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Effect of Amiloride, Ouabain and Ba^{++} on the Nonsteady-State Na–K Pump Flux and Short-Circuit Current in Isolated Frog Skin Epithelia

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Summary. Effect of amiloride, ouabain, and Ba^{++} on the nonsteady-state Na–K pump flux and short-circuit current in isolated frog skin epithelia.

The active Na^+ transport across isolated frog skin occurs in two steps: passive diffusion across the apical membrane of the cells followed by an active extrusion from the cells via the Na^+-K^+ pump at the basolateral membrane. In isolated epithelia with a very small Na^+ efflux, the appearing Na^+-flux in the basolateral solution is equal to the rate of the pump, whereas the short-circuit current (SCC) is equal to the active transepithelial Na^+ transport. It was found that blocking the passive diffusion of Na^+ across the apical membrane (addition of amiloride) resulted in an instantaneous inhibition of the SCC (the transepithelial Na^+ transport, whereas the appearing flux (the rate of the Na^+-K^+ pump) decreased with a halftime of 1.9 min. Addition of the Na^+-K^+ pump inhibitor ouabain (0.1 mM) resulted in a faster and bigger inhibition of the appearing flux than of the SCC. Thus, by simultaneous measurement of the SCC and the appearing Na^+ flux one can elucidate whether an inhibitor exerts its effect by inhibiting the pump or by decreasing the passive permeability. Addition of the K^+ channel inhibitor Ba^{++}, in a concentration which gave maximum inhibition of the SCC, had no effect on the appearing flux (the rate of the Na–K pump) in the first 2 min, although the inhibition of the SCC was already at its maximum.

It is argued that in the short period, where the Ba^{++}-induced inhibition of SCC is at its maximum and the appearing flux in unchanged, the decrease in the SCC (ΔSCC) is equal to the net K^+ flux via the Na^+-K^+ pump, and the coupling ratio (β) of the Na^+-K^+ pump can be calculated from the following equation

$$\beta = SCC_{t=0}/\Delta SCC$$

where $SCC_{t=0}$ is the steady-state SCC before the addition of Ba^{++}.

Key words frog skin · sodium flux · ouabain · amiloride · barium

Introduction

Koefoed-Johnsen and Ussing (1958) found that the apical membrane of the isolated frog skin was selectively permeable to Na^+ and almost impermeable to K^+, whereas the basolateral membrane was permeable to K^+, but almost impermeable to free Na^+. Qualitatively the same permeability properties has been found in other Na^+ transporting epithelia such as toad bladder (Macknight and Leaf, 1978), rabbit urinary bladder (Lewis, Wills & Eaton, 1978), and rabbit ileum (Schultz, 1978), etc. On the basis of these and other observations Koefoed-Johnsen and Ussing proposed the two-membrane hypothesis. According to this hypothesis, the active Na^+ transport across epithelia is thought to occur in two steps: passive diffusion of Na^+ across the apical membrane followed by an active extrusion of Na^+ across the basolateral membrane. Koefoed-Johnsen and Ussing (1958) suggested that the active mechanism was a Na–K exchange pump.

According to this hypothesis, the SCC across Na^+-transporting epithelia is carried by Na^+ across the apical membrane and by K^+ across the basolateral membrane. If the active Na^+ transport is carried out by a Na–K pump with a coupling ratio of 1.5 (3 Na/2 K) as some experiments indicate (Nielsen, 1979*a*, *b*), then two-thirds of the SCC across the basolateral membrane is carried by K^+ and one-third by Na^+ (Fig. 1). The model predicts that a prompt decrease in the Na^+ conductance of the apical membrane should give a prompt decrease in the SCC, whereas the decrease in the Na^+ flux from the cells to the basolateral bathing solution (the appearing Na^+ flux) should be slower and smaller, because it takes some time for the Na^+-K^+ pump to adjust the intracellular Na^+ concentration to the new transport situation. On the other hand, inhibition of the Na^+-K^+ pump should result in a prompt decrease in the appearing Na^+ flux via the Na^+-K^+ pump, whereas the decrease in the SCC should be slower and smaller because of the asymmetric dissipation of the ion gradients (the cells would gain Na^+ from the solution bathing the apical side of the skin and lose K^+ to the solution

