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Characterization of a Partially Degraded Na^+ Channel from Urinary Tract Epithelium

Adam Zweifach† and Simon A. Lewis

†Department of Physiology, Yale Medical School, New Haven Connecticut, 06510, and Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550

Summary. The mammalian urinary bladder contains in its apical membrane and cytoplasmic vesicles, a cation-selective channel or activating fragment which seems to partition between the apical membrane and the luminal (or vesicular space). To determine whether it is an activating fragment or whole channel, we first demonstrate that solution known to contain this moiety can be concentrated and when added back to the bladder causes a conductance increase, with a percent recovery of 139 ± 25%. Next, we show that using tip-dip bilayer techniques (at 21°C) and a patch-clamp recorder, the addition of concentrated solution resulted in the appearance of discrete current shots, consistent with the incorporation of a channel (as opposed to an activating fragment) into the bilayer. The residency time of the channel in the bilayer was best described by the sum of two exponentials, suggesting that the appearance of the channel involves an association of the channel with the membrane before insertion. The channel is cation selective and more conductive to K^+ than Na^+ (by a factor of 1.6). It has a linear *I-V* relationship, but a single-channel conductance that saturates as KCl concentration is raised. This saturation is best described by the Michaelis-Menten equation with a K_m of 160 mM KCl and a G_{max} of 20 pS. The kinetics of the channel are complex, showing at least two open and two closed states.

Since the characteristics of this channel are similar to a channel produced by the degradation of amiloride-sensitive Na^+ channels by the proteolytic enzyme kallikrein (which is released by the cortical collecting duct of the kidney), we suggest that this channel then is not synthesized by the cell but is rather a degraded form of the epithelial Na^+ channel.

Key Words epithelial Na^+ channel · cation channel · lipid bilayers · channel degradation · channel partitioning · channel kinetics

Introduction

The renal kallikrein-kinin system has long been surmised to have an important regulatory function in distal tubular mechanisms of salt and water homeostasis (Margolius, 1984; Scicli & Carretero, 1986). Recently, Lewis and Alles (1986) suggested that a possible physiological role of renal kallikrein might be to degrade amiloride-sensitive sodium channels in the apical membrane of Na^+ transporting epithelia. It was found that kallikrein and urokinase, another urinary protease, produce an irreversible inhibition of amiloride-sensitive current in the rabbit urinary bladder. Exposure of bladders mounted in vitro to physiological kallikrein activities caused both a decrease in the magnitude of amiloride-sensitive conductance and a simultaneous increase in the magnitude of an amiloride-insensitive conductance that is not selective for Na^+ over K^+. This indicates that kallikrein degrades amiloride-sensitive Na^+ channels into amiloride-insensitive leak channels. Furthermore, urinary proteases cause the conversion of leak conductances into a form that seems to partition between the mucosal bath and the apical membrane. This conductance is called the unstable leak conductance (USL). Addition of asolectin vesicles (lipid vesicles) to the mucosal bath also causes a decrease in the magnitude of the leak conductance, indicating that either a channel-activating fragment or the entire functional channel is made unstable in the apical membrane by kallikrein action and partitions between the apical membrane and the mucosal bathing solution. Since aldosterone regulates both the number of functional amiloride-sensitive Na^+ channels and the kallikrein activity (Margolius, 1984; Scicli & Carretero, 1986), it is possible that channel degradation by kallikrein may serve as a mechanism to downregulate sodium transport after stimulation by aldosterone.

The present study demonstrates that, following the addition of solution known to contain the unstable leak conductance to the bath, conductances with properties similar to the unstable leak can be incorporated into lipid bilayers. This provides evidence that the entire functional unstable leak channel partitions between the apical membrane and the bulk bathing solution.

Portions of this work have appeared in abstract form (Zweifach & Lewis, 1987).

Materials and Methods

Lipid

Phosphatidylethanolamine from *Escherichia coli* (Avanti Polar Lipids) was purchased as a 10-mg/ml solution in chloroform-methanol. A 200-μl aliquot was dried under a stream of nitrogen and resuspended in 1 ml of hexane immediately prior to use.

