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Effect of Calcium Concentration on the Transmural Potential Difference and the Intensity of the Short-Circuit Current in the Small Intestine of Rat, Chicken and Hen

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The effect of calcium concentration on the Transmural Potential Difference and the Intensity of the Short-circuit Current in rat, chicken and laying hen small intestine has been studied *in vitro* both in the presence and absence of sodium.

The results show that calcium, in the presence of sodium, produces an increase in potential and currend intensity much greater in hen intestine than in chicken and rat.

In the absence of sodium, the response of the potential as regards calcium concentration in hen intestine is parallel to the one obtained in the absence of sodium: a rise in the potential until it reaches a maximum value that corresponds to a calcium concentration of 10.8 mM in the medium, followed by a drop in the potential. However, rat intestine responds differently: the potential, negative at the serosal, becomes more and more positive with higher concentrations of calcium until it practically disappears.

There can be two kinds of mechanism for calcium transport: active transport (2, 11, 12, 17, 39) and simple or facilitated diffusion (12, 16, 31, 39).

HELBOCK *et al.* (16, 23) started a new stage in the study of calcium transport by applying to it the short-circuit current technique (35) thereby showing that calcium aborption was an active process in rat, sustained partially by the energy from cellular metabolism.

HOLDSWORTH (17, 28) in 1965 and later ADAMS (1) and WONG (40) also found calcium to be actively transported in chicken intestine, but for them the active flux of calcium was not dependent on oxidative metabolism.

The role of sodium in calcium transport has been extensively investigated. Research *in vitro* in chicken jejunum indicates (3) that when mannitol substitutes sodium, the passive flux of calcium towards the interior of the cell increases.

In rat duodenum *in vitro*, the substitution of sodium by choline or mannitol (22)

does not either change or increase calcium transport, respectively.

In 1970, ADAMS *et al.* (1) found that ouabain, a known inhibitor of Na^+-K^+-activated ATPase (50), inhibited calcium flow when it was present at the serosal side in rat intestine.

In 1974, WROBEL *et al.* (42) discovered that the presence of sodium increased calcium transport, and that ouabain inhibited sodium transport but not that of calcium (41).

BIRGE *et al.* (5-7), working with rat intestine *in vitro* produced the evidence that sodium might be a necessary cofactor at the basolateral membrane for a Ca^{2+}-K^+ dependent ATPase bound to the membrane, which could act directly on the outflux of calcium from the cell.

BARRY and EGGENTON (4) afforded new light by stablishing that the potential through the basolateral membrane in rat jejunum was not affected by changes in sodium concentration, and suggesting, therefore, that the voltage drop induced by sodium substitution took place in the transmucosal membrane. In the absence of any detectable decrease in calcium transport, they concluded that sodium was only required for a calcium outlet process at the basolateral membrane. This requirement has been confirmed and elaborated by other researchers (18, 21, 34, 41, 42).

Although the nature of the sodium effect has not been studied with rigorous precision, it seems clear enough that the mechanism for calcium outflow may be due to a sodium-calcium change at the basolateral membrane.

In this paper was studied the effect of calcium concentration at both sides on the transmural potential difference and on the intensity of the short-circuit current in the small intestine of rat, chicken and laying hen.

Materials and Methods

The animal was anesthetized subcutaneously and was kept under heating conditions for half an hour. After laparotomy the beginning of the jejunum was identified and an intestinal segment about 1 cm long was withdrawn and washed in a physiological solution at 0° C and inverted. It was then mounted on two cannulae and was used to measure the transmural potential difference and the short-circuit current.

To measure and to register the transmural potential difference two saline bridges connected each side of the intestinal wall to two Calomel electrodes, which in turn were connected to an Electrometro-Amplifier-Register.

The intensity of the short-circuit current was also measured by using two saline bridges and two Calomel electrodes connected to an external feed source of continuous current. By inserting a microamperimeter in the circuit it is possible to apply between both sides of the intestinal wall a potential exactly equal but opposite to the one generated by the tissue. In this way it is possible to measure the current intensity that corresponds to that potential. This method has been described by USSING and ZERAHN (32).

