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# Electrochemical Potential of Calcium as Index of Intestinal Permeability in Rat and Chicken

A. Vázquez, R. Jordana and J. Larralde

Departamento de Investigaciones Fisiológicas
C.S.I.C.
Universidad de Navarra
Pamplona (Spain)

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The electrochemical potential of calcium between the serosal and the mucosal sides of the small intestine in both rat and chicken have been studied *in vitro*. Techniques to measure the Transmural Potential Difference and the Short-circuit Current Intensity have been used.

Our results indicate that the permeability coefficient of calcium in rat intestine towards the serosal is sixfold over that towards the mucosal. In chicken intestine it is approximately of the same order on either side.

The permeability coefficient is defined as the ratio $\Delta Eexp/\Delta Etheor$, where $\Delta Eexp$ stands for the difference, experimentally measured, of the electrochemical potential under two distinct conditions of calcium in the medium, and $\Delta Etheor$ stands for its expected value under conditions of zero permeability.

The role of calcium on epithelial permeability has been studied in a wide variety of tissues (1, 3, 8-10, 17-19, 21, 24). CURRAN *et al.* from 1962 on (4, 5) have observed a marked decrease in the potential difference (PD) and the short-circuit current (Isc) when calcium is added to the outer bathing medium of frog skin, which has led them to the conclusion that calcium affects sodium transport by reducing the permeability of the membrane and not by a direct effect on the transport mechanism itself. If the membrane is a porous structure, calcium might bind with the negative charged centers to block partially the pores and, thus, reduce diffusion across the membrane.

By establishing a comparison between the effects of calcium and the effects of agents that on principle increase the permeability of the membrane, such as procaine and other local anesthetics, SKON and ZERAHN (23) and USSING (27) have proposed a similar mechanism to explain the resultant potassium transport increase when these agents are added to the bathing medium at the outer surface of the skin. This, besides, suggests that the transport system itself is not saturated under normal sodium concentrations.

Other experiments have shown that calcium excess inhibits the absorption of glucose (16) and other substances (6, 11, 12). It might well be that calcium reduces the relative permeability of the membrane independently of its type of transport (20, 21, 26).

Other lines of research point to the effects that calcium chelating agents produce on the epithelial cells (13, 15). They generally infer that the chelating substances, and particularly the EGTA, disorganize the intracellular communication to the point of irreversibility by annulling the electrical parameters.

## Materials and Methods

The small intestine from white Wistar rats weighing between 150 and 230 g and from chickens between two and eight weeks of age, has been used.

The animal was first anesthetized v.s.c., prior to laparotomy. Then an intestinal segment about 1 cm long was withdrawn and used to determine its potential difference and short-circuit current after the method of USSING and ZERAHN (24) as modified by HERRERA *et al.* (14).

The concentration of the medium in millimoles per litre was as follows: NaCl = 127.13; KCl = 5.10; $MgCl_2$ = 1.26; HCl = 4.10; $CaCl_2$ = 2.72 and glucose = 2.70. TrisCl replaced $CaCl_2$ isosmotically in Ca free solutions.

In some experiments 2.7 mmoles/l of calcium were added periodically to the mucosal medium which at the onset had been calcium free, while the serosal medium was kept intact. After each addition the required time for the potential to stabilize was allotted.

In other experiments 2.7 mmoles/l of EGTA (ethylene glycol tetraacetic acid) were added to mediums with $Ca^{2+}$ concentrations and without it.

## Results and Discussion

The addition of calcium to the mucosal solution always provokes a decrease in the potential difference and the short-circuit current both in rat and chicken intestine and in either the presence or absence of calcium in the initial solution on the serosal side (table I).

The levels of both the potential difference and the short-circuit current in rat intestine drop much more when calcium is absent from the serosal than in its presence. In chicken intestine the levels of the potential difference stay approximately the same in all cases. When calcium concentration on the serosal is 2.7 mM, the small increase observed after the first addition of calcium to the mucosal is not significant. However, the decreases that follow the addition of calcium are quantitatively lower in chicken intestine than in rat.

CURRAN *et al.* (4, 5) had already observed the same effect *in vitro* in frog skin, and so had DUMONT *et al.* (7) in rat intestine. They deduced that calcium effected the permeability of the membrane by hindering sodium flux across it.

An analysis of the present data leads to the following considerations: the potential registered in rat intestine, in the absence of calcium from the serosal, is 2.53 mV. This potential drops to 2.21 mV when 2.7 mmoles/l of Ca are added to the mucosal. The differences between these two values ($\Delta E$ = 0.32 mV) is attributed to effects from the added calcium.