Pipettes

Patch pipettes were pulled from 100 μl Boralex capillary tubing on a two-stage Narishige puller to a tip diameter of 2–10 μm. Prior to use, the glass was soaked overnight in nitric acid (Hamamoto & Montal, 1986). Pipettes were coated with sylgard to reduce noise and then fire polished.

Solutions

Solutions for bilayer experiments were unbuffered and contained only the ions specified. Solution pH ranged from 5.7–5.9. The solution used in the whole bladder experiments was standard mammalian Ringer's (Lewis & Diamond, 1976).

Bilayers and Single-Channel Recording

All bilayer experiments were conducted at room temperature (21°C). Bilayers were formed over pipette tips by the double-dip method (Coronado & Latorre, 1983). Briefly, 20 μl of a lipid-hexane solution is spread over the surface of a 1.67-ml Teflon chamber in which the tip of a patch pipette is already immersed. After approximately 30 sec, during which time the hexane presumably evaporates, the pipette tip is raised above the fluid surface and then, after a brief pause, lowered again. Bilayers had high resistance (50–100 GΩ) as compared with the low tip resistance (<10 MΩ) of the rather wide-tipped pipettes used. Bilayers had a slow capacitative transient that allowed them to be distinguished from tips plugged with lipid. Bilayers were selected for stability by repeated mechanical perturbations with a Teflon stir bar and observed for 10 min. Only bilayers that remained perfectly stable during the 10-min observation period were considered acceptable for further experimentation.

Single-channel currents were measured by a Yale Mk 111 patch-clamp amplifier and low pass filtered at 1 kHz on an 8-pole anti-aliasing Bessel filter (Frequency Devices). The output voltage was then converted to an AM signal on a Vetter 2D voltage-frequency converter and recorded on an Aiwa cassette deck with a 1-kHz band limit for later analysis. Amplitude and open and closed time histograms were constructed on a DEC 11/73 minicomputer using algorithms similar to those described by Colquhoun and Sigworth (1983). Current fluctuation analysis was performed on single-channel records on the DEC 11/73 using the algorithms described by Wills et al. (1984) for use on intact epithelia.

Whole Bladder Electrophysiology

Rabbit urinary bladders were dissected and mounted in temperature controlled (37°C), modified Ussing chambers specifically designed to reduce edge damage as has been described previously by Lewis (1977). Transepithelial potential, transepithelial resistance and short-circuit current were monitored by a laboratory computer (North Star Horizon II). For a more detailed description of the recording system and dissection *see* Lewis et al. (1984).

Harvesting Unstable Leak Channels

In order to obtain a source of unstable leak channels, bladders were "punched" according to the method of Lewis and deMoura (1982). Briefly, a series of hydrostatic pressure pulses is applied to the apical membrane, inducing fusion of cytoplasmic vesicles containing newly synthesized amiloride-sensitive Na^+ channels, leak channels and unstable leak channels. The reason there are unstable leak channels in subapical cytoplasmic vesicles is that channels which have partitioned from the urine into the apical membrane are internalized as the degree of stretching of the bladder decreases. The origin of these channels is considered in the Discussion. The unstable leak channels are harvested by washing the apical membrane and collecting the wash solution (ca. 200 ml in 60 sec). This wash solution was then concentrated (eight- to 10-fold) by dialyzing it against Aquacide 111 flakes (flaked polyethylene glycol) in Spectrapore dialysis tubing with a 6000–8000 mol wt cutoff. Concentrated solution was then passed over a Sephadex G50 column (5 ml) to remove bile salts. The importance of this preparative step will be discussed below.

Statistics

All values are reported as the mean ± SEM unless otherwise stated.

Results

In this section we demonstrate that (i) addition of concentrated mucosal wash solution to bladders mounted in vitro results in an increase of apical membrane conductance. This is expected whether the entire USL channel or only an activating fragment partitioned between apical membrane and solution. (ii) Addition of solution known to contain the USL (by the results mentioned above) to the bath solution of a tip-dip bilayer results in the appearance of single channel conductances. (iii) Last, some basic properties of these single channels are reported.

