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Current-voltage relations of Cs^+-inhibited K^+ currents through the apical membrane of frog skin

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Abstract. The voltage-dependence of the inhibitory effect of mucosal Cs^+ on the inward K^+ current through the apical membrane of frog skin (*Rana temporaria*) was studied by recording transepithelial current-voltage relations. Experiments were performed with skins exposed to NaCl and KCl Ringer solutions on the serosal and mucosal side respectively (control skins), as well as with tissues incubated with K_2SO_4 Ringer solutions on both sides (depolarized skins). Studies of the dose-dependence of the Cs^+ block showed that under both experimental conditions the apparent affinity of Cs^+ increased as the transepithelial potential was clamped at higher mucosal positive voltages. Under control conditions, the concentration of Cs^+ required to block 50% of the K^+ current (K_{Cs}) recorded while the transepithelial voltage was clamped at zero mV was 16 mmol/l. K_{Cs} decreased exponentially with mucosal positive voltages. The dependence of K_{Cs} on the membrane potential was analyzed with Eyring rate theory in which Cs^+ was assumed to block the K^+ transport by binding to a site within the channel. The analysis showed that this site is located at a relative electrical distance $\delta = 0.32$ of the voltage drop across the apical membrane, measured from the cytosolic side. The Hill coefficient obtained from this analysis was $n = 3.1$. Experiments with K^+-depolarized tissues showed that only inward K^+ currents recorded with positive transepithelial voltages were depressed by external Cs^+. Also under these conditions K_{Cs} showed an exponential dependence on the transepithelial potential. The analysis of these data with the rate theory revealed $\delta = 0.09$ and $n = 1.7$. The difference in δ found in control and depolarized tissues can be explained by the influence of the basolateral membrane resistance on the $I-V$ relations.

Key words: Current-voltage relations – Epithelia – K^+ current – Cesium – K^+ channel – Apical membrane – *Rana temporaria*

Introduction

Several types of potassium-selective channels in biological membranes are capable of binding cesium. The occupation of the K^+ channel by Cs^+ occludes the K^+ pathway in a voltage and concentration dependent way. The characteristics of the interaction of Cs^+ with the K^+ channel have been the subject of studies in frog skeletal muscle [8, 15, 16], in smooth muscle [3], in squid giant axon [1, 2, 4, 7], in starfish eggs [10], in tunicate eggs [13] and in the apical membrane of the cortical collecting duct [14]. A K^+ pathway with properties which resemble the characteristics of these K^+ channels was found in the apical membrane of the granulosum cells of the skin of frog species *Rana temporaria* [12, 26]. These studies showed that the K^+ channels reside in parallel with the amiloride-sensitive Na^+-transporting pathway. The K^+ pathway is sensitive to apical H^+, Rb^+, Cs^+, Tl^+, Ba^{2+} and Cd^{2+} [24, 26, 27]. Noise analysis showed that these K^+ channels switch randomly between an open and closed state [22]. Analysis of the associated fluctuation in current revealed a Lorentzian component in the power density spectrum. The blockade of the K^+ channels by Ba^{2+} was found to be competitive with K^+ [5, 23]. Ba^{2+} induced additional fluctuation of the K^+ current which resulted in a second Lorentzian in the power spectrum. In contrast to Ba^{2+}, the blockade of apical Cs^+ was not accompanied by a blocker-induced Lorentzian component. But apical Cs^+ lowered the corner frequency and plateau value of the spontaneous Lorentzian as expected from a high rate competitive blocker [22]. In this paper we describe the concentration and voltage dependence of the Cs^+ block of K^+ channels in the apical membrane of frog skin. As for many K^+ channels of excitable membranes, the block of K^+ channels in frog skin by Cs^+ is strongly potential and concentration dependent.

Materials and methods

Experiments were performed with skins of the frog species *Rana temporaria*. The animals were kept at 17° C with free access to a reservoir of tap water. During 1–4 days prior to the experiments, some of the animals were exposed to a solution containing 30 mmol/l KCl. This resulted in a large K^+ permeability of the apical epidermal membrane [19]. The skin was dissected from double-pithed frogs and was mounted in an Ussing-type lucite chamber [5]. Both sides of the tissue were continuously perfused with fresh Ringer solution with a flow rate of 5 ml/min. The skin area exposed to the bathing solutions was 0.5 cm^2. The transepithelial voltage was controlled by an automatic voltage clamp apparatus [20, 21]. During the whole experiment, the epithelium was short-circuited except during the periods where current-voltage curves were recorded. Voltage pulses of 10 mV amplitude and 1 s duration were imposed to the tissue to measure the transepithelial conductance. Current-voltage relations were recorded under voltage clamp conditions by applying a sequence of voltage pulses which amplitude

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varied from + 100 mV to −100 mV in increments of 10 mV. The pulse duration was 500 or 750 ms. The procedure of recording and processing the data and the electronic set-up for current-voltage experiments was described in detail in a previous publication [5]. The transepithelial voltages (V_t) are referred to the serosal side (serosa ground). The transepithelial currents (I_t) carried by cations moving from mucosa to serosa are considered as positive.

