REVISTA ESPAÑOLA DE FISIOLOGIA
R. esp. Fisiol., 24, n.º 4, págs. 203-206, 1968

Department of Physiology and Biochemistry (C.S.I.C.)
University of Navarra
Faculty of Sciences
Pamplona (Spain)

Effect of Dicumarol on the Intestinal Absorption of Sugars*

by

F. Ponz and M. Lluch

(Received for publication on May 10, 1968)

Recent observations made by RODRÍGUEZ-CANDELA and col. (9) revealed that Dicumarol inhibited the passage of glucose, galactose and 2-deoxyglucose into the skeletal muscle fibers (diaphragm) *in vitro*, while facilitating the penetration of xylose and arabinose, effects prevented to a great extent by the addition of vitamin K_3.

It was therefore interesting to find the effect that Dicumarol would exert on the intestinal absorption of sugars, a process different to that displayed by skeletal muscle, as it involves the active transport of several monosaccharides.

The absorption studies were performed *in vivo*, selecting a hexose that undergoes active transport, glucose, and a pentose that does not, arabinose. Dicumarol inhibits the absorption of glucose while increasing that of arabinose.

Material and Methods

Wistar adult rats, fasted for 24 hours were used in the absorption studies. In each rat, four successive absorptions were performed in the same jejunal loop *in situ*, of approximately 20 cm in lenght (11), utilizing the *in vivo* technique of successive absorptions according to SOLS and PONZ (10).

In the case of D-glucose, a 2.77 mM solution was utilized, in the same saline solution as used in the experiments with skeletal muscles (9). This saline solution was composed of 5.746 g of NaCl; 0.354 g of KCl; 0.162 g of KH_2PO_4; 0.294 g of $MgSO_4 \cdot 7H_2O$; 0.300 g of $NaHCO_3$; 1.971 g of $Na_2HPO_4 \cdot 2H_2O$; 0.488 g of $NaH_2PO_4 \cdot 2H_2O$ and distilled water recently boiled to make a solution of 1000 ml. The periods of absorption were of 20 minutes duration.

Since l-arabinose possesses a slow velocity of penetration across the intestinal barrier, the period of each absorption was of 60 minutes duration, and concentrations of 50 mM were utilized in an identical saline solution.

When Dicumarol was present, its con-

* This work has been supported in part by the «Ministerio de Educación y Ciencia».

centration in the sugar solution to be absorbed was 10^{-4} M.

The residual sugar in the intestine was assayed according to the method used by NELSON-SOMOGYI (6).

Results

1. INTESTINAL ABSORPTION OF D-GLUCOSE.

In the four successive periods of absorption, the first and third were carried out without Dicumarol while the second and fourth periods in the presence of 10^{-4} M Dicumarol. The results obtained are indicated in Table I. The average values of the first absorption, considered as control its absence in the solution of glucose and having washed the intestinal loop profusely between the second and third absorption. The addition of Dicumarol to the sugar solution in the fourth absorption increased the inhibition to 35 %.

2. INTESTINAL ABSORPTION OF L-ARABINOSE.

Table II shows the average values of L-arabinose absorbed in micromoles per cm of intestine accompanied with their corresponding standard errors. In some the experiments, Dicumarol was present in the third and fourth absorptions, while in others, as with glucose, in the second and fourth periods.

A previously referred fact (3), also observed from Table II, is that in normal

TABLE I

Effect of Dicumarol on the Active Transport of D-glucose through the Intestine of the Rat in vivo

In each rat, four successive absorptions of a 2.77 mM D-glucose in a saline solution. Dicumarol 10^{-4} M in the second and fourth absorptions, in the same solution. Absorption time: 20 minutes

Nr. Animals	1st Absorption µM/cm/20 min.	Successive Absorptions (Inhibition %)		
		2nd Absorption	3rd Absorption	4th Absorption
12	0.36 ± 0.3	23 ± 3.42	22 ± 3.95	35 ± 4.57

in all the animals, are given in micromoles of sugar absorbed per cm length of intestine (11). The corresponding values in the second, third and fourth periods are expressed as per cent deviation with respect to the first period. Each value is accompanied by its standard error (8).