The composition of the solutions used was as follows in millimolar concentration: NaCl=127.13; KCl=5.10; $MgCl_2$=1.26; Tris=4.90; HCl=4.10, and calcium, in the form of $CaCl_2$=2.7; 5.4; 8.1; 10.8, and 13.5 mM.

Sodium-free solutions differed from the previous one in that all the NaCl was substituted isosmotically by TrisCl. In all casses the solutions contained a 5.4 mM concentration of glucose.

White Wistar rats weighing from 150 to 230 g, chickens from 2 to 8 weeks of age, and laying hens aged from 12 to 18 months, were used.

The value for $A_{Ca^{2+}ser}/A_{Ca^{2+}muc}$, which corresponds to calcium activities on either side of the wall, is estimated according to Nerst's equation.

Results

Previous experiments (36) indicate that chicken intestine shows a greater degree of permeability to calcium flux than rat intestine.

The present study was undertaken to determine calcium activity in both intestines. The PD_t and the Isc in mediums with different concentrations of calcium were measured. Since the behavior of rat and chicken intestine proved to be different, the same electric measurements were taken in laying hen intestine, where the absorption of this cation ought to be highly important due to the eggshell formation. The differences observed were quite remarkable.

To find out if sodium was involved in this system, we conducted the same electric measurements at different calcium concentrations in sodium-free solutions in both rat and hen intestine.

Figure 1 and table I show the results

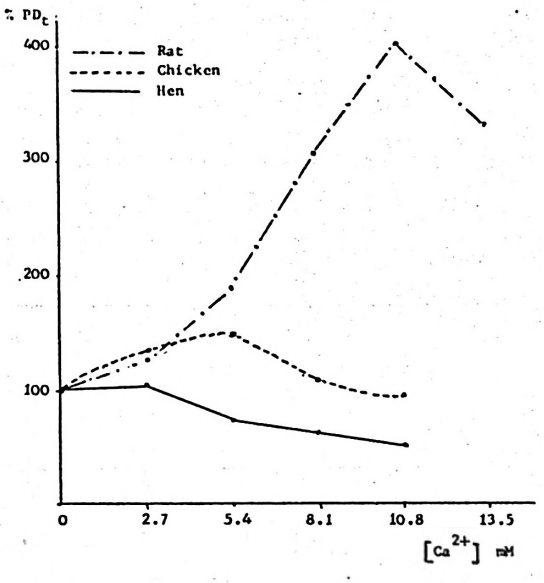

Fig. 1. *Percentage variations of the PD_t in rat, chicken and hen intestine.* Glucose 5.4 mM.

Table I. *Influence of calcium concentration on the PD_t (mV) and Isc ($\mu A/cm^2$) in rat, chicken and hen intestine.*
Sodium concentration in the medium is 127.13 mM in all cases and that of glucose is 5.4 mM.

$[Ca^{2+}]$ mM	n	PD_t mV	I_{sc} $\mu A/cm^2$	$A_{Ca^{2+}ser}/A_{Ca^{2+}muc}$
		Rat		
0	28	6.97±0.04	214.00±1.80	—
2.7	22	7.23±0.04	383.60±1.50	1.02
5.4	20	5.66±0.02	170.00±3.30	0.90
8.1	20	5.33±0.02	142.20±4.40	0.89
10.8	15	4.57±0.09	124.30±4.30	0.83
		Chicken		
0	34	5.75±0.08	103.00±2.60	—
2.7	34	7.79±0.06	156.00±4.00	1.12
5.4	34	8.12±0.03	100.90±1.60	1.20
8.1	30	5.96±0.06	112.10±1.80	1.01
10.8	25	5.45±0.03	95.10±0.50	0.96
		Hen		
0	18	1.99±0.03	9.50±0.36	—
2.7	16	2.49±0.02	21.50±0.52	1.05
5.4	16	3.80±0.02	36.60±0.70	1.15
8.1	16	6.56±0.02	63.40±0.60	1.41
10.8	16	8.00±0.02	78.80±0.70	1.58
13.5	14	6.47±0.02	61.70±0.50	1.40

obtained in solutions that contained sodium.

