REVISTA ESPAÑOLA DE FISIOLOGIA
R. esp. Fisiol., 20, n.º 2, págs. 69-75, 1964.

Department of Physiology and Biochemistry (C.S.I.C.)
Faculty of Sciences
University of Barcelona

The effect of various metabolic and sugar transport inhibitors on the oxygen uptake of the intestinal mucosa

by

F. Ponz and J. Balasch*

(Recibido para publicar el 5 de abril de 1964)

The mucosal cells of the small intestine may, through active transport, accumulate certain sugars against a gradient of concentration, absorbing them from the intestinal lumen and freeing them on the opposite serosal side, a process which requires metabolic energy. The active transport may be inhibited by a block in the cellular metabolism which has to provide the energy required, or by a block in the transport mechanism at the level of the membrane (8,21). It is interesting to know up to what point an inhibitor of the active transport excerts its action in one or other of these ways, and this sometimes depends on its concentration.

In a previous work (2) a study was made of the oxygen uptake of the intestinal mucosa of the rat in the absence of external substrate, and of the variations in such uptake induced by the presence of different sugars in the medium. In the present work we have investigated the effect of some metabolic inhibitors, well-known as inhibitors of the active transport of sugars, on the oxygen consumption of the mucosa, analysing their behaviour in accordance with their concentration, and in the presence or absence of external sugar.

Material and methods

Preparations of jejunum mucosa of the rat were used, in Krebs-Ringer-Phosphate, the oxygen uptake being measured by the manometric technique of Warburg, as described in a previous work (2).

The inhibitors were dissolved in the 2.5 ml. of the medium, in the final concentrations indicated in each case. The sugars added to the medium were glucose or fructose, both readily metabolizable. Use was made of phlorizin, dinitrophenol, dinitrocresol, sodium cyanide, sodium azide and a compound of quaternary ammonium.

Results

The results obtained are expressed in the corresponding tables in percentage

* With a grant from «Comisaría de Protección escolar».

TABLE I

Effect of Phlorizin on the oxygen uptake of the jejunum mucosa of the rat. Krebs-Ringer-Phosphate, with or without sugar, 60 minutes. Differences in percentages with respect to equal controls without inhibitor.

Preparations n.°	Phlorizin M	Sugar mM	Differences %
3	5×10^{-4}	——	— 50.5 ± 1.7
6	8×10^{-5}	——	— 2.8 ± 3.2
4	8×10^{-5}	glucose 2.77	— 25.7 ± 0.6
18	5×10^{-5}	——	+ 0.4 ± 2.5
5	5×10^{-5}	glucose 0.69	— 21.2 ± 6.5
6	5×10^{-5}	glucose 1.38	— 33.9 ± 3.9
10	5×10^{-5}	glucose 2.77	— 22.4 ± 4.5
7	5×10^{-5}	glucose 22.22	— 28.8 ± 5.9
9	5×10^{-5}	glucose 44.44	— 25.9 ± 4.8
6	5×10^{-5}	fructose 5.55	— 1.4 ± 0.6
6	5×10^{-5}	fructose 11.11	— 0.1 ± 4.5
5	5×10^{-6}	glucose 2.77	— 1.1 ± 1.8
6	10^{-6}	glucose 2.77	— 1.9 ± 2.5

differences with respect to the oxygen consumption of preparations subjected to the same experimental conditions, but in the absence of the respective inhibitor. The control values, in the presence or absence of sugar, can be found in the previous work (2).

Phlorizin. This was tested at concentrations between 10^{-6} M and 10^{-4} M, in from of the mucosa, in the absence or in the presence of glucose or fructose at different concentrations (Table I).

At the concentration of 5×10^{-4} M, the phlorizin inhibits the consumption of oxygen in the absence of substrate, which indicates that it must penetrate into the cell in proportion to its effect on the metabolism (15,26). The concentration of 8×10^{-5} M no longer inhibits respiration in the absence of substrate, while it produces inhibition when there is glucose in the medium. The same happens with phlorizin at 5×10^{-5} M. If instead of glucose there is fructose, there is no inhibition. The inhibition proved to be reversible by washing of the mucosa with Krebs-Ringer-Phosphate solution. The degree of inhibition of oxygen uptake at these levels of phlorizin 5×10^{-5} M, is aproximately 25 % and varies little or not at all when the concentration of glucose in the medium is modified, between 0.69 and 44.44 mM.

