BBA Report

BBA 41218

Pasteur effect in the *in vitro* vascularly perfused rat small intestine

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(Received May 29th, 1972)

SUMMARY

Isolated small intestine perfused *in vitro* with media with low oxygen concentration was found to contain low levels of ATP when compared with rat small intestine *in vivo*. The addition of fluorocarbon FC 75 to an erythrocyte-free perfusion medium was found to result in a high phosphate potential and a low rate of lactate production from glucose in isolated perfused small intestine, resembling the *in vivo* condition. This allowed the demonstration of a Pasteur effect in that replacement of oxygen by nitrogen (or the addition of 2,4-dinitrophenol) led to a rapid increase of the rate of glycolysis, and a decrease of the ATP concentration in the tissue.

Earlier work from this laboratory[1] showed that mitochondrial preparations, isolated from epithelial cells from rat small intestine, carried out oxidative phosphorylation, although the coupling was not tight. The possibility was considered that the high Mg^{2+}-stimulated ATPase activity of the mitochondrial preparations, contaminated with brush-border fragments, could contribute to the absence of tight coupling. Indeed, more recently we[2] were able to show tight coupling in isolated rat small intestinal epithelium mitochondria by omitting Mg^{2+} from the incubation medium. Earlier, we had given the possibility of "loose coupling" of oxidative phosphorylation serious thought, since in isolated cells and everted jejunum of the rat a clearcut Pasteur effect was found to be absent[3–5]. Similarly, no Pasteur effect was observed in intestine of other species (*cf.* ref. 6). The explanation of this phenomenon could be a low energy charge[7] in rat small intestine, since the phosphofructokinase reaction is also stimulated in small intestine, if the ATP concentration is relatively low and the ADP, AMP and P_i concentrations are relatively high[8,9]. Indeed, in earlier studies we reported[1] relatively low levels of ATP when compared with those of ADP and AMP for isolated rat small epithelial cells, as well as for everted rat small intestine, incubated in a glucose-containing and aerated incubation medium. Also,

in studies with *in vitro* (vascular) perfused rat small intestine[10], the high rate of lactate production while the intestinal lumen was empty suggested to us that the energy charge here might also have been low. In the present paper it will be shown that an insufficient oxygen supply might be the basis of these phenomena, since the situation can be altered by increasing the availability of oxygen to the perfused tissue.

TABLE I

RELATIVE ATP, ADP AND AMP CONCENTRATIONS OF JEJUNUM PERFUSED WITH DIFFERENT MEDIA

Perfusion Medium I was Krebs–Henseleit[11] bicarbonate buffer (pH 7.4), containing 1.4 mM $CaCl_2$ and 0.6 mM $MgSO_4$ and in addition 4.7% dextran (mean mol. wt 70 000), 4 mg/100 ml promethazin and 5 mM glucose. Perfusion Medium II contained fluorocarbon FC-75 (3 M Company, St. Paul, Minn.), emulsified in Medium I, in which the dextran was replaced by 3.4% fatty acid-poor bovine serum albumin (Pentex, Kankakee, Ill.). The fluorocarbon emulsion was prepared[12] with the use of a nonionic polyalkylene oxide detergent F-68 (Wyandotte Chem. Corp., Wyandotte, Mich.). 40 ml of fluorocarbon were added to 200 ml dextran-free (perfusion) Medium I, containing 4 g F-68. After sonication (Branson Sonifier, Model S 75) for 4 periods of 5 min at 4.5 A (the temperature was kept below 6 °C), the emulsion was washed 3 times with 200 ml dextran-free Medium I by centrifugation at 9000 × $g_{av.}$. The washed sediment was finally suspended in 200 ml dextran-free Medium I, containing 3.4% albumin and again sonified as above for 4 periods of 3 min. The larger fluorocarbon particles were removed by centrifugation for 5 min at 450 × $g_{av.}$ and the supernatant filtered through Whatman No. 589. Perfusions were carried out as described earlier in ref. 10. Rats were pretreated with intravenous injections of 5 mg heparin and 1 mg papaverin 2 min prior to cannulation. The temperature of the isolated intestine was kept close to 37 °C with an infrared lamp and by rinsing with warm saline. After 15 min perfusion (or when indicated *In situ*) part of the jejunum was freeze clamped[13]. The frozen tissue was pulverized in a mortar while liquid N_2 was continuously added. The powder was mixed with 5% $HClO_4$, thawed, centrifuged and the supernatant filtered through Millipore (pore size 0.4 μm), neutralized with KOH and again centrifuged. ATP[14], ADP[15] and AMP[15] were determined as indicated. The nucleotide concentrations were calculated per ml extract and the sum of [ATP] + [ADP] + [AMP] made 100%. *n* = number of experiments; average values are given.