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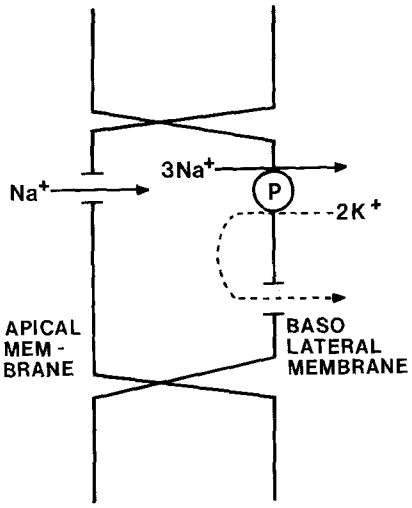

Fig. 1. The two-membrane hypothesis, drawn for a Na^+-K^+ pump having a coupling ratio of 1.5 (3Na/2K). *P*: Na^+-K^+ pump

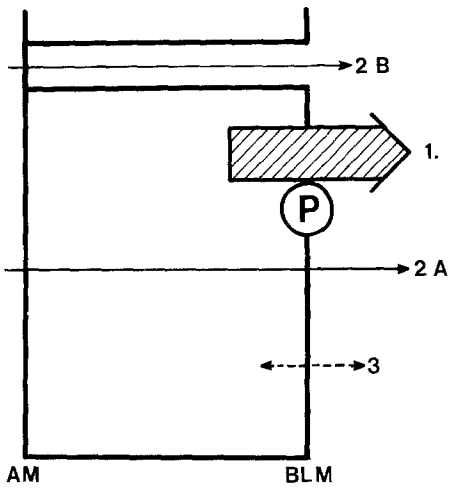

Fig. 2. Definition of fluxes. *1*, active appearing flux; *2A*, transcellular passive flux; *2B*, extracellular transepithelial passive flux; *3*, the passive flux across the basolateral membrane not included in *2*. *P*, Na−K pump. *AM*, apical membrane; *BLM*, basolateral membrane

bathing the basolateral side) this dissipation of the ion gradients would generate a current, but no appearing Na^+ flux.

Addition of Ba^{++} to the solution bathing the basolateral side of isolated epithelia resulted in a fast initial inhibition of the SCC followed by a much slower recovery. (Nielsen 1979*b*). It was suggested that the Ba^{++}-induced initial inhibition of the SCC was caused by a blockade of the K channels by Ba^{++}. If it is so, one would expect that addition of Ba^{++} to the solution bathing the epithelium should result in a prompt decrease in the SCC, whereas it initially should have a very small effect on the appearing Na^+ flux.

From the data presented here it appears that by simultaneous measurements of the SCC and the Na flux (under nonsteady-state conditions) it is possible to disclose whether the inhibition is caused by a blockade of the Na−K pump or whether it is due to a decrease in passive Na^+ or K^+ permeability. Furthermore, the data are consistent with the notion that Ba^{++} blocks the K^+ channels, and they support the idea that the coupling ratio of the Na^+-K^+ pump is 1.5.

Materials and Methods

The experiments were performed on isolated epithelia from male and female frogs *(Rana temporaria)*, except for the series of experiments shown in Table 4 which were performed on the whole isolated skin. The epithelia were dissected from collagenase-treated skins (Johnsen & Nielsen, 1978) and mounted in perspex chambers (area, 1.5 cm^2; volume, 2.5 ml) and bathed in stirred Ringer's solution ((in mM) Na^+, 115; K^+, 2.5; Ca^{++}, 1; Mg^{++}, 1; Cl^-, 117; HCO_3^-, 2.5; PO_4^{3-}, 1; glucose, 5; at pH 7.8).