Incorporation of Unstable Leak

Experiments were conducted to determine what fraction (if any) of channels could be recovered from concentrated mucosal "punch" solutions in bladders mounted in vitro. Figure 1 shows the effect of mucosal addition of this solution on short-circuit current. In one bladder, the increase in short-circuit current was 79 ± 12% of the maximum possible

increase (calculated from the equations given below). The addition of boiled concentrated mucosal wash solution did not increase short-circuit current. Lewis and Alles (1986) determined that in bladders mounted in vitro the partition coefficient for the USL was 3/2; i.e., a USL channel is 1.5 times more likely to be in the apical membrane as in the bathing solution. This value might seem unreasonably low for a hydrophobic membrane-spanning channel protein. But when the ratio of lipid volume to solution volume is accounted for, the partition coefficient becomes 30×10^6. It is possible to estimate the percent recovery (R) of USL as follows:

$$R = (I_{sc}^R/(0.6 \times I_{sc}^A)) \times 100 \quad (1)$$

where I_{sc}^R is the change in short-circuit current due to the addition of USL. The factor of 0.6 is included because for every channel detected in the apical membrane by an increase in I_{sc} there will be another 2/3 of a channel in solution. I_{sc}^A is the total possible amount of added current, and is calculated as follows:

$$I_{sc}^A = I_{sc}^T \times Va/(Vf \times 0.6) \quad (2)$$

where I_{sc}^T is the decrease in I_{sc} due to washing the mucosal chamber of a punched bladder, Vf is the volume of solution after concentration dialysis and Va is the volume of concentrated solution added to the chamber. In experiments performed on two bladders, $R = 139 \pm 25\%$ (mean ± SD, $n = 11$). This indicates that a reasonable fraction of USL, whether it is a channel or an activating fragment, can be recovered from solution.

Incorporation of the USL into Lipid Bilayers

Addition of 25–100 μl; of rabbit urine (that had been passed over a sephadex column) to the bathing solution resulted in the appearance of conductance fluctuations in previously stable bilayers. The frequency with which conductances were observed was low, on the order of 1 min of channel activity per approximately 100 min of ideal observation. Concentration of rabbit urine seemed to increase the frequency with which conductances were observed. Similar conductances were also seen in concentrated mucosal wash solution from a punched bladder. USL activity seemed to be inactivated by repeated freeze thawing, boiling, and dialysis using dialysis tubing with a mol wt cutoff of >12,000. It was not possible, however, to quantify incorporation in lipid bilayers and compare it to expected values (*see* Discussion). Nonetheless, levels of incorporation were, as expected, low. Two conductance levels were observed: 11.8 ± 3.0 pS and 3.6 ± 1.0 pS measured in symmetrical 200 mM KCl at room temperature. Since the appearance of these two conductance levels were independent of each other, it is unlikely that the smaller one is a substate of the large conductance. Figure 2 shows single-channel records of the higher conductance USL as well as an amplitude histogram.

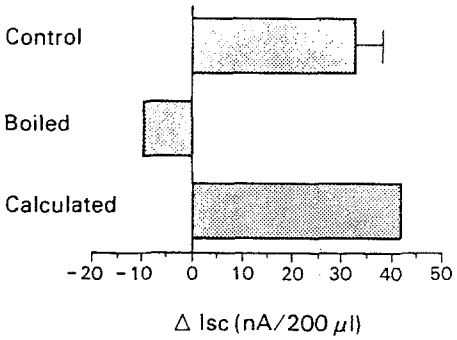

Fig. 1. The effects of addition of 200 μl aliquots of concentrated mucosal wash solution and boiled concentrated mucosal wash solution on amiloride-insensitive short-circuit current in a bladder mounted in vitro. The short-circuit current was measured 30 sec after addition to the mucosal bath solution which contained a saturating dose of amiloride. Note that the increase in I_{sc} upon addition of 200 μl of mucosal wash solution is 79 ± 12% ($n = 6$) of the maximum possible increase (bottom bar), which is calculated according to formulas presented in the text. The addition of boiled concentrated wash solution actually caused a 24 ± 0% decrease in I_{sc} 30 sec after addition ($n = 2$)

Single-Channel Properties

Quantitation of the single-channel properties of the smaller conductance was not possible due to its rapid kinetics and small amplitude. Consequently, the following section describes the single-channel properties of only the large conductance channel.