As observed in the above Table, Dicumarol evidently inhibits the intestinal absorption of glucose. In the first period that Dicumarol is present (second absorption) the inhibition is approximately of 23 %. This inhibition due to Dicumarol persist in the third absorption in spite of conditions, the absorption of arabinose decreases notably from the first to the second absorption but is maintained at a constant level in the subsequent periods.

In experiments where Dicumarol was present in the third and fourth periods, the absorption of arabinose in these periods was of the same order or superior than in the first absorption and certainly much greater than in the second absorption without Dicumarol.

When Dicumarol was present in the second and fourth absorptions, some increase in intestinal absorption of arabinose is

TABLE II

Effect of Dicumarol on the in vivo *Intestinal Absorption of L-arabinose*

In each rat, four successive absorptions of a 50 mM of L-arabinose in a saline solution. The presence of 10^{-4} M of Dicumarol in the same solution, where indicated. Absorption time: 60 minutes.

Nr. Animals	Dicumarol 10^{-4} M	Successive Absorptions (μM/cm/60 min.)			
		1st Absorption	2nd Absorption	3rd Absorption	4th Absorption
9	—	2.2 ± 0.27	1.0 ± 0.16	1.0 ± 0.14	0.9 ± 0.15
8	In 3rd and 4th Abs.	1.5 ± 0.23	0.7 ± 0.14	1.8 ± 0.28	0.8 ± 0.19
8	In 2nd and 4th Abs.	1.8 ± 0.30	2.2 ± 0.28	0.8 ± 0.14	2.1 ± 0.24

observed in the second absorption as compared to the first, implying a strong stimulation to absorption if the comparison is made in respect to the average value for the control animals in the second period. The third absorption, without Dicumarol, is of the same order as that displayed by the controls, and as such much inferior to the first absorption. In the fourth absorption, again with Dicumarol, arabinose is absorbed to a degree approximately corresponding to the second absorption and therefore in a proportion superior to what occurs in the third absorption without Dicumarol.

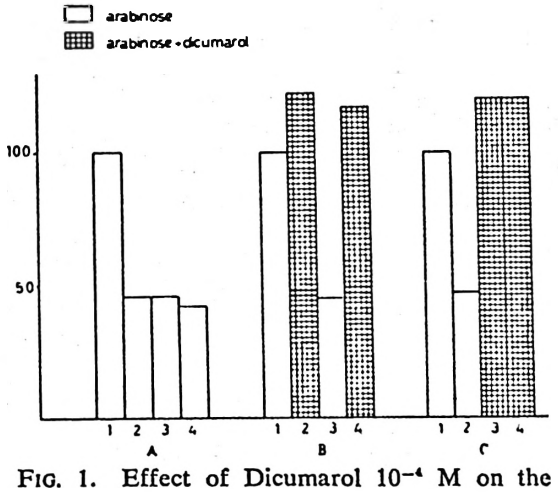

FIG. 1. Effect of Dicumarol 10^{-4} M on the intestinal absorption of L-arabinose *in vivo*. In each rat, four successive absorption with L-arabinose 50 mM in saline solution. The arabinose absorbed in the first absorption is taken as 100. Group A: Without Dicumarol. Group B: Dicumarol in the 2nd and 4th absorptions. Group C: Dicumarol in the 3rd and 4th absorptions.

The effects of Dicumarol can very well be appreciated in Fig. 1 which shows the results for the three groups of animals: The control and those that absorb arabinose in the presence of Dicumarol during the third and fourth periods or during the second and fourth periods.

A clear and intense effect of Dicumarol is shown in facilitating the intestinal absorption of arabinose.