Terms

Active Appearing Flux. Under steady-state conditions the SCC is equal to the net Na^+ flux across the isolated frog skin:

$$SCC = \text{net } Na^+_{flux} = Na^+_{influx} - Na^+_{efflux} \quad (1)$$

(Ussing & Zerahn, 1951), and the appearing flux is equal to the transepithelial Na^+ influx. Under nonsteady-state conditions one has to replace the term Na^+ influx by two other terms, namely, the disappearing flux (the flux from the solution bathing the apical side across the apical membrane into the epithelium) and the appearing flux (the flux from the epithelium into the solution bathing the basolateral side of the epithelium). The appearing Na^+ flux (Fig. 2) can be divided into three different parts:

1) the active appearing flux, which is the flux via the Na^+-K^+ pump;
2) the transepithelial, both cellular (*A*) and extracellualar (*B*), passive flux, which under steady-state conditions is equal to the Na^+ efflux; and
3) the fraction of the passive Na^+ flux across the basolateral membrane, which is not included in the efflux; the size of this Na^+ flux is unknown, but it is small compared with the other fluxes (Rick, Dörge, Arnim & Thurau, 1978, Nielsen 1982 (previous paper)).

Only isolated epithelia, in which the passive Na^+ flux (the efflux) was less than 10 % of the active Na^+ transport, were used in the experiments. Ba^{++}, ouabain, and amiloride had only relatively small effects on the Na efflux; therefore the active appearing flux can be calculated by subtracting the efflux from the appearing flux, thus:

$$\text{active appearing flux} = \text{appearing flux} - \text{efflux} \quad (2)$$

Results

As mentioned previously, a blockade of the Na^+ or K^+ channels should result in a faster and bigger reduction in the SCC than in the appearing Na^+

Table 1. Effect of amiloride (10 μM) on the appearing Na flux and SCC[a]

	Before amiloride (nmol/cm²/min)	After amiloride (nmol/cm²/min)	Δ (nmol/cm²/min)	Δ (%)
Appearing Na flux	26.3 ± 2.3	17.8 ± 1.5	8.5 ± 1.6	27.4 ± 5.3
SCC	25.7 ± 2.2	1.8 ± 0.7	23.9 ± 2.4	92.7 ± 3.2

[a] Column 1: the steady-state appearing Na^+ flux and SCC before the addition of amiloride. Column 2: the mean appearing Na^+ flux in the first 2 min after amiloride. Columns 3 and 4 give the observed changes in nmol/cm²/min and in percent, respectively. The data are the means ± SE of 7 experiments.

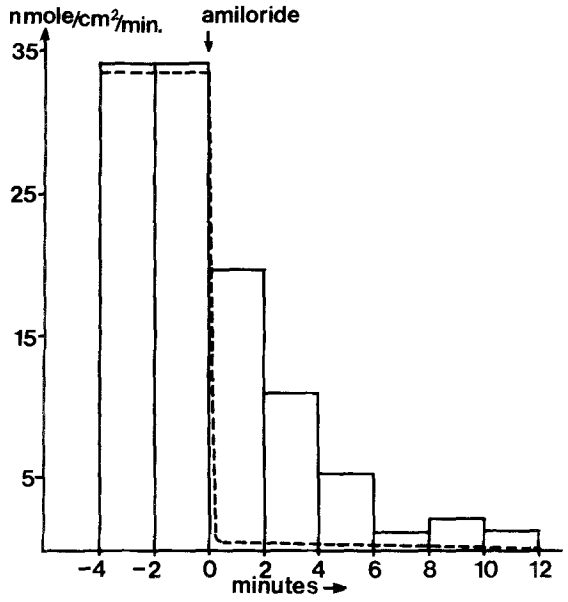

Fig. 3. Effect of amiloride (10 μM) on the SCC and the appearing Na flux. At time 0 amiloride was added to the solution bathing the apical side of the isolated epithelium. The broken line is the SCC, and the bars are the appearing Na flux