A 4.05 mV potential is registered when 2.7 mmoles/l of $Ca^{2+}$ are present on the serosal and none on the mucosal, against the 2.53 mV obtained in the absence of these cations from both sides. Their difference ($\Delta E$ = 1.52 mV) is attributed to the presence of calcium on the serosal.

If the membrane were a structure completely permeable to the flux of calcium,

Table I. *Values for the $PD_t$ and $I_{sc}$ obtained after adding calcium to the mucosal solution in rat and chicken intestine.*
Number of experiments between parentheses.

| $Ca^{2+}$ ser (mM) | Adition of $Ca^{2+}$ to mucosal (mmol/l) | Rat | | Chicken | |
|---|---|---|---|---|---|
| | | $PD_t$ (mV) | $I_{sc}$ ($\mu$A. cm$^{-2}$) | $PD_t$ (mV) | $I_{sc}$ ($\mu$A. cm$^{-2}$) |
| 0 (22) | 0 | 2.53±0.10 | 123.4± 7.4 | 4.10±0.09 | 94.0±2.3 |
| | 2.7 | 2.21±0.10 | 108.8± 6.3 | 4.01±0.06 | 90.2±6.3 |
| | 5,4 | 1.78±0.05 | 79.5± 2.1 | 3.83±0.06 | 84.0±1.3 |
| | 8.1 | 1.43±0.03 | 61.0± 0.8 | 3.20±0.05 | 64.3±1.6 |
| 2.7 (23) | 0 | 4.05±0.08 | 166.6± 6.8 | 4.15±0.06 | 127.1±6.5 |
| | 2.7 | 3.81±0.08 | 136.5± 5.3 | 4.27±0.07 | 131.6±8.1 |
| | 5.4 | 2.94±0.06 | 110.8± 3.7 | 3.97±0.08 | 120.0±7.8 |
| | 8.1 | 2.45±0.04 | 98.6± 2.3 | 3.54±0.08 | 92.7±5.7 |
| 5.4 (20) | 0 | 3.85±0.14 | 196.5±14.5 | 4.25±0.06 | 137.3±4.0 |
| | 2.7 | 3.81±0.14 | 180.7±11.2 | 4.18±0.05 | 114.5±3.9 |
| | 5.4 | 3.55±0.15 | 136.7±12.2 | 3.52±0.11 | 87.9±1.2 |
| | 8.1 | 3.14±0.16 | 112.1±11.8 | 2.93±0.06 | 60.5±1.1 |
| 8.1 (20) | 0 | 3.72±0.18 | 179.2± 9.3 | 4.50±0.10 | 91.6±4.8 |
| | 2,7 | 3.61±0.18 | 168.0±14.7 | 4.45±0.02 | 89.2±4.5 |
| | 5.4 | 3.35±0.17 | 148.3± 8.7 | 4.30±0.08 | 67.5±5.1 |
| | 8.1 | 3.05±0.15 | 139.0±10.3 | 3.60±0.07 | 64.8±3.9 |

the expected value for $\Delta E$ should equal zero:

$$\Delta E = \frac{R \times T}{Z \times F} \log 1 = 0$$

If the membrane were totally impermeable to calcium, the expected value for $\Delta E$ would be:

$$\Delta E = \frac{R \times T}{Z \times F} \log 2.7 = 12 \text{ mV}$$

The $\Delta E_{exp}/\Delta E_{theor}$ ratio will manifest the permeability degree of the membrane to calcium flux. The more its value approaches zero, the greater the permeability of the membrane will be. Thus:

Permeability from mucosal to serosal

$$Pms = \frac{0.32}{12} = 0.02$$

Permeability from serosal to mucosal

$$Psm = \frac{1.52}{12} = 0.13$$

The calcium permeability of the membrane towards serosal is, therefore, sixfold over that in the opposite direction.

Although our results coincide with those from other authors (4, 5), the decreases in the potential difference after the addition of calcium to the mucosal cannot be simply attributed to the effect of a permeability reduction of the membrane to sodium, since the calcium potential may also be counteracted by potentials from other ions and produce the overall potential decrease observed.

It cannot be stated either, for the same reason, that the presence of calcium on the serosal side causes an increase in the sodium movement.

Nevertheless, the highest absolute values for the short-circuit current obtained when calcium was present on the serosal, could well indicate that this cation plays a significant role in the movement of the other ions.