Solutions. NaCl Ringer solutions contained (in mmol/l): 115 NaCl, 2.5 $KHCO_3$ and 1 $CaCl_2$ (pH = 8.0). KCl Ringer solutions contained (in mmol/l): 115 KCl, 2.5 $KHCO_3$ and 1 $CaCl_2$ (pH = 8.0). Cs^+ was added as chloride salt to the mucosal Cl^- Ringer solution. In some of the experiments the osmolality and ionic strength were maintained constant during the Cs^+ inhibition experiments. Therefore we initially added 30 mmol/l NaCl to the mucosal solution and replaced gradually NaCl by CsCl. All these solutions in which x mmol/l CsCl and $(30-x)$ mmol/l NaCl were present, contained 50 μmol/l amiloride. The results of these experiments did not differ from experiments where no correction for osmolality was made. K_2SO_4 Ringer solutions contained (in mmol/l): 57.5 K_2SO_4, 2.5 $KHCO_3$ and 1 $CaSO_4$. Na_2SO_4 Ringer solutions contained (in mmol/l): 57.5 Na_2SO_4, 2.5 $KHCO_3$ and 1 $CaSO_4$. Cs^+ was added as Cs_2SO_4 to the mucosal SO_4^{2-} solution. All studies were done at room temperature. The data are presented as mean values ± SD.

Results

The effect of Cs^+ on the K^+ current and conductivity in non-depolarized tissues

In all the experiments the tissue was initially equilibrated with NaCl Ringer solution on both sides until the short circuit current (I_{sc}) reached a steady-state level. After this equilibration period, the mucosal NaCl Ringer solution was replaced by KCl Ringer solution. The mucosal Na^+-K^+ replacement resulted in a rapid drop of I_{sc} followed by a period of approximately 1–2 h during which I_{sc} increased slowly [24]. The Cs^+ inhibition experiments were started after I_{sc} reached a stable value. Figure 1 shows an experiment in which the inhibitory effect of Cs^+ on I_{sc} was studied by gradually increasing the mucosal Cs^+ concentration ($[Cs^+]_m$). In this experiment the ionic strength and osmolality were kept constant while $[Cs^+]_m$ was increased, by partly substituting CsCl for NaCl in such a way that $[Na^+]_m + [Cs^+]_m = 30$ mmol/l. Amiloride (50 μmol/l) was present in all mucosal solutions to block the transepithelial Na^+ currents. Up to a concentration of 6 mmol/l, Cs^+ did not affect I_{sc} significantly. Also the transepithelial conductance, which is proportional to the length of the vertical deflections of the current caused by 10 mV voltage pulses, remained approximately constant. However, 10, 20 and 30 mmol/l CsCl depressed I_{sc} markedly. With $[Cs^+]_m = 10$ mmol/l, the conductance decreased. With $[Cs^+]_m = 20$ and 30 mmol/l the voltage pulses elicited a downward deflection of the current. We shall come back to this point in the next section and show that the negative current deflections are related to the negative slope conductance resulting from the voltage dependence of the Cs^+ block. When the mucosal KCl Ringer solution was totally replaced by CsCl Ringer solution, I_{sc} was reduced to less than 6% of control which indicates that I_{sc} was nearly totally carried by K^+. After removal of Cs^+ from the mucosal solution, I_{sc} returned to 78% of its control value.

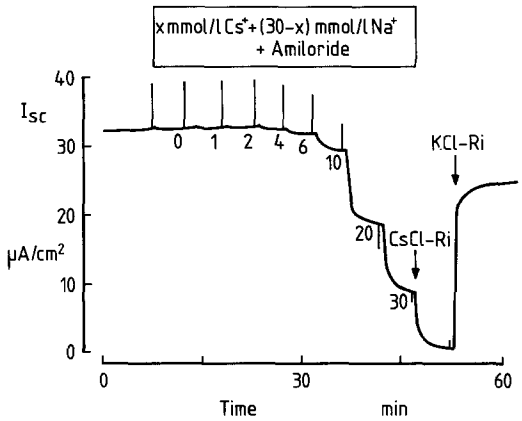

Fig. 1. Inhibition of the short-circuit current (I_{sc}) by mucosal Cs^+. Incubation media: *ser.*: NaCl Ringer solution; *muc.*: KCl Ringer solution. At the beginning of the period indicated by the *bar* at the top of the figure, 30 mmol/l NaCl plus 50 μmol/l amiloride were added to the mucosal solution. Subsequently, we increased the mucosal Cs^+ concentration by gradually replacing NaCl by CsCl. The numbers indicate the mucosal Cs^+ concentration. The vertical deflections on the current trace are caused by clamping the transepithelial potential to + 10 mV

