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Glucose Effect on PDt in Rat and Laying-Hen Small Intestine Both in Presence and Absence of Calcium

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The effect on the transmural potential from adding glucose under different conditions of sodium and calcium has been studied in rat and laying-hen intestine *in vitro* with the technique for measuring Transmural Potential Difference.

The glucose, in sodium-free mediums, has no effect on the Transmural Potential Difference in rat intestine, whether calcium be present or absent. As to hen intestine, the PDt increases when calcium is present in the medium, both in the absence and presence of sodium.

Glucose absorption was also determined *in vivo* by using mediums with/without calcium. In rat intestine glucose absorption was not affected by the presence of calcium, while its presence enhanced glucose absorption in hen intestine.

The close relation between sodium transport and the transport of sugars has been known for a long time (6-8, 15).

CLARKSON *et al.* (5) observed that adding glucose to the mucosal solution of *in vitro* preparations in rat intestine led to a quick rise in the Transmural Potential Difference.

SCHULTZ and ZALUSKY (16, 17) showed in 1964 that the addition of sugars and aminoacids accelerated sodium transport.

From the above considerations it is obvious that sodium and sugar can move when bound in all the processes of net transport across the tissue.

It has also been shown and confirmed (9, 13, 14) that the mechanism for sodium outflux across the basolateral membrane is a reogenic process, and that the currents generated on one side can affect the Electrical Potential Difference on the other (9, 14).

Research on chicken jejunum *in vitro* (1) has shown that the passive flux of calcium towards the cell interior increases after the substituting of sodium for manitol. The calcium flux, however, is not modified by this substitution in rat duodenum *in vitro* (12).

BAR *et al.* (2) suggested in 1969 that calcium transport was highly dependent on the cellular metabolism of hen intestine

in vivo. They found in 1970 (22) that calcium uptake in hen intestine *in vitro* was a lineal function of the calcium concentration in the medium. This lineality, due perhaps to a diffusion process, could also be assigned to the presence of a transport carrier when the medium is far from the saturation point.

It has been equally well established and corroborated (10, 11, 19, 23, 24) that sodium is only required for the process of calcium outflux at the basolateral membrane in rat intestine.

As to hen intestine the following results have been found *in vitro* (21): *a*) a lineal dependence of the Transmural Potential Difference with calcium concentrations of up to about 9.5 mM in the medium; *b*) maximum Transmural Potential value with concentrations of calcium at 10.8 mM and glucose at 5.4 mM, both in the presence and absence of sodium. A. Ca^{2+}-glucose pass mechanism, sodium independent, has been suggested.

Finding the amplitude of each pass mechanism for Na^{+}-glucose, Na^{+}-Ca^{2+}-glucose, and Ca^{2+}-glucose in hen and rat intestine is the object of the present study.

Materials and Methods

White Wistar rats weighing 150 to 230 g and laying-hens from 12 to 18 months of age have been used.

The animal was first anesthetized subcutaneously with urethane at 12.5 % and was then kept under heating conditions for half an hour. After laparotomy the jejunum initium was identified and an intestinal segment about 1 cm long was withdrawn, washed in a physiological solution at 0° C and inverted. It was then mounted on two cannulae and its Transmural Potential Difference measured following a previously described technique (20, 21).

The intestinal absorption of sugars for *in vivo* experiments was done according to the SOLS and PONZ (18) technique.

Four types of solutions were used, the composition of which in millimolar concentration was as follows:

a) Tris-Cl = 127.13; KCl = 5.10; $MgCl_2$ = 1.26.
b) Tris-Cl = 127.13; KCl = 5.10; $MgCl_2$ = 1.26; $CaCl_2$ = 10.81 hen and 2.70 (rat).
c) NaCl = 127.13; KCl = 5.10; $MgCl_2$ = 1.26.
d) NaCl = 127.13; KCl = 5.10; $MgCl_2$ = 1.26; $CaCl_2$ = 10.80 hen and 2.70 (rat).

All these solutions were buffered with Tris 4.90 mM and HCl 4.10 mM. Glucose was added to all the mediums at different stages of the experiments up to a 5.4 mM concentration.

Results

The Transmural Potential Difference has been measured in four types of solutions both in the absence and presence of glucose (fig. 1).

