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## Inhibition of $K^+$ conductance by $Ba^{2+}$ at the apical membrane of rabbit gallbladder epithelium

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**Fisiologia.** — *Inhibition of* $K^+$ *conductance by* $Ba^{2+}$ *at the apical membrane of rabbit gallbladder epithelium* (*). Nota di GIULIANO MEYER, CARLO ROSSETTI e DARIO CREMASCHI, presentata (**) dal Corrisp. V. CAPRARO.

RIASSUNTO. — Le vie conduttive per il $K^+$ presenti nelle membrane apicali dell'epitelio di cistifellea di coniglio vengono inibite dal $Ba^{2+}$ in concentrazioni mM. Esse sembrano perciò diverse dalle vie basolaterali degli epiteli a bassa ed alta resistenza e dai canali del $K^+$ delle membrane eccitabili, che sono molto più sensibili al $Ba^{2+}$.

## INTRODUCTION

The apical and basolateral membranes of gallbladder epithelium in rabbit as well as in Necturus exhibit a high $K^+$ conductance [7 , 12, 13, 14]. Whereas a large basolateral $K^+$ conductance is general in the epithelial cells, the apical highly conductive pathways for $K^+$ are limited to some leaky epithelia [5, 6, 8] and seem to have different properties with respect to the former. Basolateral $K^+$ channels seem to be quite sensitive to $Ba^{2+}$ [10, 13] and under this aspect they are similar to those of the plasma membranes of excitable tissues [3]; conversely, the apical pathways are inhibited only by larger concentrations of the divalent cation [4, 9, 13].

The aim of this paper is to examine the sensitivity to $Ba^{2+}$ of the apical $K^+$ channels in the epithelium of rabbit gallbladder.

## METHODS

Gallbladders from New-Zealand rabbits were excised from the animal (killed by a blow on the head), washed free from bile with Krebs-Henseleit solution and opened lengthwise. The technique used to mount gallbladders in lucite chambers (window: 0.196 $cm^2$) for the subsequent recording of apical membrane potential difference and apical/basolateral resistance ratio was as described previously [2, 7].

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The control saline used had the following composition (mM): $Na^+$, 134; $K^+$, 5.9; $Ca^{2+}$, 2.5; $Mg^{2+}$, 1.2; $Cl^-$, 149.8; Tris OH/Tris Cl buffer, 9, pH 7.4. When $K^+$ was removed it was replaced by $Na^+$; when $Ba^{2+}$ was added, it substituted $Na^+$.

Statistical analysis was performed by *t*-test on paired or non-paired data as indicated. In the Tables asterisks label experiments statistically different from controls (*: $p < 0.05$; **: $p < 0.01$). Results are reported as means $\pm$ standard errors (number of experiments in parenthesis).

## Results

The effect of 5 or 10 mM $Ba^{2+}$ on the apical membrane potential difference ($V_m$) and on the apical/basolateral resistance ratio ($R_m/R_s$) are reported in Table I. Solutions were changed during single impalements. At both concentrations the cation depolarizes $V_m$ by about 9 mV in a highly significant way ($p < 0.01$); on the contrary, no significant difference is observed between the two depolarizations. Their value was taken in steady-state condition about 2-3 min after the saline change; they are both completely reversible as it is shown in Table I.

### Table I.

*Effect of* 5 *or* 10 *m*M $Ba^{2+}$ *on the apical membrane potential difference* $V_m$ (*the variation is indicated as* $\Delta$) *and on the apical/basolateral resistance ratio* $R_m/R_s$.

Statistical analysis on paired data for $V_m$ and $\Delta$.

| | $V_m$ (*m*V) | $\Delta$ (*m*V) | $R_m/R_s$ |
|---|---|---|---|
| Control | $-$ 64.0 $\pm$ 0.5 (7) | | 1.26 $\pm$ 0.15 (5) |
| 5 mM $Ba^{2+}$ | | + 8.6 $\pm$ 1.8 (7)** | 2.90 $\pm$ 0.14 (4) ** |
| Control | | + 0.6 $\pm$ 0.6 (7) | |
| Control | $-$ 61.2 $\pm$ 0.8 (6) | | 1.10 $\pm$ 0.15 (5) |
| 10 mM $Ba^{2+}$ | | + 9.0 $\pm$ 0.3 (6) ** | 3.80 $\pm$ 0.30 (5) ** |
| Control | | 0.0 $\pm$ 1.0 (6) | |

If the effects on $R_m/R_s$ ratio are examined, one can observe a highly significant increase of the ratio under both conditions (Table I), denoting a large rise in $R_m$. However, in this case 10 mM $Ba^{2+}$ exhibits a stronger action than that observed at the lower concentration used ($p < 0.05$).

In order to shed some light on the contradictory results concerning $V_m$ and $R_m/R_s$, the experiment was repeated by impaling during luminal perfusion with 5 mM $Ba^{2+}$ and by introducing the saline with 10 mM $Ba^{2+}$ during

the impalement. By this more delicate procedure one can observe a small (+ 2.1 mV) but highly significant depolarization parallel to the increase in the resistance ratio (Table II). One can conclude that the effects obtained with 5 mM $Ba^{2+}$ are not maximal.

TABLE II.

*Effect of* 10 mM $Ba^{2+}$ *on the apical membrane potential difference* $V_m$ *(variation indicated as* $\Delta$*) and on the apical/basolateral resistance ratio* $R_m/R_s$ *of cells pretreated with* 5 mM $Ba^{2+}$.