In order to determine this last point with greater precision, fragments of the same mucosa were incubated in the presence or phlorizin 5×10^{-5} M, some with glucose 0.69 mM and others with glucose 44.44 mM. The respective means (7 experiments) of oxygen uptake were 115.9 and 117.4 μl O_2/100 mg. f. w., thus the inhibition in the two cases do not present significant differences.

Dinitrophenol. It was tested at concentrations between 10^{-4} M and 10^{-6} M. (Table II.)

TABLE II

Effect of Dinitrophenol on the oxygen uptake of the jejunum mucosa of the rat. Krebs-Ringer-Phosphate, with or without sugar, 60 minutes. Differences in percentages with respect to equal controls without inhibitor.

Preparations n.°	Dinitrophenol M	Sugar mM	Differences %
9	10^{-4}	——	— 66.2 ± 2.5
6	5×10^{-5}	——	— 42.1 ± 3.2
3	2×10^{-5}	——	— 3.8 ± 1.2
9	10^{-5}	——	+ 16.5 ± 5.1
9	10^{-5}	glucose 2.77	+ 22.8 ± 7.2
6	10^{-5}	fructose 5.55	+ 11.0 ± 3.0
6	10^{-6}	——	+ 0.2 ± 3.6
6	10^{-6}	glucose 2.77	— 1.1 ± 3.1

Dinitrophenol 5×10^{-5} M still strongly inhibits the respiration of intestinal mucosa, in the absence of external substrate. It is only on reaching 2×10^{-5} M that this effect no longer appears. On the other hand, Dinitrophenol 10^{-5} M provokes an increase in oxygen uptake whether in the presence or in the absence of sugar in the medium. At 10^{-6} M, it no longer has any effect.

Dinitrocresol. This agent acts, like DNP, also uncoupling the oxidative phosphorylation and presents similar effects to those found with DNP. (Table III.)

Dinitrocresol, at 10^{-5} M and higher concentrations, inhibits the oxygen consumption of the mucosa in the absence of substrate to a degree which depends on the concentration. On the other hand, with glucose 2.77 mM in the medium, DNC 10^{-5} M increases the respiration.

Sodium cyanide. The inhibition of the oxygen uptake commences above 10^{-4} M and increases with the concentration. For the same concentration of cyanide, the inhibition is higher when there is sugar in the medium (Table III.)

Sodium azide. Azide 5×10^{-4} M inhibits the oxygen consumption in the presence of glucose 2.77 mM in the medium, but it has no effect in the absence of substrate. In greater concentrations there is a marked inhibition in both cases. (Table III.)

Quaternary ammonium. We have utilized the diisobutyl-phenoxy-etoxy-ethyl-dimethyl benzylammonium chloride.

At concentrations higher than 10^{-4} M, the quaternary ammonium inhibits respiration in the absence of substrate, the inhibition increasing with the concentration of inhibitor. However, at 10^{-4} M there is no effect in the absence of external substrate, while the increase in oxygen uptake due to the presence of glucose 2.77 mM is inhibited by 26.8 %. This inhibition is not produced if the mucosa is incubated with the compound of quaternary ammonium and then washed with Krebs solution, before determining the oxygen consumption in the presence of sugar.

TABLE III

Effect of several inhibitors on the oxygen uptake of the jejunum mucosa of the rat. Krebs-Ringer-Phosphate, with 2.77 mM glucose (G) or without it, 60 minutes. Differences in percentages with respect to equal controls without inhibitor.

Prep. n.°	Inhibitor M	Sugar	Differences %
A. Dinitrocresol			
4	10^{-4}	—	— 66.7
6	5×10^{-5}	—	— 54.4 ± 0.6
13	10^{-5}	—	— 12.5 ± 3.7
10	10^{-5}	G	+ 23.0 ± 0.7
B. Cyanide			
6	10^{-3}	—	— 50.9 ± 4.1
6	10^{-3}	G	— 74.0 ± 4.0
14	5×10^{-4}	—	— 15.0 ± 3.5
10	5×10^{-4}	G	— 29.5 ± 4.4
6	10^{-4}	—	+ 3.8 ± 2.6
C. Azide			
6	10^{-4}	—	— 56.4 ± 1.0
6	10^{-3}	G	— 45.9 ± 5.9
9	5×10^{-4}	—	+ 0.63 ± 2.2
6	5×10^{-4}	G	—29.5 ± 5.5
6	10^{-4}	—	+ 3.9 ± 0.6
6	10^{-3}	G	+ 1.3 ± 1.4
D. Quaternary ammonium			
6	10^{-3}	—	— 46.0 ± 0.8
6	5×10^{-4}	—	— 20.7 ± 7.7
12	10^{-4}	—	+ 1.1 ± 3.7
12	10^{-4}	G	— 26.8 ± 5.2