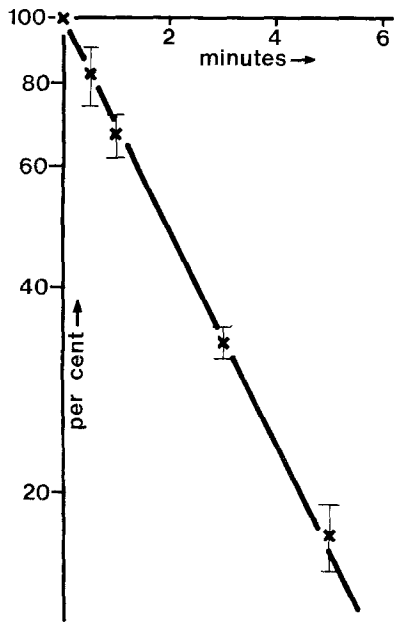

Fig. 4. Effect of amiloride (40 μM) on the active appearing Na^+ flux. *Abscissa:* time in minutes after addition of amiloride. *Ordinate:* active appearing flux expressed in percent of the steady-state active appearing flux before the addition of amiloride (100 % = 26.2 nmol/cm²/min). The points are average of 6 experiments. The bars denote ± SE. The active appearing fluxes were calculated for each sampling period and plotted against time at the midpoints of each sampling period

flux, whereas a blockade of the $Na^+ - K^+$ pump should have the opposite effect both on SCC and appearing Na^+ flux. In order to test this hypothesis, experiments were carried out on isolated epithelia, which had been preincubated for at least 15 min with the isotope (in an Ussing-type chamber) before measurement of the appearing flux was started. All the experiments were carried out under short circuited conditions.

Amiloride

Addition of amiloride (an inhibitor of the Na^+ channels) resulted in a prompt decrease in the SCC, whereas the decrease in the appearing Na^+ flux was much slower (Fig. 3). The mean decrease in the SCC in the first 2 min after addition of amiloride was 23.9 ± 2.4 nmol/cm²/min, whereas the decrease in the appearing Na^+ flux was 8.5 ± 2.4 nmol/cm²/min (Table 1). The decrease in the active appearing flux after addition of amiloride could be described as a first-order process with a halftime of 1.9 min (Fig. 4). The active appearing flux was calculated from Eq. (2). The efflux was calculated from the steady-state Na^+ influx and the SCC (Eq. (1)), since amiloride has no significant effect on the passive Na^+ flux (Baba et al., 1968; Nielsen & Tomlinson, 1970) it is justified to calculate the passive Na^+ flux in this way. Furthermore, the correction was small (the passive flux in these series of experiments was only 1.8 % of the influx), so it does not affect the conclusion drawn. Thus, blockade of the Na^+ channels resulted initially (as the model (Fig. 1) predicted) in a greater

and faster decrease in the SCC than in the active appearing Na^+ flux.

According to the model (Fig. 1) one might expect that it could be possible to estimate the Na^+ transport pool by measuring the amount of tracer Na^+ leaving the epithelium after complete blockade of the Na^+ channels with amiloride. By graphical integration of the active appearing Na^+ flux in the first 6 min after addition of amiloride (which according to the halftime found 1.9 min should represent about 90 % of the Na^+ in the pool), a Na^+ pool of 58.0 ± 6.9 neq/cm^2 was found. When the Na^+ transport pool in the same experiments was estimated from the SCC measured just before addition of amiloride (by using Fig. 4 of the previous paper, which shows the correlation between the SCC and the Na^+ transport pool) the Na^+ transport pool was found to be 31.4 ± 2.7 neq/cm^2.

Thus there is an apparent discrepancy between the size of the pool under these two different experimental conditions.

The Na^+ transport pool was in the previous paper estimated by measuring the build-up of the tracer influx on epithelia kept under steady-state conditions. Parsons and Hoshiko (1971) and Candia and Reinach (1977) have shown that there is a slow-exchanging Na^+ pool in isolated epithelia. The Na^+ in this pool will be partially labeled during the preincubation used in the present experiments. The Na^+ in the slow exchanging Na^+ pool cannot at all, or only a little, contribute to the transepithelial Na^+ transport because the transepithelial Na^+ transport is practically in a steady state after 5-min incubation (Nielsen, 1982). Blockade of the transepithelial Na^+ transport results in a shrinkage of the cells (Voûte & Ussing, 1970*a*), a shrinkage of the cisternae of endoplasmic recticulum (Voûte, Møllgård & Ussing, 1975) and in a collapse of the intercellular spaces in the isolated epithelium (Carasso, Favard, Jard & Rajerison, 1971, Voûte & Ussing, 1970*b*). Therefore one may suggest that the reason for the apparent discrepancy between the Na^+ pools might be due to the fact that the addition of amiloride results in a release of Na^+ from the slower exchanging cellular and extracellular compartments. If this is the case, one cannot get a correct estimate of the transport pool by measuring the appearing flux after blockade with amiloride.