Duration of Conductance Events

Figure 3 shows a cumulative histogram of the distribution of USL channel residence times in bilayer membranes. The histogram is best described by the sum of two exponentials, with time constants of 25 and 160 sec. It must be noted that these are underestimates since there is a delay between the initial observation of channel activity and recording of the events. The presence of two residence times suggest that the original model proposed by Lewis and Alles (1986), is an oversimplification. This point will be addressed in the discussion in more detail.

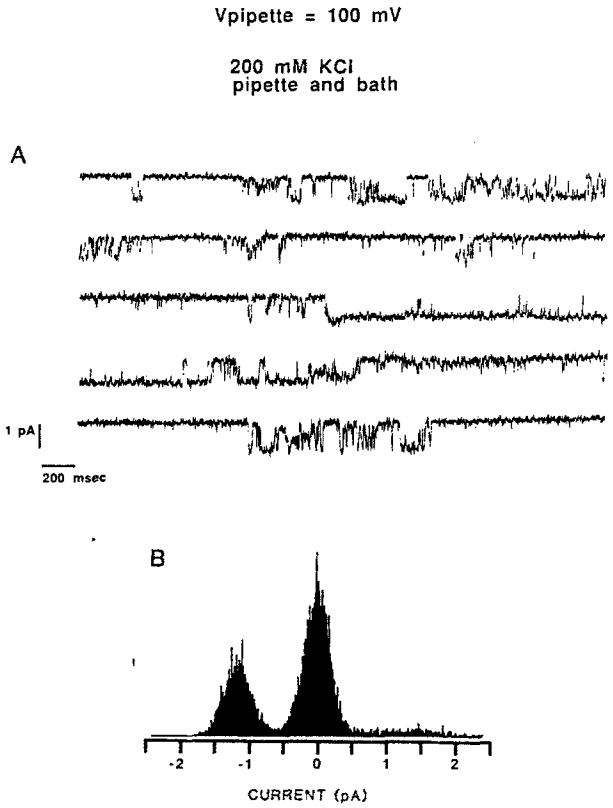

Fig. 2. (*A*) Single-channel recording from a typical experiment in which 20 μl of rabbit urine was added to the bath solution. Pipette and bath solutions were 200 mM KCl. Note the two modes of kinetic behavior, i.e., closed and bursting open and open and bursting closed. A downward transition is an opening. (*B*) Amplitude histogram constructed from a longer segment of the record shown above. The single-channel conductance of this particular channel is 12 pS, and the open time probability is 0.34

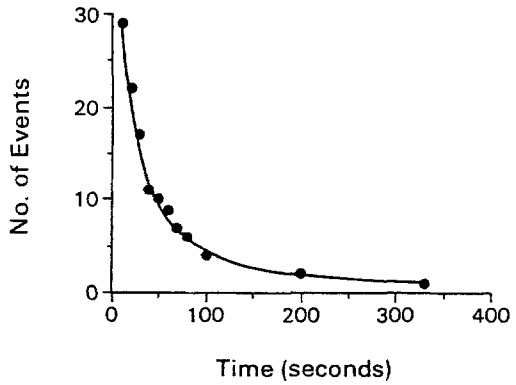

Fig. 3. Cumulative histogram of the duration of conductance events. Only bilayers with a single high conductance channel were used. The smooth curve is the sum of two exponentials with time constants of 25 and 160 sec, and intercepts of 33 and 7 events, respectively. The existence of two exponentials suggests an intermediate step between the channel, being only in the solution or the membrane. Such a step might be an association with the membrane, at which point the channel can either partition into the membrane or back into the solution