Discussion

Phlorizin. The inhibition by this glucoside of the absorption of glucose, galactose and other actively transported sugars has been amply confirmed «in vivo» and «in vitro» (8,21). On the other hand, it has no effect on the absorption of pentose (27) or sorbose (6). Neither does it inhibit the absorption of fructose (5,11). Some observations made us think of the effect of the glucoside in low concentrations, which would be exercised on the penetration of the glucose at the level of the membrane (22-23). These concentrations, of about 10^{-4} M, inhibit the absorption, while they do not inhibit the respiration of the mucosa (17,25). The effect of the phlorizin has been interpreted as one of competitive inhibition between the glucoside and the sugar by a carrier in the membrane (1).

In our experiments we have compared the effect of the phlorizin on the oxygen consumption in the absence and presence of substrate. It has been determined that the concentration 5×10^{-4} M still strongly inhibits (50 %) the oxygen uptake in the absence of substrate, and that it is only on reaching 8×10^{-5} M that it proves ineffective on the endogenous respiration. The respiration of the preparations of mucosa is thus more sensitive than that of the everted sacs of intestine used by Smyth and his colleagues (17). On the other hand, the consumption of oxygen in the presence of glucose in different concentrations is inhibited by between 20 and 30 % with phlorizin 5×10^{-5} M, which may be attributed to the fact that, in the presence of the glucoside, the level of endocellular sugar diminishes by inhibition of the penetration of the sugar, and with this the elevation of oxygen uptake due to the external glucose is less than in the absence of phlorizin. This effect, however, does not appear with fructose in the medium, since the penetration of this sugar is not inhibited by phlorizin (5,11).

As the phlorizin seems to produce its effect through competition with the sugar (1), we planned experiments with very different external concentrations of glucose. Contrary to what might have been expected, the inhibition of oxygen consumption with phlorizin 5×10^{-5} M is amply independent of the external concentration of the sugar. This, at first sight, does not support the view that the inhibition by the phlorizin is competitive, for in this case the inhibition would necessarly be much less with high concentrations of glucose (44.44 mM) than with those over 60 times lower (0.69

mM). The explanation might be that our experimental period of 60 minutes was long enough to reach endocellular levels of glucose in the presence of phlorizin which were not very different, in spite of the notable differences in external concentration.

Dinitrophenol. It is known that DNP in concentrations of about 2×10^{-4} M and 5×10^{-5} M uncouples the oxidative phosphorylation of the respiration (13). DARLINGTON and QUASTEL (10) showed that dinitrophenol 10^{-4} M notably inhibited the active transport of glucose through the intestine, and attributed it to the fact that this concentration inhibited the oxidative phosphorylation, but not the respiration. This same concentration of DNP has been used by several other authors to investigate the dependence of the active transport of a substance on the process of oxidative phosphorylation.

We have found that, at 10^{-4} M and even 5×10^{-5} M, DNP inhibits the respiration of the mucosa in the absence of substrate. It is only on reaching concentrations lower than 2×10^{-5} M that we find the typical effect of elevation of the oxygen consumption by blocking of the oxidative phosphorylation. Therefore, the inhibitions of absorption found at concentrations higher than 2×10^{-5} M cannot be attributed solely to inhibition of the oxidative phosphorylation, but might be due to the less specific effects of the inhibition of the respiration. With 10^{-6} M of DNP we have no longer observed any effect.

The stimulation of the oxygen uptake in the absence of substrate at 10^{-5} M concentration also appears when there is glucose or fructose in the medium with slight quantitative differences. Probably the inhibition of the transport of sugar at this concentration is such that it hardly modifies the availability of oxidable substrate, so that the typical respiratory increase can make its appearance.