Effect of Ouabain

Addition of the Na–K pump inhibitor ouabain (0.1 mM) to the isolated epithelia resulted in a fast initial inhibition of the SCC and the appearing Na^+ flux and caused a 30 % increase in the Na^+ efflux

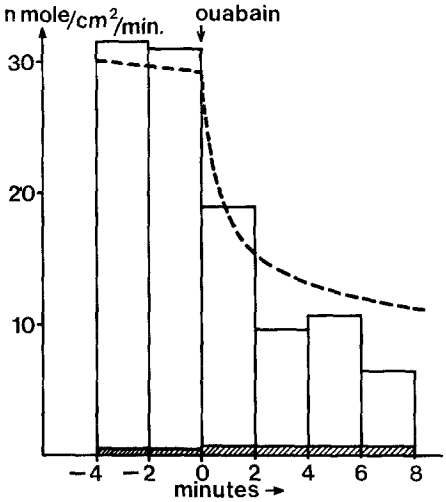

Fig. 5. Effect of ouabain (0.1 mM) on the SCC, the appearing Na^+ flux and the Na^+ efflux. At time 0 ouabain was added to the solution bathing the basolateral side of the epithelium. The broken line is the SCC, the bars are the appearing Na^+ fluxes, and the hatched bars are the Na effluxes

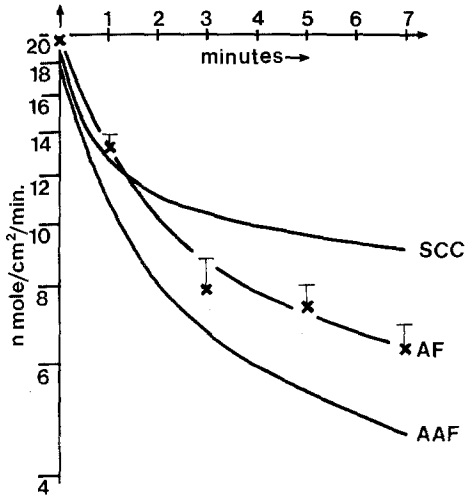

Fig. 6. Effect of ouabain (0.1 mM) on the short-circuit current (curve marked SCC), the appearing flux (curve marked *AF*), and the active appearing flux (curve marked *AAF*). The appearing Na^+ fluxes were calculated for each sampling period and plotted against time at the midpoints of each sampling period. ($x-x$): appearing Na flux ±SE ($n=9$). The active appearing flux was calculated by subtracting the efflux from the appearing flux

(Fig. 5). The inhibition of the SCC could be described by two first-order processes (Fig. 6): 38.7 ±3.1 % of the inhibition had a halftime of 0.487 ±0.038 min, and 61.3 ±3.1 of the inhibition had a half time of 22.0 ±4.5 min ($n=9$); thus the changes in SCC could be described by the following empirical function:

$$SCC = 18.6\,[0.387 \cdot \exp(-1.42t) + 0.613 \cdot \exp(-0.0315t)]$$

Table 2. Effect of ouabain (0.1 mM) on the appearing Na^+ flux and SCC[a]

	Before ouabain (nmol/cm²min)	After ouabain (nmol/cm²/min)	Δ (nmol/cm²/min)	Δ (%)
Appearing Na^+ flux	19.95±2.29	12.92±1.38	7.07±1.22[b]	34.2±3.4
SCC	18.56±2.37	13.23±1.62	5.17±0.82[b]	27.8±2.2

[a] Column 1: the steady-state appearing Na^+ flux and SCC before the addition of ouabain. Column 2: the mean appearing flux and SCC in the first 2 min after ouabain. Columns 3 and 4 give the observed changes in nmol/cm²/min and in percent. The data are the means ±SE of 9 experiments.
[b] $0.01 > P > 0.005$ t test for nonindependent samples of the difference $(7.07-5.17)=1.90 \pm 0.53$ nmol/cm²/min.