Effect of the transepithelial potential on the inhibition of I_K by Cs^+

The negative deflection of the transepithelial current in response to 10 mV transepithelial pulses observed with $[Cs^+]_m = 20$ and 30 mmol/l, suggested that the *I-V* curves recorded in this condition would exhibit a region of negative slope. Similar negative slope conductances were observed in current-voltage (*I-V*) experiments of Ba^{2+}-inhibited transepithelial K^+ currents through frog skin [5], skeletal muscle [17], squid axon [6] and starfish egg [9]. These studies showed that the negative slope conductance is indicative of a blocker effect which is concentration as well as voltage dependent. In order to investigate the voltage dependence of the Cs^+ block we recorded *I-V* relations at different $[Cs^+]_m$. The results are shown in Fig. 2A. In the absence of Cs^+ the *I-V* relation was nearly linear. Small deviations from the linear behaviour were observed at the most positive and negative voltages. However, with all mucosal Cs^+ concentrations, strong nonlinear regions became visible in the *I-V* curves. In the presence of Cs^+, I_t increased with V_t, reached a maximum and then declined. This gave rise to a region in the *I-V* curves where the slope conductance was negative. This part of the *I-V* curve shifted to less positive voltages as $[Cs^+]_m$ was increased. At 20 and 30 mmol/l $[Cs^+]_m$, the negative conductance was obtained around zero mV. This explains the negative current deflections observed with $[Cs^+]_m = 20$ and 30 mmol/l in the transepithelial conductance measurements while the epithelium was short-circuited (Fig. 1). For $[Cs^+]_m = 10$ mmol/l and larger the *I-V* relations approached the same line at the highest voltages. Regression analysis of the *I-V* data in this linear part of the *I-V* diagram (between 60 and 100 mV), revealed that the regression line passed through the origin (dashed line). This suggests that at voltages above 60 mV the currents recorded with

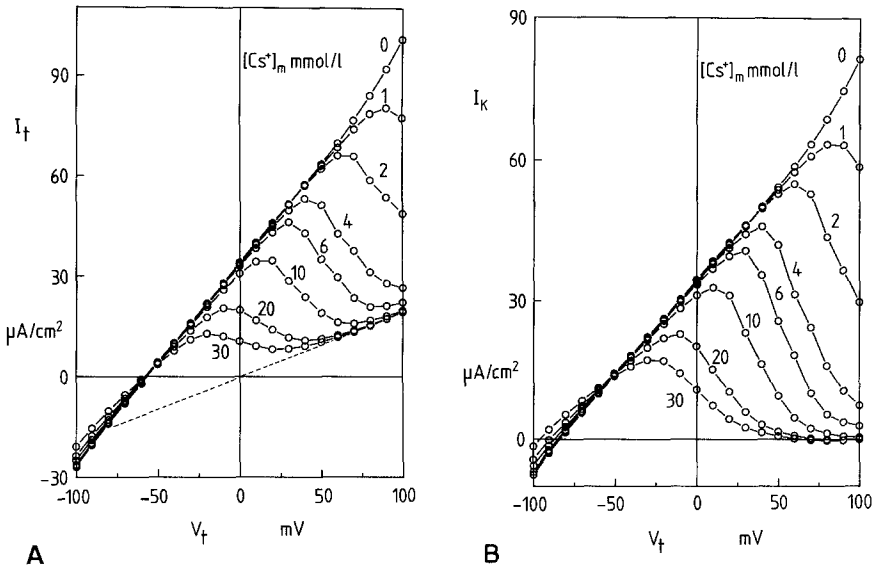

Fig. 2 A, B. Transepithelial current-voltage relations of the experiment shown in Fig. 1. The transepithelial potentials are referred to the serosal side (serosa ground). Solutions: serosal side: NaCl Ringer solution, mucosal side: KCl Ringer solution. Panel **A**: Transepithelial current (I_t) as a function of the transepithelial voltage (V_t). The *dashed line* is obtained from linear regression analysis of the *I-V* data between 60 and 100 mV recorded with $[Cs^+]_m = 30$ mmol/l. Panel **B**: Cs^+-blockable transepithelial current (I_K), obtained by subtraction of the shunt current (*dashed line*, in **A**) as a function of the transepithelial voltage (V_t)