Dinitrocresol. This presents a form of action similar to that of DNP. It is also a potent uncoupling agent of the oxidative phosphorylation (7). At a concentration of 3×10^{-4} M it inhibits the absorption of glucose «in vivo» (24) and, at 10^{-4} M, it completely inhibits the active transport «in vitro» (9, 3, 4).

In our experiments, at 10^{-4} M and even at 5×10^{-5} M it still exerts an important inhibition of the respiration in the absence of substrate. At 10^{-5} M a slight inhibition is produced in the absence of substrate and an increase of oxygen uptake when there is glucose in the medium. According to these results, the inhibition of the transport of sugar at 10^{-4} M —as we have seen for DNP— should be interpreted by the general effects of the blocking of the metabolism of the mucosa cells, and not by an exclusive uncoupling effect of the oxidative phosphorylation.

Sodium cyanide. The inhibiting effect of cyanide on the respiration, which acts principally on the cytochromo-oxydase, has long been well-known. DARLINGTON and QUASTEL (10) showed that cyanide at 10^{2} M completely inhibits the active transport of glucose «in vitro», without altering the diffusion of sorbose. «In vivo» the effect of cyanide on the absorption of glucose is difficult to separate from the general toxic effect (19).

Ours results show that, at 5×10^{-4} M and higher concentration, inhibition of the respiration is produced both in the absence and in the presence of substrate. With 10^{-2} M, the concentration used by DARLINGTON and QUASTEL, the respiratory inhibition of the oxygen uptake is very considerable, which explains why in fact active transport of glucose, depending on metabolic energy, is not effected.

Sodium azide. This presents an action analogous to that of cyanide and is active at the same concentrations. At 3×10^{-3} M, it inhibits «in vivo» the intestinal absorption of glucose (18,23) and at 10^{-2} M it completely inhibits the active transport of this sugar «in vitro» (10). As we have seen, azide 10^{-3} M inhibits the respiration in the absence and in the presence of glucose, while at 5×10^{-4} M there is no inhibition of the endogenous respiration, but there is inhibition of the increase in oxygen uptake provoked by the presence of the sugar in the medium. These effects may be produced through the inhibition of several enzymatic processes, as blocking of the cytochromo-oxydase (12) and the uncoupling of the oxidative phosphorylation (14).

The concentration 10^{-2} M employed by DARLINGTON and QUASTEL (10) does not permit to discriminate between these effects, since at this level such a considerable metabolic inhibition is produced that the active transport is necessarily blocked.

Quaternary ammonium. The compound utilized inhibits the fermentation of glucose by yeast (16) at concentrations which have no effect on the endogenous respiration, by a preferential effect at the membrane itself on the penetration of the sugar. It also inhibits the intestinal absorption «in vivo» (20).

We have not observed effects on the respiration of the intestinal mucosa in the absence of substrate with concentrations of 10^{-4} M, while the consumption of oxygen is inhibited in the presence of glucose. This inhibition is reversible by washing of the mucosa with Krebs solution, which seems to confirm that, in the intestinal mucosa also, its effect at this concentration is exerted at the level of the membrane and not directly upon the cellular metabolism. At 5×10^{-4} M and higher concentrations inhibition of the respiration can already be observed in the absence o substrate, which indicates that compound already enters into the cell in an appreciable proportion.

Summary

A study has been made of the effect of various metabolic inhibitors on the oxygen uptake of the jejunum mucosa of the rat (Warburg method, Krebs-Ringer-Phosphate), in the absence or presence of metabolizable sugars in the medium.

Phlorizin inhibits the endogenous respiration at 5×10^{-4} M (50 %). At $5 \times \times 10^{-5}$ M, it no longer inhibits the endogenous respiration, but it does inhibit the oxygen uptake in the presence of glucose, independently of the external concentration of the sugar (between 0.69 and 44.44 mM). It has no effect in the presence of fructose. At 10^{-6} M, it has no effect, with or without glucose in the medium.

Dinitrophenol inhibits the endogenous respiration still at 5×10^{-5} M (42 %). At 10^{-5} M, it increases the endogenous respiration and the consumption of oxygen in the presence of glucose or fructose. It has no action at 10^{-6} M. The blocking described of the active transport of sugars at 10^{-4} M must refer to respiratory inhibition and not solely to uncoupling of the oxidative phosphorylation.