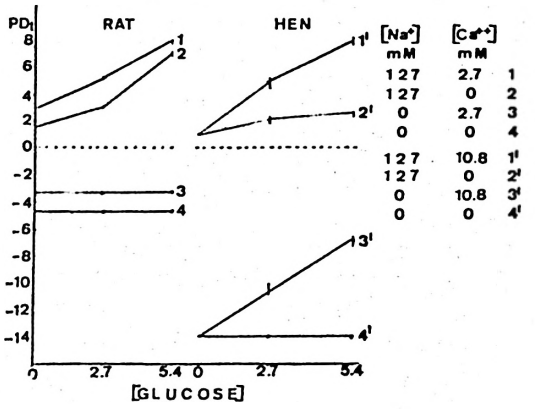

Fig. 1. *Effect of glucose on the Transmural Potential Difference under distinct conditions of calcium and sodium concentration in rat and laying-hen intestine.*
[Glucose] = mM. PDT = mV.

Table I. *Percent of potential values for each individual ion in relation to the total potential generated for sodium and calcium combined.*

Values are calculated from data of figure 3. Potential due to $Ca^{2+} - Na^{+}$ is $34.1 - 27.3 = 6.8$ % or $72.7 - 65.9 = 6.8$ %.

	%
Potential due to Ca^{2+} in the absence of Na^{2+}	34.1
Potential due to Ca^{2+} in the presence of Na^{+}	27.3
Potential due to Na^{+} in the absence of Ca^{2+}	72.7
Potential due to Na^{+} in the presence to Ca^{2+}	65.9

Solution a) *Sodium-free and calcium-free.* Rat: the potential was not altered after adding glucose; it stayed at —4.82 ± 0.33 mV.

Hen: the potential remained at —14.00 ± 0.19 mV.

In sodium and calcium free mediums glucose did not affect the potential either in rat or hen.

Solution b) *Sodium-free with calcium.* Rat: Adding glucose did not affect the value of the potential which stayed at —3.53 ± 0.46 mV.

Hen: The addition of glucose produced a potential increase from —14.10 ± 0.22 mV to —6.70 ± 0.15 mV.

Solution c) *Calcium free with sodium.* Rat: The addition of glucose raised the potential from 1.63 ± 0.14 mV to 6.77 ± 0.13 mV.

Hen: The potential rose from 0.63 ± 0.03 mV to 2.00 ± 0.03 mV after sugar addition.

Solution d) *With sodium and calcium.* Rat: On adding glucose to the medium the potential increased from 1.70 ± 0.09 mV to 7.17 ± 0.07 mV.

Hen: The adding of glucose raised the potential from 0.72 ± 0.03 mV to 8.00 ± 0.03 mV.

Experiments carried out *in vivo* for glucose absorption both in the absence and presence of calcium, show (fig. 2) that calcium enhances by 62 % the passage of glucose across the intestinal wall in hen, but it produces no modifications in rat.

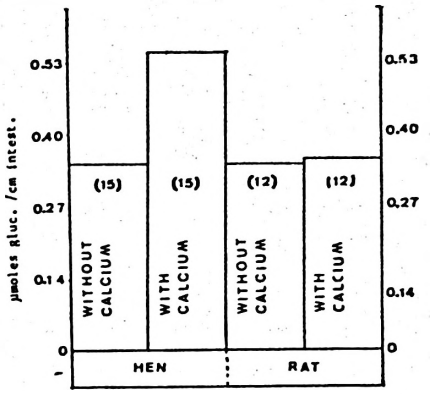

Fig. 2. *Glucose absorption* in vivo *in hen and rat intestine.*

Absorption time 10 min. Glucose concentration in lumen 2 mM. Calcium concentration, 10.8 mM for hen, and 2.7 mM for rat. Absorption dates in µmol gluc./cm intestine. ( ) = number of experiments.

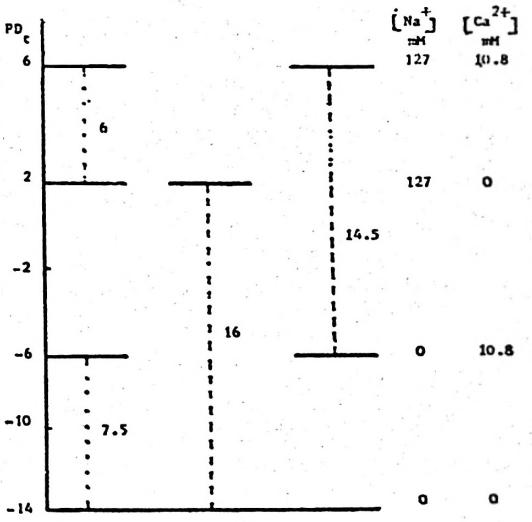

Fig. 3. *Diagram of the potential for distinct concentrations of Na^{+} and Ca^{++} in hen intestine.*

Values taken from figure 1. Glucose 5.4 mM.