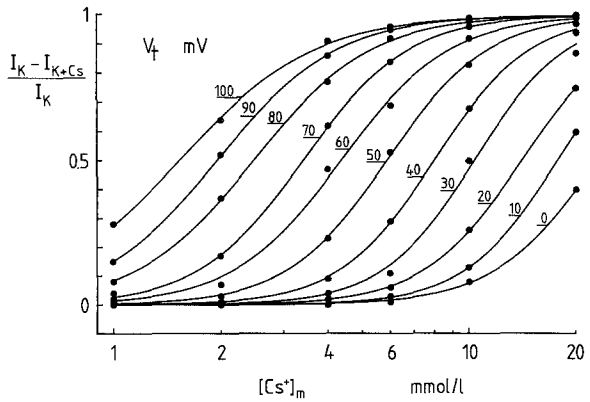

Fig. 3. Concentration-inhibition diagrams at different transepithelial voltages (V_t) obtained from the data in Fig. 2B. The *solid lines* represent the fractional current $[(I_K - I_{K+Cs})/I_K]$ calculated by fitting Eq. (1) to the experimental data

Table 1. Parameters obtained from $I_{sc}-[Cs^+]_m$ and *I-V* experiments in non-depolarized tissues

No.	I_{sc} μA/cm²	R_t kΩ · cm²	K^0_{Cs} mmol/l	n	δ
1	21.3	3.09	8.09	1.3	0.34
2	21.4	2.92	8.43	2.0	0.36
3	27.8	2.18	5.42	2.1	0.24
4	29.4	2.17	8.88	1.8	0.30
5	33.4	1.35	21.70	3.2	0.34
6	50.3	1.14	24.44	4.3	0.35
7	72.5	0.69	36.17	6.9	0.33
mean	36.6	1.90	16.16	3.1	0.32
SD	17.2	0.84	10.6	1.8	0.04

I_{sc}: short-circuit current; R_t: transepithelial resistance; K^0_{Cs}: Cs^+ concentration to block 50% of I_K calculated with Eqs. (1) and (2); n: Hill coefficient; δ: apparent electrical distance of the Cs^+ binding site from the cytosolic end of the channel

$[Cs^+]_m \geq 10$ mmol/l pass through a non-selective, possibly paracellular pathway. The resistance of this non-selective pathway calculated from the slope of the dashed line was 5.12 kΩ · cm². We assumed therefore that over the entire voltage range the extrapolated dashed line represents the shunt current. Under this assumption we calculated the K^+ current through the apical K^+ channels by subtracting this shunt current from the I_t values recorded at different $[Cs^+]_m$. In this way we obtained the relation between the K^+ current, I_K, and V_t depicted in Fig. 2B. At the highest voltages, the corrected currents vanish for all Cs^+ concentrations. Moreover, in this voltage range we did not observe a deviation of I_t from the extrapolated shunt current, as could be expected if Cs^+ was forced to go through the K^+ channels, relieving the block. Such a relief of Cs^+-block at high voltages has been observed for example in Ca^{2+}-activated K^+ channels from smooth muscle [3] and in K^+ channels in the squid giant axon [7].

The voltage-dependence of the Cs^+ block was analyzed by plotting the corrected currents (like Fig. 2B) in inhibition-concentration diagrams such as that shown in Fig. 3. The ratio between the amount of current blocked by Cs^+, $(I_K - I_{K+Cs})$, and the current recorded in the absence of Cs^+ at the same potential, I_K, is plotted as a function of the logarithm of $[Cs^+]_m$ at different V_t. At all transepithelial potentials, the Michaelis-Menten type equation as used in our previously reported *I-V* experiments with Ba^{2+} [5] was fitted to the experimental data (continuous curves at the different V_t):

$$\frac{I_K - I_{K+Cs}}{I_K} = \frac{1}{1 + \left[\frac{K_{Cs}}{[Cs^+]_m}\right]^n}. \quad (1)$$

K_{Cs} is equal to $[Cs^+]_m$ at which 50% of the potassium current is blocked by Cs^+ and n is the Hill coefficient. The values for n thus calculated from 7 experiments are shown in Table 1.

