp technique, although a rectifying chloride channel having similar conductance has been incorporated into planar lipid bilayers from cardiac sarcolemma vesicles (18). The cardiac channel shows somewhat different kinetics, opening in bursts that have a time scale of seconds at -50 mV; its selectivity between different anions was not reported.

Except for somewhat higher selectivity, the high-conductance anion channel \( g_{362} \) is strikingly similar to voltage-dependent anion channels (VDACs) from mitochondrial outer membrane (19, 20) and to anion channels recently described in primary cultures of rat muscle, rabbit urinary bladder epithelium, and rabbit glial cells and in several continuous cell lines (embryonic carcinoma, A6, and MDCK cells) (21–27). Nearly all plasmalemma VDACs reported to date have been from cultured cells, and all eventually become inactive at potentials outside the range ±20 mV; hence, their physiological function, if any, is a mystery. A developmental role might explain their widespread distribution in a variety of cultured cells; however, the VDACs described in this study were from uncultured, fully differentiated cells from...
adult rabbits, and similar channels have been reported in peritoneal macrophages (28), which are also presumably differentiated. We usually observed low-conductance anion channels in excised patches immediately before VDACs became active. This finding, combined with their identical selectivities and the fact that both channels are DIDS- and voltage-sensitive, hints that the plasmalemma VDAC in rabbit urinary bladder might be a relative of the 64 pS anion channel or perhaps a breakdown product of it.

Regardless of this possible relationship, g_{64} is active at normal membrane potentials and so would be responsible for basolateral membrane chloride conductance in the rabbit urinary bladder. Cation selectivity of this channel (0.043) is remarkably similar to earlier macroscopic estimates of P_{Na+}/P_{Cl-} for the basolateral membrane (0.038), so g_{64} may also account for basolateral permeability to sodium ions in this epithelium (11).

The voltage dependence of g_{64} (deactivation at hyperpolarized potentials) and the channel's weak selectivity among anions are qualitatively similar to the characteristic of chloride conductance in amphibian skin, which have been studied by transepithelial voltage clamping (29, 30). The chloride conductance of toad skin characterized by Larsen and coworkers (e.g., ref. 31) is thought to reside in the apical membrane of the mitochondria-rich cells and is activated when the transepithelial potential is clamped outside-negative; i.e., when the apical membrane would be depolarized. If the intracellular potential in short-circuited toad skin is -70 to -100 mV as it is in the frog skin (32), the voltage dependent activation of chloride conductance could be accounted for by channels resembling g_{64}. There is evidence that the basolateral membrane of frog skin has a similar anion pathway that is sensitive to membrane voltage and cell volume (33). Further studies are needed to establish whether g_{64} is responsible for high basolateral chloride conductance in other epithelia, notably the thick ascending limb of Henle’s loop (3) and collecting duct (34, 35), and whether it is present in membranes of such diverse tissues as cornea (1), insect hindgut (36), trachea (2), and Necturus gallbladder (37), where chloride conductance is known to be strongly modulated.

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