Rat. In calcium-free solutions, the potential registered was 6.97 mV; with a calcium concentration of 2.7 mM, the value obtained was 7.23 mV. For higher concentrations, the values for the potential become lower and lower. The same happens to the Isc.

The ratio $A_{Ca^{2+}ser}/A_{Ca^{2+}muc}$ also decreases as the amount of calcium in the medium increases, so that with calcium concentrations above 2.7 mM the values obtained are lower than one.

Chicken. In chicken intestine the potential increases according to the calcium concentration until it reaches a maximum

value at a calcium concentration of 5.4 mM. Therefrom higher concentrations bring about a fall in the potential. The same happens to the Isc and to the ratio of calcium activities on either side of the intestinal wall.

Hen. In hen intestine the highest value for the PD_t Isc and $A_{Ca^{2+}ser}/A_{Ca^{2+}muc}$ is obtained with a calcium concentration of 10.8 mM in the medium. It is worth remarking that the percentage increase of the potential in hen intestine is much higher than in rat and chicken for their respective optimum concentrations of calcium (fig. 1).

Figure 2 shows the results from experiments performed in sodium-free solutions.

Rat. The response of the potential against a calcium concentration differs from the one observed in the presence of sodium. The potential, negative on the serosal, keeps rising as the calcium concentration in the medium increases, reaching nearly 0 mV values with high concentrations of this cation.

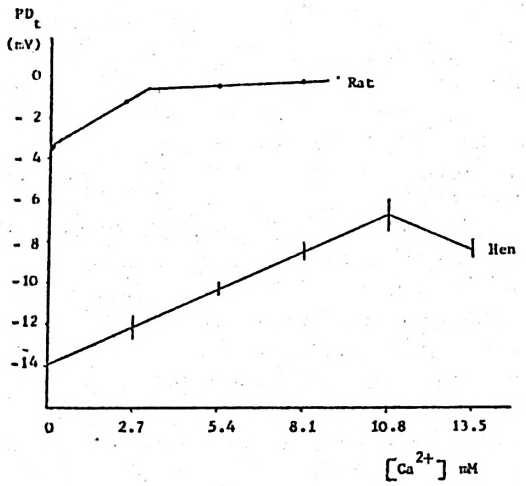

Fig. 2. *Variation of PD_t (mV) in rat and hen intestine with calcium concentration in sodium free solutions.*
Glucose 5.4 mM.

Hen. The behavior of hen intestine against a concentration of calcium implies a potential response parallel to the one obtained in the presence of sodium. The potential increases from —13.80 mV in the absence of calcium to —6.49 mV with a calcium concentration of 10.8 mM.

Table II. *Relation between calcium concentration and electric flux.*
Na^+ = 127.13 mM; glucose = 5.4 mM. Data for hen intestine have been taken from Table 1. $I_{Ca^{2+}}$ = Current Intensity due to the calcium ion. $I_{Na^+Ca^{2+}}$ = Current Intensity due to sodium and calcium ions crossing the membrane together. I = total current intensity. The values of current intensity are expressed in $\mu A/cm^2$.

$[Ca^{2+}]$ mM	i	$I_{Ca^{2+}}$ / $I_{Na^+Ca^{2+}}$	$I_{Ca^{2+}}$ / $I_{Na^+Ca^{2+}}/I$
0	9.50	0	0
2.7	21.50	12.0	0.53
5.4	36.60	27.1	0.73
8.1	63.4	53.9	0.84
10.8	78.8	69.3	0.87
13.5	61.7	52.2	0.83

Table III. *Influence of calcium concentration on the relation of calcium activities on either side of the intestinal wall in the absence of sodium.*
Rat and hen intestine. Glucose = 5.4 mM.