Figure 3 clearly demonstrates that when V_t is increased, the inhibition-concentration curves shift to the left. This indicates that at higher voltages a smaller $[Cs^+]_m$ is required to block 50% of I_K. So K_{Cs} decreases with increasing V_t. This is shown in Fig. 4 where K_{Cs} is represented in a semi-logarithmic diagram as a function of the transepithelial voltage. This diagram reveals a linear relation between the logar-

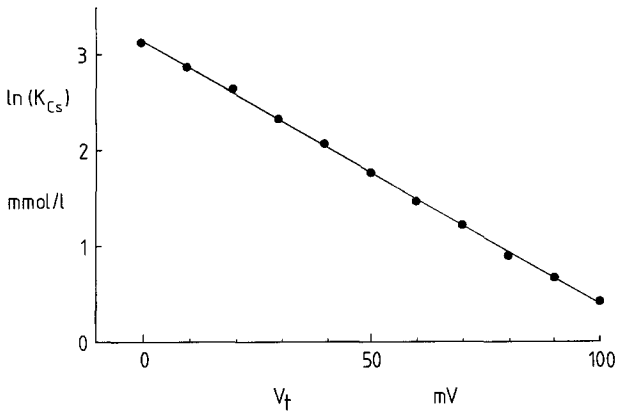

Fig. 4. Dependence of K_{Cs} on the transepithelial voltage V_t. The K_{Cs} values were calculated from the fit of Eq. (1) as depicted in Fig. 3. The *straight line* was obtained by linear regression analysis

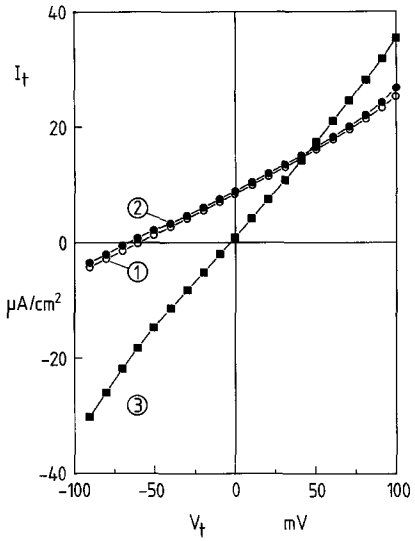

Fig. 5. *I-V* curves recorded before and during K^+ depolarization of the basolateral membrane. *Curve 1* and *2* compare the effect of the substitution of Cl^- for SO_4^{2-} while *curve 3* illustrates the effect of the serosal Na^+ by K^+ replacement. Ringer solutions used to record the *I-V* curves: *curve 1*: muc.: KCl, ser.: NaCl; for *curve 2*: muc.: K_2SO_4, ser.: Na_2SO_4; for *curve 3*: muc.: K_2SO_4, ser.: K_2SO_4

ithm of K_{Cs} and V_t which indicates that K_{Cs} depends exponentially on V_t. This can be interpreted in terms of a model which assumes that Cs^+ blocks the K^+ currents by binding to a site inside the channel. The voltage dependence of K_{Cs} can be expressed in an equation derived from a rate theory analysis of the entry of Cs^+ into the channel [11]:

$$K_{Cs} = K_{Cs}^{\circ} \exp\left[\frac{(\delta-1)zF}{RT} f_a V_t\right]. \quad (2)$$

In this expression, δ represents the fraction of the potential difference across the apical membrane experienced at the binding site for Cs^+, referred to the cytosolic side: K_{Cs}° is K_{Cs} at $V_t = 0$ mV; $f_a = R_a/(R_a + R_b)$ is the fractional resistance with R_a and R_b the resistance of the apical membrane and basolateral membrane respectively; z is the valence of the blocking ion ($z = 1$) and F, R and T have their usual meaning. Taking into account that in frog skin incubated with mucosal KCl Ringer solutions, R_a is much larger than R_b [12], f_a approaches in many experiments a value of 1. For the analysis of the data obtained with non-depolarized skins, we assumed that the entire transepithelial potential dropped across the apical membrane and that as a first approximation we could assume $f_a = 1$. From linear regression analysis of the $\ln(K_{Cs})$ and V_t data in Fig. 4 we obtained the values for δ and K_{Cs}° as shown in Table 1. The mean values are $\delta = 0.32 \pm 0.04$ and $K_{Cs}^{\circ} = (16 \pm 11)$ mmol/l.

I-V relations in depolarized tissues

An important problem which arises in the interpretation of the above described results is that we utilized the transepithelial voltage V_t instead of the voltage drop across the apical membrane, V_a. Because of the serial arrangement of both membranes of the epithelium, only a fraction of the transepithelial voltage ($f_a \cdot V_t$) is sensed across the apical membrane. To overcome this problem we performed *I-V* experiments in K^+ depolarized tissues. Because of the high permeability for K^+ of the basolateral membrane of the frog skin, the replacement of serosal Na^+ by K^+ will result in a depolarization of the intracellular potential and will reduce the basolateral membrane resistance considerably [18]. As a result, most of the imposed transepithelial potential V_t will drop across the apical membrane. Moreover the intracellular potential recorded under short-circuit conditions has been shown to approach zero [18]. Current-voltage analysis was difficult in Cl^- Ringer solutions because of the very large increase in shunt conductance occurring during K^+ depolarization. As a consequence the currents recorded during an *I-V* experiment passed largely through the shunt, instead of through the apical K^+ channels. The correction of the *I-V* curves as described above by subtracting the shunt current became very inaccurate. For this reason we performed the experiments using tissues incubated with SO_4^{2-} Ringer solutions.