Statistical analysis on paired data for $V_m$ and $\Delta$.

| | $V_m$ (mV) | $\Delta$ (mV) | $R_m$/RS |
|---|---|---|---|
| Contro (5 mM $Ba^{2+}$) . | − 54.7 ± 0.3 (5) | | 2.6 ± 0 (5) |
| 10 mM $Ba^{2+}$ . . . . | | + 2.1 ± 0.4 (5)** | 3.7 ± 0 (5) ** |
| Control (5 mM $4a^{2+}$) . | | + 0.5 ± 0.4 (5) | |

Table III shows that $K^+$ removal from the lumen during an impalement hyperpolarizes the apical membrane p.d. by about 10 mV in highly significant way; however, the presence of 10 mM $Ba^{2+}$ during $K^+$ removal not only abo-

TABLE III.

*Effects on the apical membrane potential difference* ($V_m$) *of* $K^+$ *removal or* $K^+$ *removal and* $Ba^{2+}$ *addition (in the lumen).*

Statistical analysis on paired data.

| | $V_m$ (mV) | $\Delta$ (mV) |
|---|---|---|
| Control . . . . . | − 67.8 ± 1.4 (25) | |
| $K^+$ = 0 mM . . . . . | | − 9.8 ± 0.6 (25) ** |
| Control . . . . . | | − 0.7 ± 0.4 (25) |
| Control . . . . . | − 64.0 ± 1.2 (11) | |
| $K^+$ = 0 mM, $Ba^{2+}$ = 10 mM . . . . . | | + 11.2 ± 1.2 (11) ** |
| Control . . . . . | | + 0.8 ± 0.4 (11) |

lishes this effect, but also causes a highly significant depolarization. The latter is not significantly different from the depolarization raised by 10 mM $Ba^{2+}$ alone so that no algebraic additivity seems to occur between the effects of $Ba^{2+}$ and $K^+$ removal.

## Discussion

The results here reported are evidence that $Ba^{2+}$ blocks $K^+$ channels in the apical membrane. In fact, $K^+$ conductance is dominant in the apical membrane and the nearly 4 times increase in resistance raised by $Ba^{2+}$ can be accounted for only by a strong reduction of the dominant conductance. Moreover, the hyperpolarization caused by $K^+$ removal is abolished by $Ba^{2+}$ and $Ba^{2+}$ depolarizes $V_m$ to the same extent both at normal and at zero $K^+$ concentration in the lumen.

Our results are in agreement with those obtained in other leaky epithelia with high apical conductance for $K^+$ [4, 9, 13] and confirm that these $K^+$ channels are already inhibited by mM $Ba^{2+}$ concentrations.

Since $K^+$ conductance is dominant in the apical membrane of rabbit gallbladder [7] and $Ba^{2+}$ depolarizes $V_m$ only by about 10 mV, the degree of inhibition might seem rather low. Conversely, the following discussion will show that $Ba^{2+}$ effects are quite large.

Since the epithelium is leaky, a change in the apical electromotive force ($\Delta E_m$) and in the resistances of the microcircuit set up by the cell and the paracellular pathway modifies the circulating current. The variation of the current raises a potential drop on $R_m$ ($V'_m$) which is opposite to the variation of $E_m$. The change in the apical membrane p.d. measured after $Ba^{2+}$ treatment ($\Delta V_m$) is then:

$$\Delta V_m = \Delta E_m - V'_m \simeq 10 \text{ mV} .$$

If one supposes reasonably that the basolateral resistance and electromotive force are not affected by the luminal treatment and that the paracellular resistance and electromotive force remain negligible with respect to cellular resistance and e.m.f., the potential drop on the mucosal membrane resistance is:

$$V'_m = E'_t \frac{R'_m}{R'_t} - E_t \frac{R_m}{R_t} = (E_t + \Delta E_m) \frac{R'_m}{R'_t} - E_t \frac{R_m}{R_t} =$$

$$= E_t \left( \frac{R'_m}{R'_t} - \frac{R_m}{R_t} \right) + \Delta E_m \frac{R'_m}{R'_t} = E_t \left( \frac{R'_m}{R'_m + R_s} - \frac{R_m}{R_m + R_s} \right) + \Delta E_m \frac{R'_m}{R'_m + R_s}$$

where $E_t$, $E'_t$, $R_m$, $R'_m$, $R_t$, $R'_t$, are respectively the total net e.m.f.'s of the circuit, the apical and the total cellular resistance before and after treatment.

The value of $E_t$ is — 10.8 mV (1) and

$$\frac{R'_m}{R'_m + R_s} \quad , \quad \frac{R_m}{R_m + R_s}$$

are deducible from $R_m/R_s$ obtained in the presence or absence of $Ba^{2+}$ (10 mM) and reported in the Tables I and II.

Thus:

$$V'_m = -10.8 \left( \frac{3.75}{3.75 + 1} - \frac{1.18}{1.18 + 1} \right) + \Delta E_m \frac{3.75}{3.75 + 1} = -2.7 + \Delta E_m \, 0.79$$

and

$$\Delta V_m = \Delta E_m - (\Delta E_m \, 0.79 - 2.7)$$

$$\Delta E_m \simeq 35 \text{ mV} .$$

As a conclusion the $V_m$ depolarization of about 10 mV corresponds to a much larger $E_m$ depolarization of about 35 mV, a result which is in accordance with a large effect of $Ba^{2+}$ on the dominant $K^+$ conductance.

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