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Effect of Inhibitors on the Intestinal Active Transport of Glucose in Tortoise

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Glucose intestine active transport in tortoise *Testudo hermanni robertmertensi* Wermuth has been studied *in vitro* in the presence of known sugar active transport inhibiting substances. The final Serosal/Mucosal gradient is practically the same in aerobic and anaerobic conditions. A 10^{-4} M concentration of DNP inhibits active transport of glucose and increases O_2 uptake; a 10^{-3} M concentration reinforces transport inhibition and lowers O_2 uptake to normal values. Nullification of glucose active transport was not achieved by any of the DNP essayed concentrations. NaF greatly inhibits both glucose active transport and O_2 uptake, whereas phlorizin inhibits transport and does not affect respiration.

Tortoise intestine is able to obtain the required energy for its glucose active transport through both aerobic and anaerobic metabolism. Besides its oxidative phosphorylation uncoupling action, DNP also appears to affect glycolysis. Glycolysis inhibition and intestinal epithelial alteration may be responsible for the strong inhibition caused by NaF. Phlorizin seems to inhibit sugar transport by competence on the sugar carrier, at membrane level, without disturbing cellular metabolism.

Active transport of sugars may be blocked at the level of cellular metabolism supplying the required energy or at that of the transport system itself located in the membrane (2, 11).

It has been shown that glucose is actively transported by the intestine of *Testudo hermanni robertmertensi* Wermuth, whereas galactose is not (10). In this paper, the effect of known metabolic inhibitors and other glucose active transport affecting substances has been studied by *in vitro* intestinal preparations from this species.

Materials and Methods

Tortoise specimens of *Testudo hermanni robertmertensi* Wermuth, measuring from 10 to 17 cm in shell length have been

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used. Four intestinal segments, prepared according to WILSON-WISEMAN's method for everted sacs (15), and suspended in a Krebs-Ringer-Tris saline medium (13) adapted to reptile tissue, were obtained from each animal. A detailed explanation of the experimental procedure has been described in a former paper (10). At the beginning of each experiment the concentration of glucose and that of the employed inhibitor were the same in both serosal and mucosal compartments. Initial glucose concentration was 0.5 mM, and the mucosal volume, 4 ml. Incubation was conducted in Warburg flasks at 30° C in O_2 atmosphere in all the experiments, excepting those in anaerobiosis where N_2 substituted O_2. Each experiment lasted 60 minutes.

Oxygen uptake was measured by WARBURG's direct method (14), and D-glucose(u)-^{14}C was determined by liquid scintillation (6). Glucose transport is expressed by Sf/Mf ratio, where Sf and Mf represent the number of counts per minute (c.p.m.) in the serosal and the mucosal compartments at the end of the incubation period.

Results

Active transport of D-glucose in anaerobiosis. The solution for the mucosal and serosal compartments was brought to anaerobic condition through gasification with N_2 during 10 minutes before initiation of the experiment. After preparing the sacs, the Warburg flasks were again gasified with nitrogen for other 5 minutes and remained in N_2 atmosphere during the experiment.

As table I shows, glucose was actively transported in the presence as well as in the absence of O_2, without any significant variations in the final gradients. This reveals that the tortoise anaerobic metabolism easily yields the required energy for the active transport of glucose.

Effect of 2-4-dinitrophenol (DNP). A 10^{-5} M concentration of this inhibitor did not alter active transport of glucose, but 10^{-4} M and 10^{-3} M concentrations inhibited it 61 and 72 % respectively. Its

Table I. *Active transport of D-glucose and O_2 uptake by tortoise intestine* (T. hermanni robertmertensi *Wermuth) in the presence of different inhibitors.*

Everted intestinal sacs were suspended in KRT solution at the initial glucose concentration of 0.5 mM in both compartments. The Sf/Mf ratio represents the c.p.m. relation between serosal and mucosal at the end of the incubation period. Oxygen uptake by tissue refers to wet weight. Mean values are given with their standard errors. In parenthesis, number of experiments. P values correspond to the statistical analysis of the difference between the means of control and experiment values. Every experiment lasted one hour.

Experiment characteristics	Final gradient Sf/Mf	O_2 uptake μl O_2/100 mg w.w.
Control	1.81±0.41(5)	19.60±1.21(5)
Anaerobiosis	1.81±0.20(11) $p < 0.9$*	—
Control	2.53±0.25(40)	20.35±0.45(46)
DNP 10^{-5} M	2.57±0.25(9) $p < 0.9$*	26.28±2.37(9) $p < 0.02$
DNP 10^{-4} M	1.59±0.16(18) $p < 0.02$	32.85±1.08(18) $p < 0.001$
DNP 10^{-3} M	1.42±0.07(10) $p < 0.05$	19.81±0.44(10) $p < 0.5$*
NaF 10^{-2} M	1.22±0.11(9) $p < 0.02$	26.51±0.83**(9) $p < 0.7$*
NaF 9×10^{-2} M	0.94±0.01(6) $p < 0.02$	14.80±1.46(6) $p < 0.001$
Phlorizin 10^{-6} M	1.17±0.10(8) $p < 0.02$	20.12±1.97(8) $p < 0.8$*
Phlorizin 10^{-5} M	1.11±0.11(8) $p < 0.02$	19.37±1.37(8) $p < 0.7$*

* = not significant.
** The control for this group was 25.64 ± 1.22.

complete nullification, however, was never achieved.