Figure 5 compares the *I-V* curves recorded with Cl^- and SO_4^{2-} Ringer solutions as incubation media on both sides of the skin, with K^+ and Na^+ as principal cation on the mucosal and serosal side, respectively. It is clear that the anion exchange has only a small effect on the *I-V* relation. However, substituting serosal Na^+ for K^+ results in a shift of the open circuit potential V_m from about -60 mV to -2 mV. The *I-V* curve passes approximately through the origin of the *I-V* plot. Besides the shift in V_m, there is also a large increase in the slope of the *I-V* curve.

Figure 6 shows the *I-V* curves recorded in the presence of mucosal Cs^+ concentrations up to 20 mmol/l. It is clear from this plot that in K^+ depolarized tissues Cs^+ blocks only the positive or inward K^+ currents. A reduced affinity of Cs^+ at negative transepithelial voltages was also observed in non-depolarized skins (see Fig. 2). We determined the shunt current in these experiments with K^+-depolarized tissues also by assuming that 20 mmol/l mucosal Cs^+ totally blocked the transcellular K^+ current at high voltages. We furthermore assumed that as in the non-depolarized skins, the shunt current is linearly related to the transepithelial voltage and becomes zero during short-circuit conditions. Under these assumptions the shunt current can be represented by the dashed line which passes through the origin and equals I_t at $[Cs^+]_m = 20$ mmol/l and $V_t = 100$ mV. After correction for the shunt current, the same procedure as in the non-depolarized experiments was followed to obtain the

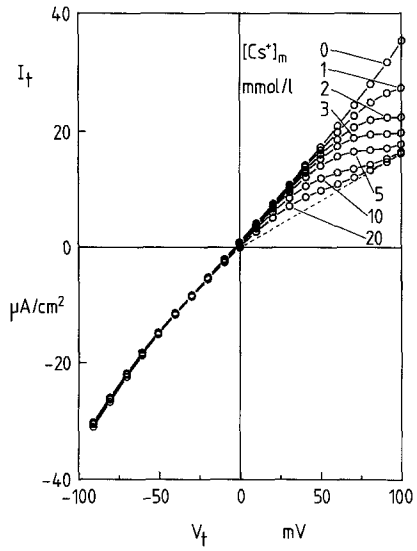

Fig. 6. Effect of mucosal Cs^+ on the transepithelial *I-V* relations recorded under depolarized conditions. K_2SO_4 Ringer solution on both sides. External Cs^+ concentrations varied from 0 to 20 mmol/l

dissociation constant K_{Cs} as a function of V_t. Also in these experiments we found a linear relation between $\ln(K_{Cs})$ and V_t. From 4 experiments we obtained $\delta = 0.09 \pm 0.06$, $n = 1.7 \pm 0.2$. K_{Cs} (50 mV) determined at 50 mV was 21 ± 2 mmol/l.

Discussion

The main conclusion of the present study is that Cs^+ blocks K^+ conductance through apical K^+ channels in frog skin in a strongly voltage and concentration-dependent way. This block has similar characteristics as the blockage of these channels by Ba^{2+} [5]. The *I-V* curves show a strong non-linearity: I_K increases with V_t, reaches a maximal value at $V_t = V_{max}$ and declines thereafter. At voltages above V_{max} the *I-V* curves have a negative slope up to the most positive voltages (+100 mV). The lack of an increase in I_K at $V_t > V_{max}$ suggests that Cs^+ cannot pass through the K^+ channel in the observed voltage range [3] and relieve the block.