Table 3. Effect of Ba^{++} (4.53 mM) on the appearing Na^+ flux and SCC.[a]

	Before Ba^{++} (nmol/cm²/min)	After Ba^{++} (nmol/cm²/min)	Δ (nmol/cm²/min)	Δ (%)
Appearing Na flux	17.2±2.8	16.0±2.2	1.2±1.0	4.2±4.9
SCC	16.5±2.9	7.5±2.9	7.5±1.1	52.9±2.0

[a] Column 1: the steady-state appearing Na^+ flux and SCC before the addition of Ba^{++}. Column 2: the mean appearing flux and SCC in the first 2 min after ouabain. Columns 3 and 4 give the observed changes in nmol/cm²/min and in percent. The data are the means ±SE of 7 experiments.

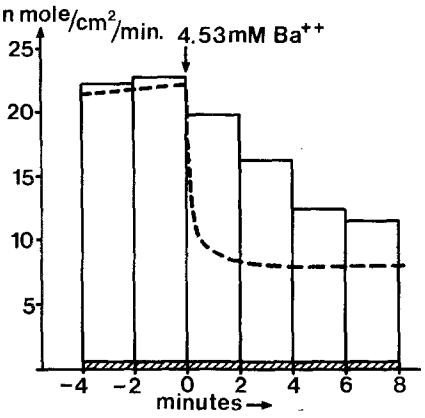

Fig. 7. Effect of Ba^{++} (4.56 mM) on the SCC, the appearing Na flux, and the Na^+ efflux. At time 0 Ba^{++} was added to the solution bathing the basolateral side of the epithelium. The broken line is the SCC, the bars are the appearing Na^+ fluxes, and the hatched bars are the Na^+ effluxes

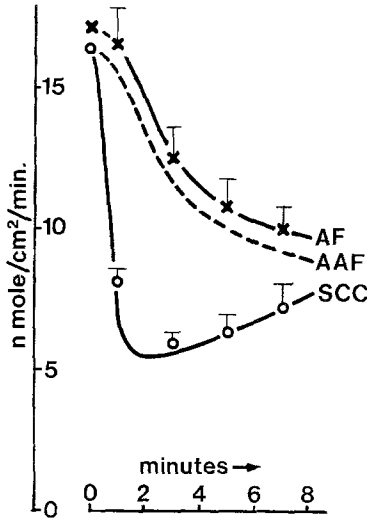

Fig. 8. Effect of Ba^{++} (4.53 mM) on the SCC (curve labeled *SCC*), appearing Na^+ flux (curve labeled *AF*), and active appearing flux (curve labeled *AAF*). At time 0 Ba^{++} was added to the solution bathing the basolateral side of the epithelium. The values given at time 0 are the steady-state values found before the addition of Ba^{++}. The mean appearing flux and SCC were calculated for each period and plotted against time at midpoints of each sampling period. Values are means ±SE ($n=6$)

where SCC is given in nmol/cm²/min and t is time in min (Fig. 6). The inhibition of the appearing Na^+ flux (Fig. 6, *AF*) and the active appearing Na^+ flux (Fig. 6, *AAF*) could also be described by two first-order processes, described by the following empirical function

$$AF = 20.0\,[0.52 \cdot \exp(-0.894t) + 0.48 \cdot \exp(-0.0578t)]$$

$$AAF = 18.1\,[0.58 \cdot \exp(-0.956t) + 0.48 \cdot \exp(-0.0693t)]$$

where *AF* and *AAF* are given in nmol/cm²/min and t is time in min. The mean decrease in the appearing Na^+ flux in the first 2 min after ouabain was 7.07 nmol/cm²/min, whereas the decrease in the SCC was 5.17 nmol/cm²/min (Table 2), the difference between the appearing Na^+ flux and the SCC 1.90 ± 0.53 nmol/cm²/min was significant

Table 4. Comparison of the net Na^+ flux and the short-circuit current across the frog skin before and 1 hr after the addition of Ba^{++} (4.53 mM)[a]