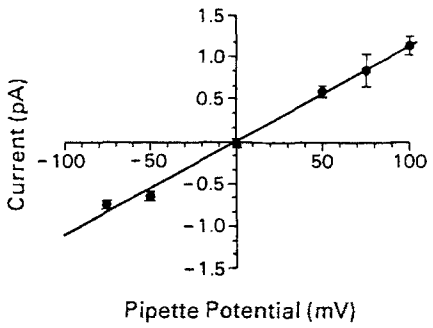

Fig. 4. Current-voltage relation in symmetrical 200 mM KCl. Since it was not possible to construct *I-V* curves for each patch, results from 23 patches were pooled. Each point represents the mean ± SEM of at least three patches. Slope conductance is 10.8 pS

Current Voltage Relation

Figure 4 shows the current-voltage relationship for the higher conductance channel in symmetrical 200 mM KCl. The figure was compiled from the results of amplitude histograms constructed at the indicated holding potentials. Because channels only reside in the bilayers for short periods of time (*see above*), it was not possible to construct complete *I–V* curves for each patch. Instead, results were pooled from 23 patches from which it was possible to derive histograms. It is interesting to note that current was a linear function of voltage over the range measured.

Selectivity

Figure 5 shows the current voltage relationship of the higher conductance channel with a 10-fold concentration gradient imposed across the patch, from which $P_K : P_{Cl}$ was calculated to be >12 using the Goldman-Hodgkin-Katz equation. Note that despite the concentration gradient imposed across the bilayer there is little or no evidence of Goldman rectification. This is most likely because the K_m (the concentration for half maximal saturation) of single-channel conductance is about 160 mM (*see below*). P_K/P_{Na} was estimated to be 1.6 by comparing the conductance in 1 M KCl and 1 M NaCl solutions. The conductance of the channel in 1 M NaCl was 9.9 ± 0.4 pS. Thus the higher conductance USL channel has the following selectivity: $P_K/P_{Na}/P_{Cl}$ = 13 : 7 : 1.

Concentration Conductance Relationship

The conductance of the larger channel in symmetrical solutions was found to be a saturating function

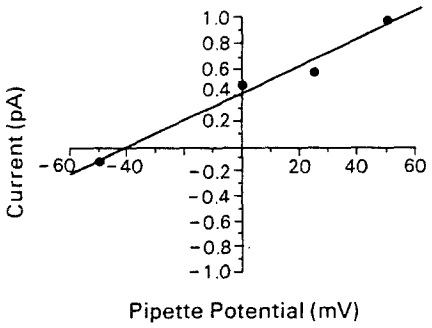

Fig. 5. Current-voltage relationship in asymmetrical conditions. The pipette solution is 1 M KCl and bath solution is 100 mM KCl. Each point is the mean, and the vertical bar is the SEM of at least three patches. The reversal potential was estimated to be approximately −40 mV. Selectivity was calculated using the Goldman-Hodgkin-Katz equation and demonstrates that this channel is at least 12 times more selective for K as compared to Cl

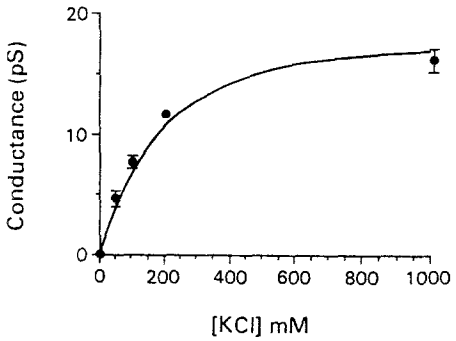

Fig. 6. Saturation of single-channel conductance as a function of increasing KCl concentration. Each point is the mean ± SEM of at least three patches. The smooth curve was generated using the Michaelis-Menten equation and yielded values of 160 mM for the K_m and 20 pS for the G_{max}

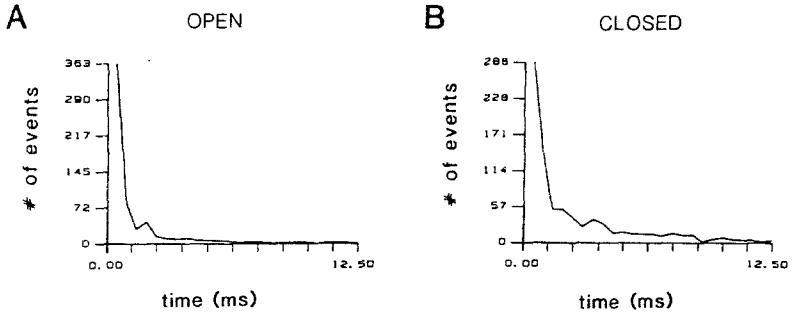

Fig. 7. (*A*) An example of an open time histogram for the USL. Note that it is composed of at least two exponentials. The time constants are given in the text. (*B*) An example of a closed time histogram for the USL. As in the open time histogram, the closed time histogram is the sum of two exponentials. The time constants are given in the text