The voltage dependence of the block is consistent with the idea that Cs^+ binds to a site within the channel itself, and that this site senses a fraction of the transmembranal electrical potential. As a consequence, the on- and off-rate by which Cs^+ enters and leaves the site in the channel are voltage dependent and thus also K_{Cs}. According to a model based on Eyring rate theory [11, 25], K_{Cs} is expected to be an exponential function of the voltage across the apical membrane V_a (referred to the cytosolic side):

$$K_{Cs} = B \cdot \exp\left[\frac{(\delta-1)zF}{RT} V_a\right] \tag{3}$$

where B is K_{Cs} at $V_a = 0$ mV. As V_a is unknown in our experiments, we utilized a relation between K_{Cs} and V_t as discussed in a previous paper [5]. V_a and V_t are related by:

$$V_a = f_a V_t + f_a E_b + (f_a - 1)E_a \tag{4}$$

where E_a and E_b are the K^+ equilibrium potentials across the apical and basolateral membrane and $f_a = R_a/(R_a + R_b)$ is the fractional resistance with R_a and R_b the resistances of the apical and basolateral membrane respectively. From Eqs. (3) and (4) follows

$$K_{Cs} = B \cdot \exp\left[\frac{(\delta-1)zF}{RT}(f_a E_b + (f_a - 1)E_a)\right] \cdot \exp\left[\frac{(\delta-1)zF}{RT} f_a V_t\right]. \tag{5}$$

The first exponential factor in Eq. (5) does not depend on V_t. We therefore defined

$$K^o_{Cs} = B \cdot \exp\left[\frac{(\delta-1)zF}{RT}(f_a E_b + (f_a - 1)E_a)\right] \tag{6}$$

so that Eq. (5) becomes:

$$K_{Cs} = K^o_{Cs} \cdot \exp\left[\frac{(\delta-1)zF}{RT} f_a V_t\right]. \tag{7}$$

We assumed f_a to be nearly equal to 1 in non-depolarized skins and we neglected its influence. Figure 4 shows that Eq. (7) can be fitted very well to the data. δ was found to be 0.32. This value is larger than the value obtained in depolarized tissues $\delta = 0.09$. A possible origin of this discrepancy could be the attenuation of the imposed transepithelial voltage by the presence of the basolateral membrane resistance in non-depolarized (ND) skins. In experiments with these tissues we found that the slope of the relation between $\ln(K_{Cs})$ and V_t, $A_{ND} \equiv (\delta-1)zFf_a/RT$ [Eq. (6)] was -0.0270 ± 0.0015 ($n = 7$). From experiments with depolarized (*D*) tissues we obtained $A_D \equiv (\delta-1)zF/RT = -0.0365 \pm 0.0022$ ($n = 4$). Assuming that the position of the binding site within the channel is not displaced by depolarization, the ratio of A_{ND} and A_D provides an estimate for $f_a{:}f_a = 0.74 \pm 0.06$. From microelectrode experiments (muc.: KCl-Ringer's; ser.: NaCl-Ringer's) we found $f_a = 0.68 \pm 0.13$ ($n = 7$) (unpublished experiments) which is in agreement with the above obtained result. In our calculations of δ we assumed $f_a = 1$. Therefore the values of δ obtained in non-depolarized tissues are an overestimation of the real value.

The present results obtained with non-depolarized skins show that Cs^+ binds to a site which is located at a relative distance of 0.32 from the cytosolic side of the channel whereas our previous study [5] under the same experimental conditions demonstrated that the binding site for Ba^{2+} is at a relative distance of 0.72. This suggests that Cs^+ has to enter deeper into the channel than Ba^{2+} to reach its binding site. Zeiske and Van Driessche [27] found in experiments with Tl^+, which can also pass through the K^+ channel, that I_{sc} shows an anomalous mole fraction behavior when mucosal K^+ is successively replaced by Tl^+. From this study it was concluded that the channel has at least two different binding sites. The present results confirm this conclusion from the finding of different δ's for Ba^{2+} and Cs^+.

The Cs^+ concentration required to block 50% of the K^+ current in non-depolarized tissues at $V_t = 0$ mV is 16 mmol/l, which is much larger than the Ba^{2+} concentration required to inhibit the same amount of current: 135 μmol/l. This indicates that the chemical affinity of Cs^+ for the binding site is low compared with Ba^{2+} so that Cs^+ is an effective K^+ channel blocker only at high mucosal potentials. Table 1 shows that K^o_{Cs} varied considerably among the different experiments. Moreover, we found the