Discussion

Rat. In a previous work (21) the relative importance of the ionic flux for calcium-sodium and for calcium was found to be about 44 % with respect to total ionic flux in rat:

$$A) \quad \frac{I_{Na-Ca} + I_{Ca}}{I} \times 100 = 44, \text{ where}$$

I_{Na-Ca} stands for the electric flux generated by bound sodium and calcium ions crossing the membrane together; I_{Ca}, the flux generated by the calcium ion on crossing the intestinal wall by itself; I, total electric flux.

The value 44 % is obtained in mediums that contain 5.4 mM glucose, 127 mM sodium and 2.7 mM calcium.

If on one hand sodium is a necessary cofactor at the basolateral membrane for a Ca^{2+}—Na^{+} ATPase which can act directly on the outflux of calcium from the cell, as BIRGE *et al.* (3, 4) indicated, and on the other hand we find that the absence of sodium does not modify the potential when adding glucose (fig. 1), this means that a Ca^{2+}—glucose coupling does not occur independently of sodium and that, therefore, the I_{Ca} component defined in the previous expression for the transmural flux of calcium is nil.

When sodium is present in the medium the addition of 5.4 mM/l glucose generates a potential of about 5 mV, both in the absence and in the presence of calcium. The response to glucose addition, however, in a calcium-free medium is not parallel to the one obtained in a calcium medium. This means that the presence of both calcium and sodium is important, and that the I_{Na-Ca} compound in the previous expression would represent 44 % of the total ionic flux.

Hen. Adding 5.4 mM/l glucose to a calcium without sodium produces a 7.5 mV increase in the PDt; the increase is only 6 mV in a medium with both calcium and sodium (fig. 3). The difference between the two increased values is, therefore, 1.5 mV.

On the other hand, sodium without calcium increases the PDt in 16.0 mV, and sodium with calcium, in 14.5 mV. The difference between these two values is also 1.5 mV (fig. 3). This 1.5 mV could, therefore, be generated by sodium in the absence of calcium or by calcium in the absence of sodium.

In hen the relative importance of the ionic flux for Ca^{2+}—Na^{+} and for Ca^{2+} (21) is about 87 % with respect to total ionic flux:

$\frac{I_{Na-Ca} + I_{Ca}}{I} \times 100 = 87$. All the terms have the significance indicated in the expression *A*).

The I_{Ca} component is not void in this case since the PDt increase (fig. 1), obtained in the absence of sodium, is only due to the presence of calcium.

The importance of each component in the global expression might, therefore, be established as follows: with 5.4 mM glucose, sodium and calcium combined generate a total potential of 22 mV (fig. 3) of which the percentage that corresponds to each individual ion are expressed in table I.

In a medium with complete sodium and calcium values, therefore, calcium generates approximately 27 % of the potential, sodium 66 % and sodium-calcium 7 % (27 + 66 + 7 = 100).

If the relation PDt — ionic flux is taken to be linear (21) then the following relations can be established:

$$\frac{I_{Ca}}{I_{Na}} = 0.41$$

$$\frac{I_{Ca-Na}}{I_{Na}} = 0.10$$

When 1 mole of sodium, therefore, crosses the membrane, 0.4 mole of calcium and 0.10 mole of bound calcium and sodium cross the membrane along with it.

Finally, the *in vivo* experiments show that glucose disappears from the intestinal

lumen at a rate 62 % greater in mediums with calcium concentrations of 10.8 mM than in mediums without calcium. These differences, however, are not observed in rat.

Resumen

Utilizando la técnica de medidas de Diferencia de Potential Transmural, se han realizado experimentos en intestino de rata y gallina ponedora en los que se estudia el efecto de la adición de glucosa en el potencial transmural para diferentes condiciones de sodio y calcio.

La glucosa, en medios sin sodio, no ejerce ningún efecto sobre la Diferencia de Potencial Transmural (DPt) independientemente de la presencia o ausencia de calcio en intestino de rata. En cuanto al de gallina, aumenta la DPt siempre que el calcio esté presente en el medio, tanto en ausencia como en presencia de sodio.

También se realizaron determinaciones de absorción de glucosa *in vivo* utilizando medio con y sin calcio. En intestino de rata, la absorción de glucosa no se ve afectada por la presencia de calcio. Sin embargo, en intestino de gallina, la presencia de calcio favorece la absorción de glucosa.

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