Oxygen uptake increased 67 % with 10^{-4} M DNP in the medium, as it is usual with uncoupling agents of the oxidative phosphorlyation, and returned to normal values at a ten times higher DNP level (table I).

Effect of sodium fluoride. Fluoride is a well known glycolytic inhibitor. As CaF_2 is insoluble, the $CaCl_2$ salt present in the physiological solution was omitted in these experiments. This omission did not affect active transport of glucose.

At 10^{-2} M concentration of NaF, glucose transport was practically non-existent, and it was totally blocked on substituting all NaCl in the medium for NaF (table I).

On the other hand, oxygen uptake was not affected by 10^{-2} M NaF but a 9×10^{-2} M concentration of inhibitor decreased it greatly.

Effect of phlorizin. Active transport of glucose was strongly inhibited at 10^{-6} M or higher concentrations of phlorizin. The presence of this substance did not significantly affect the O_2 intestinal uptake in tortoise (table I).

Discussion

The final gradients of glucose obtained under anaerobic conditions were not significantly different from those under aerobiosis. This result shows that the required energy for the active transport can be easily obtained by tortoise intestine from anaerobic metabolism.

Several publications have brougth to light the great tolerance of tortoise to anoxia (4, 5). SCHILB *et al.* (12) and KLAHR *et al.* (7, 8) have proved that a Na^+ «pump» placed in tortoise bladder acts efficiently during extended periods of time in the absence of O_2. Tortoise intestine can evidently use the oxidative process, but it is partially, and sometimes totally, dependent on glycolysis for the obtention of the required energy for its active transport of glucose.

DNP is a well known uncoupler of oxidative phosphorylation. A 10^{-5} M concentration of DNP did not seem to alter active transport of glucose, but it did increase slightly (29 %) O_2 uptake. DNP concentrations ten times higher clearly showed its uncoupling effect by increasing O_2 uptake to 67 % maximum and by strongly inhibiting at the same time active transport of glucose. A 10^{-3} M concentration of DNP reinforced transport inhibition and reduced O_2 uptake to normal values. None of the essayed concentrations produced a nullification of transport. In rat (11) on the contrary, a 10^{-5} M concentration of DNP completely nullifies *in vitro* glucose transport and increase to maximum O_2 uptake. This result is obvious since the active transport of sugars in rat entirely depends on the energy derived from oxidative metabolism, while this is not the case in tortoise.

KLAHR *et al.* (8) observed active transport of Na^+ in tortoise bladder in aerobiosis as well as in anaerobiosis, yet DNP inhibited active transport of Na^+. These authors suggested that DNP could inhibit a hypothetical highly energetic intermediary derived from glycolysis itself or from the glycolytic ATP.

Whatever the case, it can be said that active transport sensitivity to DNP in tortoise intestine is markedly inferior to that observed in mammals; a fact that partly coincides with the results previously obtained in snail (3), a species with similar characteristics concerning O_2 dependence.

Fluoride inhibits active transport of glucose and O_2 uptake, perhaps through a glycolysis inhibition that lessens energy production. A marked tissue desquamation, and mucosal liquid turbidity were further observed at the end of the incubation period.

Phlorizin markedly inhibits active transport of glucose in tortoise intestine. At

10^{-6} M and 10^{-5} M concentrations, active transport is inhibited 88 and 93 % respectively. Oxygen uptake, on the other hand, does not change. For this reason, the possible effect of this substance on cellular metabolism can be discarded, and its action may be explained by competitive inhibition on the sites of the sugar carrier, as it occurs in other species (1).

Resumen

Ha sido estudiado *in vitro* el transporte activo de glucosa por el intestino de *Testudo hermanni robertmertensi* Wermuth en presencia de determinadas sustancias, conocidas como inhibidoras del transporte activo de azúcares. El gradiente de acumulación de glucosa es el mismo en aerobiosis que en anaerobiosis. El DNP 10^{-4} M inhibe el transporte activo de glucosa al mismo tiempo que incrementa el consumo de O_2. A concentración 10^{-3} M aumenta la inhibición del transporte sin llegar a anularlo y se normaliza el consumo de O_2. El FNa inhibe fuertemente el transporte de glucosa y también el consumo de O_2. La florricina inhibe el transporte activo de glucosa, sin afectar al consumo de O_2.

En el intestino de tortuga, la energía para el transporte activo de glucosa puede obtenerse tanto del metabolismo aerobio como del anaerobio. El DNP además de actuar como desacoplante de la fosforilación oxidativa debe inhibir de alguna forma la glicolisis. La inhibición por el FNa se atribuye a inhibición de la glicolisis y a la alteración del epitelio intestinal. La florricina inhibe el transporte a nivel de la membrana sin alterar el metabolismo celular.

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