The calcium permeability values for chicken intestine, estimated as those for rat, are as follows:

$$Pms = 0.010 \quad \text{and} \quad Psm = 0.015$$

This little difference in calcium movement in either direction may explain, on one hand the similarity in the initial values obtained in all cases and, on the other hand the smaller potential decreases observed after the periodic additions of calcium.

Furthermore, if the membrane resistance is estimated by the quotient of the ratio «potential difference/short-circuit current» from the data on table I, it is evident that the resistance to the ionic flux across the rat intestinal wall is approximately the same regardless of the concentration of calcium on the serosal side (table II). In chicken intestine, however, the resistance seems to be smaller with $Ca^{+2}$ concentrations between 2.7 and 5.4 mM.

The data from the present experiments show the different behavior of rat and chicken intestine after the addition of calcium. Their analysis brings out two fundamental differences.

The permeability to calcium is much greater towards the serosal than towards the mucosal in rat intestine. In chicken intestine it is approximately the same in either direction.

The resistance offered by the membrane to the ionic flux is practically the same no matter what the concentration of calcium on the serosal side is; in chicken intestine, however, the resistances is smallell with $Ca^{2+}$ concentration between 2.7 and 5.4 mM.

Table II. *Electric resistance of the small intestine in rat and chicken under various calcium concentrations on the serosal side.* $[Ca^{2+}]_{mucosal} = 0$ mM. Resistance values are expressed as $K\Omega \times cm^2$.

| $[Ca^{2+}]$ muc. (mM) | 0 | 2.7 | 5.4 | 8.1 |
|---|---|---|---|---|
| Rat | 0.020 | 0.023 | 0.019 | 0.020 |
| Chicken | 0.043 | 0.032 | 0.031 | 0.048 |

When EGTA is added to the solution there follows an almost 50 % decrease of the initial value of the potential, about 18 min after the addition in rat intestine, and after 10 min in chicken.

In either case and both in the absence and in the presence of calcium the nulling is complete after 40 min. The potential values are in no case restored by a washing without EGTA (fig. 1).

The present results, strengthened by those of HARRY and FISHMAN (13), in frog skin *in vitro* and by those of BAR *et al.* (2) who found an accumulation of chelated calcium in the intestinal epithelium of birds, point to two possibilities: either the EGTA binds itself to the components of the membrane forming complexes of a high stability constant which would affect negatively the ionic permeability of the wall, or the EGTA bound to calcium and to other ions in the medium, penetrates the pores of the membrane and blocks them so that the washing solution ions have no access to the inner complex of the pore.

The fact that EGTA acts more quickly in chicken intestine than in rat, could be explained by a greater permeability of the intestinal epithelium to calcium in chicken

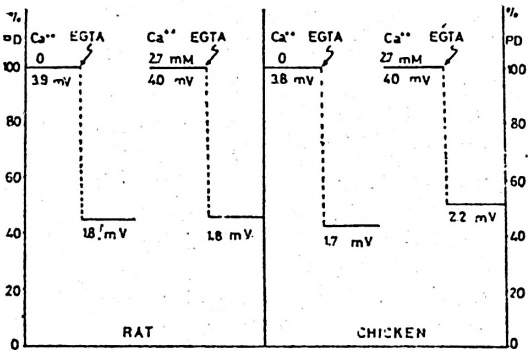


Fig. 1. *Effect on the transmural potential difference (PD) from the addition of EGTA. Inhibition of the PD 18 minutes after the addition of EGTA in rat intestine, and afted 10 minutes in chicken.*

than in rat, at least as to its action in mediums with concentrations of calcium.

## Resumen

Utilizando la técnica de medidas de Diferencia de Potencial Transmural y de Intensidad de Corriente de Cortocircuito, se ha estudiado el potencial electroquímico del calcio entre el lado serosal y mucosal del intestino delgado de rata y pollo *in vitro*.

Los resultados indican que el calcio en el intestino de rata tiene un coeficiente de permeabilidad hacia el lado serosal del orden de seis veces superior al que tiene hacia el lado mucosal. En el intestino de pollo es aproximadamente del mismo orden en los dos lados.

El coeficiente de permeabilidad está definido como la relación ΔEexp/ΔEteor, en donde ΔEexp es la diferencia medida experimentalmente para el potencial electroquímico entre dos condiciones distintas de calcio en el medio y ΔEteor es la que cabría esperar en condiciones de permeabilidad nula.

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