	Influx (nmol/cm²/min)	Efflux (nmol/cm²/min)	Mean short circuit current (nmol/cm²/min)	Mean net sodium flux (nmol/cm²/min)
Control	11.18 ± 1.03	0.25 ± 0.09	10.72 ± 0.69	10.92 ± 0.68
1 hr after Ba^{++}	9.80 ± 0.89	0.47 ± 0.14	9.67 ± 1.02	9.33 ± 0.92

[a] The flux period was 1 hr. The control values are the steady-state values found before the addition of Ba^{++}. Measurement of the fluxes in the presence of Ba^{++} was started 1 hr after the addition of Ba^{++}. The influx was measured with $^{24}Na^+$ and the efflux simultaneously with $^{22}Na^+$. Values are the mean ± SE of 6 experiments.

($0.01 > P > 0.005$). Thus, inhibition of the $Na^+ - K^+$ pump resulted, as the model predicted, in a greater decrease in the active appearing Na^+ flux than in the SCC. During incubation with ouabain the relative discrepancy between the SCC and the appearing Na^+ flux increased (Figs. 5 and 6); this is probably caused by the asymmetric dissipation of the ion gradients (the cells have a high K^+ and a low Na^+ concentration, whereas the bathing solution has a high Na^+ and a low K^+ concentration).

Effect of Ba^{++}

Addition of Ba^{++} to the isolated epithelia resulted initially in a faster and bigger decrease in the SCC than in the appearing Na^+ flux, but had no effect on the Na^+ efflux in this period (Fig. 7). The mean decrease in the SCC the first 2 min after addition of Ba^{++} was 52.9 ± 2.0 %, whereas the decrease in the appearing Na^+ flux was only 4.2 ± 4.9 % (Table 3). This indicates that addition of Ba^{++} causes a decrease in the passive Na^+ or K^+ permeability of the epithelium. From Fig. 8, which shows the time course of the SCC and the active appearing flux after the addition of Ba^{++}, it is seen that the active appearing Na^+ flux continues to decrease for the first min after Ba^{++}, whereas the SCC starts to recover.

Table 4 shows a number of double labeling experiments, where the steady-state Na^+ influx, Na^+ efflux, and SCC were measured simultaneously before and 1 hr after the addition of Ba^{++}. Both in the control period and in the presence of Ba^{++} there was a good agreement between the net Na^+ flux and the SCC.

Thus continuous incubation with Ba^{++} resulted in a nearly complete recovery in the SCC and the appearing Na^+ flux, so the discrepancy between the SCC and the appearing flux vanishes.

Discussion

The model presented above (Fig. 1) predicts that a sudden decrease in Na^+ permeability of the apical membrane (addition of amiloride) or a sudden decrease in the passive K^+ permeability of the basolateral membrane (addition of Ba^{++}) should result in a larger and faster reduction in the SCC than in the active appearing Na^+ flux. That this is so is seen from Tables 1 and 3 and from Figs. 2, 3, 7 and 8. The model also predicts that inhibition of the $Na^+ - K^+$ pump (addition of ouabain) should result in a bigger inhibition of the active appearing Na^+ flux than of the SCC. This is seen to be the case from Table 2 and Figs. 5 and 6. Thus, by simultaneous measurement of the SCC and the active appearing Na^+ flux it is possible to disclose whether an inhibitor has an effect on the $Na^+ - K^+$ pump or on the passive Na^+ or K^+ permeability.

After blockade of the Na^+ channels with amiloride, the decrease in the active appearing Na^+ flux could be described by a first-order process with a halftime of 1.9 min (Fig. 4). This is in agreement with what was found in the previous paper, where it was shown that there was a nearly linear relationship between the active Na^+ transport and the cellular Na^+ concentration, when the cellular Na^+ concentration was below 10 mM.

The decrease in the active appearing Na^+ flux (the pump flux) after addition of ouabain could formally be described by two first-order processes. This does not necessarily mean that the cells contain different types of $Na^+ - K^+$ pumps, since blockade of some pump sites results in an increase in the rate of the noninhibited pumps, because of the increase in cellular Na^+ concentration. Furthermore, the cells lose K^+ during ouabain poisoning, which would increase the K^+ concentration in the extracellular space. An increase in the extracellular K^+ concentration reduces the binding of ouabain to the $Na^+ - K^+$ pump (Cala, Cogswell & Mandel, 1978). Since these secondary changes would influence the rate of ouabain binding to the $Na^+ - K^+$ pump and the rate of the pump, complex changes in the active appearing flux during ouabain poisoning has to be expected.