Discussion

Glucose absorption to the blood, from a 2.77 mM luminal solution, requires an active transport. This process remains partially inhibited by 10^{-4} M Dicumarol. The mechanism of its action can not be related to its hypothrombinemic effects, owing to the latency period required to take such effects. Emphasis should therefore be laid on its action on membrane transport or on the epithelial cell metabolism. The fact that the inhibition persists even after profusely washing the in-

testinal loop in order to eliminate any residual Dicumarol demonstrates that the action is not due to competition with the glucose entry. The persistency of the effect may be due to the penetration of Dicumarol into the epithelial cells or due to its attachment in sites on the membrane thereby impairing the active transport of glucose. The diverse actions of Dicumarol on the respiratory chains and oxidative phosphorylation (1, 2, 4, 5) can explain its inhibitory effect on the intestinal absorption of glucose, in like manner to other uncoupling agents, such as dinitrophenol, dinitrocresol and attractyloside, which are also inhibitors (7).

The action of Dicumarol on the intestinal absorption of arabinose is of particular interest. As this pentose is not actively transported across the intestinal wall, it is independent of the utilization of metabolic energy and therefore not affected by the various uncoupling agents of oxidative phosphorylation (7). The results obtained evidently demonstrate that Dicumarol does not inhibit the intestinal absorption of arabinose, but, unlike the other uncoupling agents, facilitates the intestinal absorption of arabinose. The absorption of arabinose is doubled when Dicumarol is present in the solution, than during its absence.

On the other hand, contrarily to what happens to the inhibition of the glucose absorption, the facilitation of arabinose absorption does not continue if after one period of absorption with Dicumarol the subsequent one is performed on its absence in the sugar solution. This suggests that the stated action can not be attributed to metabolic alterations in the epithelial cells owing to the entered Dicumarol, but to some other direct effect on the membrane which makes it more permeable to the pentose.

It is otherwise of great interest that Dicumarol has similar effects on the intestine as on the diaphragm, inhibiting the penetration of glucose and facilitating that of arabinose in both the systems.

Summary

The *in vivo* technique of successive absorption was utilized to investigate the effect of Dicumarol on the intestinal absorption of D-glucose and L-arabinose.

A 10^{-4} M concentration of Dicumarol inhibits the absorption of glucose. This effect persists in a successive absorption without Dicumarol.

On the contrary, the absorption of L-arabinose is enhanced by Dicumarol when present in the sugar solution.

References

1. CHANCE, B.: In *Regulation of Cell Metabolism*, Ciba Foundation Symposium. Ed. Little and Brown, Boston, Mass., p. 91, 1959.
2. CHANCE, B., and WILLIAMS, C. R.: *Advances in Enzymol.*, **17**, 65, 1956.
3. LARRALDE, J., and GIRÁLDEZ, A.: *R. esp.* Fisiol., **13**, 253, 1957.
4. MARTIUS, C.: In *Regulation of Cell Metabolism*, Ciba Foundation Symposium. Ed. Little and Brown, Boston, Mass., p. 194, 1959.
5. MARTIUS, C.: *Biochem. Z.*, **329**, 450, 1957.
6. NELSON, N.: *J. Biol. Chem.*, **153**, 375, 1944.
7. PONZ, F., and LLUCH, M.: *R. esp. Fisiol.*, **16**, sup. I, 297, 1960.
8. PONZ, F., and LLUCH, M.: *R. esp. Fisiol.*, **18**, 123, 1962.
9. RODRÍGUEZ-CANDELA, J. L., and SALINAS, M.: *Proceed. VIth I.D.F. Congress*, Estocolmo, 1967.
10. SOLS, A, and PONZ, F.: *R. esp. Fisiol.*, **3**, 207, 1947.
11. VIDAL-SIVILLA, S., SOLS, A., and PONZ, F.: *R. esp. Fisiol.*, **6**, 195, 1950.