of both KCl (Fig. 6) and NaCl (*not shown*) concentrations. When fit to the Michaelis-Menten equation, the K_m in KCl was 160 mM, and the maximum conductance, G_{max}, was 20 pS.

Kinetics

USL channels demonstrated complex kinetics. There was no apparent voltage dependence. Kinetics did not depend on ionic species or concentration. Open time histograms (Fig. 7*A*) were best fit as the sum of two exponentials with rate constants of 0.18 ± 0.04 $msec^-$ and 1.11 ± 0.2 $msec^-$ (n = 11). Closed time histograms (Fig. 7*B*) were also fit as the sum of two exponentials with rate constants of 0.28 ± 0.07 $msec^-$ and 1.34 ± 0.17 msec (n = 11). Because open and closed time distributions are the sum of exponentials, it is not possible to estimate mean open and closed times by simply taking the inverse of the time constants. Furthermore, because the USL partitions between the bilayer membrane and the bathing solution, it is difficult to estimate mean open and closed times in a straightforward manner without making unjustified assumptions about whether the channel partitions into the bilayer in a particular conformational state. The probability that the channel is open was estimated from the amplitude histograms and was 0.41 ± 0.03 (n = 37).

Current Fluctuation Analysis

One of the hallmarks of the USL in the in vitro bladder was that the power spectral density of this conductance had the form of *1/f* noise where the power at 1 Hz was linearly related to the I_{sc}, and the slope was 1.2. The power spectral density of the single-channel USL was also of the form *1/f* with the power at 1 Hz being (200 ± 56) × 10^{-15} amp^2 sec/A (n = 5) and the slope being 1.34 ± 0.06 (n = 5). The significance of these results will be discussed below.

Discussion

We consider in turn four topics: (i) that the brief appearance of unitary conductance events in lipid

bilayers is due to the partitioning of a channel into and out of the membrane and not an artifact; (ii) a comparison of this channel to other cation-selective channels reported in the literature; (iii) evidence that this channel is a partially degraded amiloride sensitive Na^+ channel; and (iv) the physiological significance and source of this channel.

This Conductance is not an Artifact

Although the presence of artifacts constitutes a hazard in any experiment involving lipid bilayer membranes, and is especially problematic in our experiments due to the lack of a pharmacological blocker of the USL channel, the points listed below rule out the possibility that this conductance is an artifact. There are three general classes of artifacts that plague lipid bilayers. These are seal resistance artifacts, "detergent" channels, and other artifacts of unknown origin. Seal resistance artifacts can be distinguished by an exponential time course of openings and closings, due to the distributed impedance of the membrane-seal-glass complex (Cooper et al., 1986). We observed square conductance events with unresolvably fast openings and closings. Thus the conductances we observed were not seal resistance artifacts. Care was also taken to avoid contamination of the bilayer and apparatus with detergents. Also, detergent channels would be expected to persist stably in bilayers, whereas our channel resided in membranes for only short times. The above points suggest that it is unlikely that the conductances we observe were detergent channels. Other noteworthy points are that an artifact in a lipid bilayer membrane would not consistently demonstrate the unitary conductance, kinetic properties, selectivity and saturation of single-channel conductance that we observed in a large number of patches.

Finally, samples known to contain USL channel activity could be concentrated in dialysis tubing and rendered inactive by three different methods, which are: freeze-thawing, boiling, and use of dialysis tubing with a molecular weight cutoff of >12,000 daltons to concentrate the solution. Taken together, the above points argue against this conductance being an artifact.