Ba^{++} reduces the K^+ conductance in frog heart (Hermsmeyer & Sperelakis, 1970), in frog muscle

(Henderson, 1974), and the passive K^+ permeability of the basolateral membrane of the isolated frog skin (Nagel, 1979; Nielsen 1979*b*). These observations are in agreement with the data presented here (Table 3, Figs. 7 and 8). Under steady-state conditions the net K^+ flux via the Na^+-K^+ pump is (according to the model (Fig. 1)) equal to the K^+ current via the K^+ channels. The model predicts that a fast complete inhibition of K^+ channels initially should result in a reduction in the SCC which is equal to the net K^+ flux via the K^+ channels, whereas the rate of the Na^+-K^+ pump (the active appearing flux) initially should remain unchanged. Thus, in this short period, where the K^+ channels are completely closed and the appearing flux is unchanged, the correct coupling ratio (β) of the Na^+-K^+ pump can be found from the initial inhibition in the SCC

$$\beta = \frac{SCC_{t=0}}{\Delta SCC} \tag{3}$$

where $SCC_{t=0}$ is the steady-state short-circuit current just before the addition of Ba^{++} and ΔSCC is the initial decrease in the SCC caused by the blockade of the K^+ channels.

The addition of Ba^{++} to the solution bathing the basolateral side of the isolated epithelium resulted in a fast inhibition of SCC (Fig. 7) followed by a much slower recovery. The maximal initial inhibition of the SCC, which was obtained 30–120 sec after addition of Ba^{++} amounted to 2/3 (65.0 $\pm$1.5 %) of the SCC (Nielsen, 1979*a*). On basis of this and other observations (Nielsen, 1979*b*), it was suggested that the coupling ratio of the Na^+-K^+ pump was 1.5 (3 Na/2 K). From Fig. 8 it is seen that the active appearing flux was nearly unchanged during the first 2 min of incubation with Ba^{++}, although the SCC had reached maximal inhibition; consequently (if Ba^{++} completely blocks the K^+ channels), it should be correct to calculate the coupling ratio from the observed decrease in the SCC (*see* Eq. (3)).

The model predicts that a blockade of the K^+ channels should depolarize the cells as observed by Nagel (1979). A depolarization of the cells should, according to the Goldman-Hodgkin-Katz equation (Hodgkin & Katz 1949), reduce the net Na^+ flux from the apical solution into the cells; consequently, the cellular Na^+ concentration should decrease. The active appearing Na^+ flux decreases in the first 8 min after addition of Ba^{++} (Fig. 8); this indicates that the cellular Na concentration decreases, because in the previous paper it was found that there was a nearly linear relationship between the intracellular Na^+ pool and the active appearing flux, when the Na-flux was smaller than 40 nmol/cm^2/min.

The SCC starts to recover 30 sec to 2 min after the addition of Ba^{++}, whereas the active appearing Na^+ flux continues to decrease the first 8 min (Fig. 8). A recovery in the SCC, without a concomitant increase in the active appearing Na^+ flux, is most likely caused by the formation of a Ba^{++}-insensitive K^+ pathway in the basolateral membrane. Such a recovery in the K^+ permeability results in an increase in the SCC and a repolarization of the cells. Consequently, the net Na^+ flux across the apical membrane into the cells increases, too, which results in an increase in the cellular Na^+ concentration and the appearing Na^+ flux. The data in Table 4 shows that after 1 hr of incubation with Ba^{++} both the SCC and the appearing Na^+ flux have nearly recovered and the discrepancy between the SCC and the active appearing Na^+ flux has vanished.

In order to maintain electroneutrality in the cells during the blockade of the K^+ channels, the cells have to either (i) reduce the Na^+ flux across the apical membrane by the amount of K^+ which is pumped into cells, or (ii) the cells have to expel cations or gain an equivalent amount of anions (Nielsen, 1981).

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