$[Ca^{2+}]$ mM	n	$A_{Ca^{2+}ser}/A_{Ca^{2+}muc}$
	Rat	
0	12	—
1.3	12	1.21
2.7	12	1.33
5.4	8	1.45
	Hen	
0	18	—
2.7	16	1.16
5.4	16	1.36
8.1	16	1.58
10.8	16	1.78
13.5	14	1.57

Discussion

Previous studies (8-10) showed that rat and chicken intestine behaved differently in the presence of calcium. This difference can be clearly seen in the following fact: The addition of galactose to the mucosal solution in chicken intestine produced a PD_t increase proportionally greater in a medium that contained calcium than in one without it. In rat intestine the PD_t increase was the same independently of calcium presence or absence.

Some authors working with rat intestine with the technique of USSING and ZERAHN (35) have proved the existence of a mucosal-serosal transport of calcium against a chemical gradient of concentration, when calcium concentrations in the medium are about 1 mM (1, 16, 20, 23, 29, 37, 40).

The Isc values obtained for calcium concentrations higher than 2.7 mM (170, 142, 124 $\mu A/cm^2$) are lower than those obtained in the absence of calcium (214 $\mu A/cm^2$). This indicates that calcium, at such concentrations, provokes a decrease of the membrane ionic permeability (21, 24, 33). Besides, the ratio $A_{Ca^{2+}ser}/A_{Ca^{2+}muc}$ which remains lower than one during these high calcium concentrations, hints at the impossibility for calcium to cross over to the serosal side.

In chicken intestine the ratio $A_{Ca^{2+}ser}/A_{Ca^{2+}muc}$ reaches its highest value (1.20) when calcium concentration is 5.4 mM (fig.1, table I).

The representation PD_t vs Isc (E vs I) responds to a straight line equation:

$$E = 1.5 + 0.04\ I \qquad (1)$$

On the other hand the relation E — $[Ca^{2+}]$ is:

$$E = 5.7 + 1.07[Ca^{2+}] - 0.11([Ca^{2+}])^2 \qquad (2)$$

Carrying over (1) to (2) and clearing I:

$$I = 1/0.04\ (4.2 + 1.07\,[Ca^{2+}] - 0.11\,[Ca^{2+}]^2)$$

$$dI/d[Ca^{2+}] = 1/0.04\ (1.07 - 0.22\,[Ca^{2+}]) = 0$$

$$[Ca^{2+}] = 4.8$$

Maximum ionic flux therefore corresponds to calcium concentrations of 4.8 mM in the medium.

WASSERMAN and KALLFELTZ (39) observed in chicken intestine *in vivo* that maximum absorption of calcium occurred when the luminal concentration of that ion was 5.2 mM.

It is clear, therefore, that calcium absorption reaches its maximum values in mediums where calcium concentration is about 5 mM.

As to chicken intestine negative effects of calcium on the permeability of the membrane become significant at calcium concentrations higher than 8.1 mM.

In hen intestine the highest value for the ratio $A_{Ca^{2+}ser}/A_{Ca^{2+}muc}$ takes place when the calcium concentration is 10.8 mM (1.58). And, as before, the relation E — I is:

$$E = 1.1 + 0.08\ I \qquad (3)$$

I stands for the intensity of the current originating from all the ions in the medium, which can be broken down like this:

$$I = I^\circ + I_{Na^+} + I_{Ca^{2+}} + I_{Na^+Ca^+} \qquad (4)$$

I° = current intensity from all the ions in the medium except sodium and calcium. No interferences are supposed to exist between sodium and calcium ions and all the others.