This Cation Channel is not Unique

None of the properties of the USL is in itself unique. The literature contains many reports of cation-selective ion channels (Hillyard, Zeiske & Van Driessche, 1982; Latorre & Miller, 1983; Maruyama, Moore & Petersen, 1985; Van Driessche & Zeiske, 1985). In fact, the cation-selective pore forming antibiotic gramicidin is commonly known to exhibit similar properties to those of the USL. Gramicidin added to bath solutions partitions into membranes and forms conductive channels when two gramicidin molecules associate in a head-to-head orientation across the bilayer (Gomperts, 1977). Although the partition coefficient of the USL channel is much lower than gramicidin, and so gramicidin is not normally thought to partition out of membranes, in principle the USL channels is no different than gramicidin. Our report, however, is the first demonstration that an ion channel of vertebrate origin can exhibit such behavior.

Is the USL Channel an Enzymatically Degraded Form of the Na^+ Channel?

The impetus for this study came from the observation by Lewis and coworkers that replacement of the mucosal solution of the in vitro rabbit urinary bladder epithelium with identical solution resulted in a decrease in current flow and conductance of the apical membrane (Lewis & Alles, 1986; Lewis & deMoura, 1982; Lewis et al., 1984). Further experiments suggested that this decrease was due to a partitioning of a conductive pathway out of the apical membrane and into the bulk solution.

The following observations suggest that the USL channel is in fact the same moiety as found in the apical membrane of the bladder epithelium.

1. The channel containing solution added to the bilayer was obtained from mucosal bathing solutions (punched bladder wash solution and urine) known to contain the conductance.

2. The selectivity of the USL in the apical membrane of the rabbit urinary bladder epithelium for Na^+ to K^+ is 0.5 to 0.7 : 1 and in the lipid bilayer is 0.54 : 1.

3. The residency time constant of the USL in the apical membrane is 129 sec and in the bilayer is 25 and 160 sec. Considering the differences in lipid composition and temperature, these results are in good agreement.

4. The amount of incorporation of the USL into lipid bilayers was (as expected) low. Due to the infrequent occurrence of channel insertion and finite time of bilayer stability (typically 1 hr), it was not possible to quantitate incorporation of the USL into lipid bilayers and compare it to theoretical values.

The relationship governing incorporation is:

$$\text{No. of channels in memb.} = 30 \times 10^6 \times \frac{\text{volume of bilayer}}{\text{volume of solution}} \times \text{No. of channels in soln.} \quad (3)$$

where 30×10^6 is the partition coefficient for the USL (Lewis & Alles, 1986) between a lipid and aqueous solution of equal volume. This relation means that:

a) The amount of incorporation into a lipid bilayer then depends upon the ratio of lipid volume to solution volume (2×10^{-15} for a patch pipette with a radius of 0.5 μm) and the number of channels added to the aqueous phase (20×10^3 to 200×10^3 channels). Thus for concentrated mucosal wash solution, it would be expected that the bilayer would exhibit conductance activity for 0.1–1 min per every 100 min of "ideal" experimental observation. Concentrated rabbit urine might be expected to give more incorporation than nonconcentrated urine, and indeed this seemed to be true. However, the predicted incorporation would not be more than 5 min channel activity per 100 min of "ideal" observation, which is still far too low to be measured reliably. Finally, in experiments with urine and concentrated urine the number of channels in the urine collected is not known, and when urine is concentrated, many other substances, including kallikrein may also be concentrated, which could further degrade channels and reduce channel number.

b) The amount of incorporation is a function of the area of the lipid bilayer, which is not known with any precision because the location of the bilayer in the pipette tip is not known. Also, calculations of expected incorporation assume a well-stirred bulk solution phase. Since the bilayer may be formed part way up the shank of the pipette, it is not known whether an unstirred diffusion space exists between the bulk solution and the bilayer. In addition, the extent of extraneous nonbilayer lipid domains is not known. Thus quantitation of incorporation of the USL into lipid bilayers is not possible at present because of low expected incorporation and difficulty in defining relevant lipid volume. The fact that observed incorporation is qualitatively similar to what is expected supports the contention that this channel is an enzymatically degraded Na^+ channel.