largest K^o_{Cs} values in experiments with low transepithelial resistance and high control I_{sc} values. It is reasonable to assume that f_a is small in skins in which the apical membrane is highly permeable for K^+, whereas f_a values will approach 1 in skins with a small apical K^+ permeability. Indeed Eq. (6) shows that K^o_{Cs} depends on f_a so that the scatter of the values of K^o_{Cs} could be due to the variation in fractional resistance. Moreover, Eq. (6) demonstrates that K^o_{Cs} depends also on E_a and E_b, which vary strongly with the transport capacity of the epithelium. This will cause an additional scatter of the K^o_{Cs} values. This is illustrated by calculations of the exponential factor in Eq. (6) (K^o_{Cs}/B) with values for E_a, E_b and f_a obtained from microelectrode experiments which were performed in a separate experimental project (unpublished observations): In 6 experiments K^o_{Cs}/B varied from 0.085 to 0.535. Using the same microelectrode data we checked the effect of f_a by calculating the ratio of $K^o_{Cs}(f_a = 1)$ obtained with $f_a = 1$ and $K^o_{Cs}(f_a < 1)$ calculated with f_a from the microelectrode experiments. Data from 6 microelectrode experiments showed that all individual values of $K^o_{Cs}(f_a = 1)/B$ were smaller than $K^o_{Cs}(f_a < 1)/B$. The mean value of the ratio was 0.72. So in all these experiments, an increase in f_a caused a decrease of K^o_{Cs}. Furthermore, our calculations showed that smaller K^o_{Cs}/B values were found with larger f_a values. On the other hand, our results obtained with K^+-depolarized skins showed that the affinity of Cs^+, studied at positive transepithelial voltages, did not vary among different frogs like in the non-depolarized tissues. Indeed, the mean value of K_{Cs} obtained at $V_t = 50$ mV was 20 mmol/l with a SD of 2 mmol/l ($n = 4$) which is much smaller than the SD for K_{Cs} in non-depolarized skins (see Table 1).

The fit of the data with Eq. (1) provides an estimation of the Hill-coefficient n (Table 1). A value $n > 1$ as found in our experiments gives rise to the large steepness of the dose-response relationship. $n > 1$ suggests that more than one Cs^+ ion is required to block the K^+ channel. Table 1 also demonstrates that n varies largely among the different experiments with non-depolarized tissues. On the other hand, such a large scatter is not found in experiments with depolarized epithelia ($n = 1.7 \pm 0.1$). This observation might suggest that the fractional resistance also affects the calculation of n.

To compare our findings with other K^+ channels we define δ^* as the apparent electrical distance to the blocking site from the point of entry of the blocker into the channel. In squid giant axon external Cs^+ can block inward, but not outward K^+ currents. Adelman and French [1] found that δ^* varied with the outer Cs^+ concentration from 0.6 (5 mmol/l) to 1.3 (200 mmol/l). δ^* values larger than 1 are explained with a multi-site channel model in which δ^* is the sum of the electrical distances of the different sites [3]. Internal Cs^+ can block outward moving K^+ currents in squid giant axon. At sufficiently positive internal potentials, internal Cs^+ is able to pass the channel. δ^* is also concentration dependent [7] and increases from 0.45 to 0.91 when the inner Cs^+ concentration is raised from 5 to 100 mmol/l. In the starfish egg cell membrane [10] as well as in the tunicate egg cell membrane [13] external Cs^+ blocks the inward K^+ current. Cs^+ cannot pass itself through the channel. δ^* was found to be larger than one in both cases: $\delta^* \approx 1.45$ in the starfish egg and $\delta^* = 1.46$ in the tunicate egg. Internal as well as external Cs^+ can block K^+ conductance of the Ca^{2+}-activated K^+ channel from smooth muscle [3]. Internal Cs^+ was found to bind to a site located at an electrical distance of $\delta^* = 0.54$. Internal Cs^+ behaves as an impermeable blocker. On the other hand, the voltage dependence of the channel blockade by external Cs^+ is a function of the Cs^+ concentration as was found in the squid axon. δ^* was 1.4 at 10 mmol/l Cs^+. External Cs^+ can pass the channel at large internal negative voltages. The characteristics of this block were explained by a two-site "knock-on" model. The K^+ inward rectifier in frog skeletal muscle can also be blocked by Cs^+ in a voltage dependent way [15, 16]. The kinetics of the block were explained with a two-site multi-ion model with the sites located at $\delta^* = 0.25$ and $\delta^* = 0.5$. In our experiments we found for the block of the K^+ channels by external Cs^+: $\delta^* = 1 - \delta = 0.91$ (depolarized tissues). Even at high external positive values Cs^+ cannot pass the channel. Our experiments with K^+-depolarized skins showed that Cs^+ does not block the transepithelial currents recorded at negative voltages and that therefore in frog skin external Cs^+ can only block inward K^+ currents. These results correspond with the results obtained for the block of inward K^+ currents by external Cs^+ in squid axon, starfish egg and tunicate egg. However, we found no dependence of δ^* on the external Cs^+ concentration. Also δ^* was smaller than 1. However, while in all the above mentioned experiments the data were fitted with a model assuming a 1:1 block (the Hill coefficient n was taken equal to 1), we had to fit our results with a mean value of $n = 1.7$ which indicates that we deal with a model which is more complicated than a simple one blocker-one site model.

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