I_{Na^+} = current intensity due to sodium when it crosses the membrane alone. $I_{Ca^{2+}}$ = the one due to calcium. $I_{Na^+Ca^{2+}}$ = the one due to sodium — calcium if they cross the membrane together. According to this:

For $Ca^{2+} = 0$

$I^\circ + I_{Na^+} = 9.5\ \mu A\ cm^{-2}$ (see table I)

Substituting this value in (4)

$$I = 9.5 + I_{Ca^{2+}} + I_{Ca^{2+}Na^+} \qquad (5)$$

Carrying over (5) to (3)

$$E = 1.1 + 0.08\ (9.5 + I_{Ca^{2+}} + I_{Na^+Ca^{2+}}) \qquad (6)$$

$$E = 1.9 + 0.08\ (I_{Ca^{2+}} + I_{Ca^{2+}Na^+})$$

This gives the relation of dependence of E, not on the total ionic flux, but on that of calcium when it crosses the membrane alone and when it crosses it together with sodium.

It was highly interesting to know the relative importance of both fluxes, and to this purpose table II was elaborated (data for chicken intestine were extracted from table I).

The relative importance of the ionic $I_{Ca^{2+}} + I_{Na^{+}Ca^{2+}}$ flux with respect to total ionic flux increases as the calcium concentration in the medium augments, reaching the order of 87 % when calcium concentration is 10.8 mM. At higher concentrations negative effects on the permeability of the membrane are supposed to intervene.

As to rat intestine at its optimum concentration (2.7 mM) the relation $I_{Ca^{2+}} + I_{Ca^{2+}Na^{+}}$ is 44 %, i.e. the ionic flow of calcium by itself and bound to sodium is much more important in hen intestine as compared with total ionic flux than in rat (44 %). It may be that in rat intestine the first of the two components of the relation $I_{Ca^{2+}} + I_{Ca^{2+}Na^{+}}$ is not significant with respect to the other one.

In hen intestine the relation of calcium activities is higher in mediums without sodium (table III) than in mediums with sodium (table I). In both cases the highest value for that relation is obtained when calcium concentration in the medium is 10.8 mM.

Some authors had found in rat (14, 15) and chicken intestine (19) higher transmural transport of calcium in a medium without sodium than in one with sodium. Three hypotheses have been proposed to explain these facts: Competition for a commom carrier (26, 27); competition between Na — Ca at the level of the cellular membrane (25); a calcium effect on a metabolic intermediary (30) which would alter cellular permeability to calcium.

The fact that in rat in the absence of sodium the quotient $A_{Ca^{2+}muc}/A_{Ca^{2+}ser}$ increases according to calcium concentration does not imply that a greater calcium flux towards the serosal side is taking place. It is rather more probable that, due to the permeability difference of the membrane sides and to their negative effects on the Isc, calcium enters the cell from the mucosal side faster than from the serosal side, affecting thus the transmural potential. In hen intestine, the changes observed in the potential are not to be attributed to this effect for, as figure 2 shows, after a calcium concentration of 10.8 mM the ratio $A_{Ca^{2+}ser}/A_{Ca^{2+}muc}$ decreases, a phenomenon not seen in rat.

Therefore the behaviour of either intestine in the presence of calcium seems to be distinct. This can be related to the mechanism for calcium transport, which possesses various transport systems, at least one is different in hen intestine.

Resumen

Se estudia el efecto de la concentración de calcio sobre la diferencia de potencial transmural e intensidad de corriente de cortocircuito *in vitro* en intestino delgado de rata, pollo y gallina ponedora, en presencia y en ausencia de sodio.

Los resultados indican que, en presencia de sodio, el calcio produce un aumento del potencial mucho mayor en el intestino de gallina que en los de pollo y rata.

En ausencia de sodio, la respuesta del potencial frente a la concentración de calcio en el intestino de gallina es paralela a la que se obtiene en presencia de sodio: aumento del potencial hasta un valor máximo que corresponde a una concentración de calcio en el medio de 10,8 mM, y luego disminución de aquél. Sin embargo, el intestino de rata responde de un modo diferente: el potencial, negativo en serosal, va haciéndose cada vez más positivo hasta casi su anulación.

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