5. Important confirmation that the higher conductance channel described in this study is the USL channel comes from the results of power spectral analysis. Lewis et al. (1984) made the empirical observation that the power at 1 Hz (β) is directly proportional to the magnitude of the USL current in the intact epithelium. They derived a relationship between β and current of 150×10^{-15} A^2 sec/A. This value is in excellent agreement with the value of β determined from spectral analysis of single-channel recording of 200×10^{-15} A^2 sec/A. Furthermore, the slope of the spectrum derived from single channels was 1.34, which is close to the values reported for the intact epithelium (1.14–1.26). Thus the higher conductance channel described here can completely account for the macroscopic observations of Lewis et al. (1984). When one considers the complex kinetics of this USL (two open and two closed times) it is not surprising (and indeed predicted) that the power spectral density will have the form of $1/f$ (Sauve & Szabo, 1985).

In a recent communication, Lewis and Alles (1986) demonstrated that kallikrein, a urinary protease, caused a sequential degradation of the amiloride-sensitive Na^+ channel in the apical membrane of the rabbit urinary bladder epithelium into a form which is non-amiloride sensitive, cation selective and stable in the membrane and then into a form which is non-amiloride sensitive, cation selective and unstable in the apical membrane. This latter conductance (produced by degradation of the Na^+ channel) has the same selectivity, a similar residency time in the membrane, and the relationship between the power at 1 Hz and short-circuit current as the conductance induced in bilayers by solutions that should contain the USL. Thus the identical fingerprints of the USL channel in the bladder epithelium and the conductance induced in bilayers strongly suggests that the channel reported in the present study is indeed a hydrolyzed form of the Na^+ channel. The single-channel properties of the USL have both similarities and differences with the amiloride-sensitive Na^+ channel. First, at physiological NaCl concentrations the USL will have a single-channel conductance of approximately 6 pS (at room temperature), similar to that reported by Palmer and Frindt (1986) of 5 pS for the Na^+ channel. Second, both channels saturate as the cation concentration is increased, the USL has a K_m of 160 mM (for symmetric KCl) and the Na^+ channel is approximately 75 mM (for NaCl in cell-attached patches). They differ in that the Na^+ channel has been shown to exhibit very slow transitions between open and closed states, with mean open times on the seconds time scale, and amiloride induces a fast flickering block which reduces the mean open times to the millisecond time scale. The USL is not influenced by amiloride and spontaneously flickers between open and closed states in a complex kinetic process. In spite of these kinetic differences, P_o for both channels is 0.41.

Physiology of Channel Degradation

It seems clear that this unstable leak pathway is the end result of the degradation of an amiloride-sensitive Na^+ channel produced by the proteolytic enzyme kallikrein. Since kallikrein is known to be released from the renal cortical connecting duct (Margolius, 1984; Scicli & Carretero, 1986) which is

located immediately proximal to the cortical collecting duct, a nephron segment known to have a high density of amiloride-sensitive Na^+ channels, we suggest that some of the USL channels found in the urine, apical membrane and presumably in the subapical vesicles of the urinary bladder epithelium are of tubular origin. Lewis and Alles (1986) proposed that a physiological role of the renal kallikrein system might be as a downregulator of apical membrane Na^+ permeability. Using a serial model for channel degradation, S.A. Lewis et al. (*unpublished observation*) calculated that the USL channel should have a conductance of about 12 pS (as compared to the value of 6 reported here). Considering the number of assumptions required in the model calculation, we feel that a sequential model for channel degradation is a good approximation for the degradation of amiloride-sensitive Na^+ channels.

In summary, the present study demonstrates that a conductance with identical functional properties to the USL channel (Lewis & Alles, 1986; Lewis et al., 1984) appears in bilayer membranes following the addition of solution known to contain this component to the bath. This incorporation into a bilayer suggests that the entire functional USL channel is made unstable in the apical membrane of Na^+ transporting tissues by the action of renal proteases. Since aldosterone is known to increase kallikrein activity, this provides confirmation of the hypothesis that a physiological role of renal kallikrein is to degrade amiloride-sensitive Na channels into a form that is unstable in membranes, thus downregulating the natriuretic response to aldosterone.

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