Dinitrocresol has an action similar to that of DNP, but at 10^{-5} M it does not elevate but still slightly inhibits the consumption of oxygen in the absence of substrate, while it increases it somewhat in the presence of glucose.

Cyanide still inhibits the endogenous respiration at 5×10^{-4} M (15 %), and also inhibits the oxygen uptake with glucose in the medium (30 %). At 10^{-4} M it has no effect.

Azide, at 5×10^{-4} M, no longer inhibits the respiration in the absence of

substrate, and inhibits it when there is glucose in the medium; it has no effect at 10^{-4} M.

A quaternary ammonium compound at 10^{-4} M does not inhibit the consumption of oxygen without external sugar, while it does inhibit it when there is glucose present.

Phlorizin, at 5×10^{-5} M, and the quaternary ammonium compound, at 10^{-4} M, must exert their inhibition at the level of the cellular membrane, impairing the entrance of glucose. The other inhibitors tested, and these same two at higher concentrations, block the metabolism and with it the oxygen uptake.

Bibliografía

(1) ALVARADO, A. and R. K. CRANE : *Biochim. Biophys. Acta*, **56**, 170, 1962.

(2) BALASCH, J. y F. PONZ : *R. esp. Fisiol.*, **19**, 177, 1963.

(3) BILHER, I. and R. K. CRANE : *Fed. Proceedings*, **20**, 1, 1961.

(4) BILHER, I., A. K. HAWKINS and R. K. CRANE : *Biochim. Biophys. Acta*, **59**, 94, 1962.

(5) BOGDANOVE, E. M. and S. B. BARKER : *Proc. Soc. Exptl. Biol. Med.*, **75**, 777, 1950.

(6) BRÜCKNER, J. : *Helv. Physiol. et Pharmacol. Acta*, **9**, 259, 1951.

(7) CLOWES, G. H. A., A. K. KELTCH, C. F. STRITTMATER and C. P. WALTERS : *J. Gen. Physiol*, **33**, 555, 1950.

(8) CRANE, R. K. : *Physiol. Rev.*, **40**, 789, 1960.

(9) CRANE, R. K. and P. MANDELSTAM : *Biochim. Biophys. Acta*, **45**, 460, 1960.

(10) DARLINGTON, W. A. and J. H. QUASTEL : *Arch. Biochem. Biophys.*, **43**, 194, 1953.

(11) FRIDHANDLER, L. and J. H. QUASTEL : *Arch. Biochem. Biophys.*, **56**, 412, 1955.

(12) HORECKER, B. I. and J. N. STANNARD : *J. Biol. Chem,*, **172**, 589, 1948.

(13) LOOMIS, W. F. and F. LIPMANN : *J. Biol. Chem.*, **173**, 807, 1948.

(14) LOOMIS, W. F. and F. LIPMANN : *J. Biol. Chem.*, **179**, 503, 1949.

(15) LOTSPEICH, W. D. and D. M. KELLER : *J. Biol Chem.*, **222**, 843, 1956.

(16) PARÉS, R. : *R. esp. Fisiol.*, **12**, 153, 1956.

(17) PARSONS, B. J., D. H. SMYTH and C. B. TAYLOR : *J. Physiol.*, **144**, 387, 1958.

(18) PONZ, F. : *R. esp. Fisiol.*, **8**, 261, 1952.

(19) PONZ, F. : *R. esp. Fisiol.*, **9**, 277, 1953.

(20) PONZ, F. : *R. esp. Fisiol.*, **12**, 43, 1956.

(21) PONZ, F. : *Rapports Vies. Journées Biochimiques Latines*, Genève, 1961.

(22) PONZ, F. y J. LARRALDE : *R. esp. Fisiol.*, **8**, 71, 1952.

(23) PONZ, F. y M. LLUCH : *R. esp. Fisiol.*, **11**, 267, 1955.

(24) PONZ, F. y M. LLUCH : *R. esp. Fisiol.*, **16**, Suppl. I, 297, 1960.

(25) PONZ, F. y A. PEÑALVER : *Actas Soc Esp. Fisiol.*, 311, 1955.

(26) SHAPIRO, B. : *Biochem. J.*, **41**, 151, 1947.

(27) WERTHEIMER, E. : *Arch. Ges Physiol.*, **233**, 514, 1934.