Perfusion medium	*Flow rate (ml/min)*	*n*	*% of adenine nucleotides*		
			ATP	*ADP*	*AMP*
I	15.8	2	78.5	18.8	2.7
I	4.8	3	70.0	22.5	7.5
II	10.8	2	84.0	14.2	1.8
In situ	–	4	82.1	15.9	2.0

It can be seen from Table I that perfusion with a modified Krebs–Henseleit[11] bicarbonate buffer, containing 5 mM glucose and saturated at 37 °C with O_2–CO_2(95:5,v/v), especially when the flow rate is low, leads to a lower energy charge than that of the rat small intestine *in situ* (non-resorptive state; rat under Nembutal narcosis). The situation *in situ* can be approached during perfusion *in vitro* of the isolated small intestine, when a fluorocarbon emulsion is added to the perfusion medium. The fluorocarbon-containing medium carries considerably more oxygen than the medium without fluorocarbon, as can

be calculated from the data of Ruigrok and Elbers[12]. Another series of experiments in which, in addition to ATP, ADP and AMP, creatine phosphate was determined and in which the values were expressed in nmoles per mg DNA, is seen in Table II.

TABLE II

CONCENTRATIONS OF ATP, ADP, AMP AND CREATINE PHOSPHATE IN VASCULARLY PERFUSED RAT SMALL INTESTINE UNDER DIFFERENT CONDITIONS

The perfusions were carried out with Medium II (see Table I). Creatine phosphate was determined in the ATP determination[14] after the exhaustion of ATP, by adding 3.6 units creatine kinase. DNA[16] was determined in the residues of $HClO_4$ extracts (*cf.* Table I). The values (nmoles per mg DNA) represent the mean of two separate experiments. The mean flow during vascular perfusions was 10.8 ml/min.

	In situ	*Aerobic perfusion (10 min)*	*Anaerobic perfusion (10 min)*
ATP	216.5	202.6	85.9
ADP	40.8	38.4	51.5
AMP	4.9	4.2	43.9
Creatine phosphate	245.1	276.6	10.6
$\Sigma \sim P$	718.9	720.2	233.9

Now that we have a means of increasing the energy charge of the tissue by the addition of fluorocarbon to the aerated perfusion medium, we reinvestigated aerobic glycolysis during perfusions *in vitro* . It can be seen from Table III that a Pasteur effect can be observed: in the presence of oxygen, the rates of glucose utilization and of lactate productions are low; when oxygen is replaced by nitrogen, or when the uncoupler of oxidative phosphorylation 2,4-dinitrophenol is added, the rates of glucose utilization and lactate production are strongly increased. These effects are very rapid, as can be concluded from the experiments shown in Fig. 1. The fact that in the presence of nitrogen the amount of lactate formed is less than twice the amount of glucose utilized (Table III) may be due to incomplete anaerobiosis, since the tissue remains exposed to air.

From these results it may then be concluded that proper oxygenation of the tissue leads to a high energy charge resulting in limitation of the rate of lactate production. The intestine *in vivo*, even during glucose transport, also shows a very low rate of lactate production[17] and so does the rat intestine perfused *in vitro* with a solution containing erythrocytes as well as norepinephrine and dexamethasone that results in an adequate perfusion pressure, as so elegantly worked out by Windmueller and Spaeth[18].

The authors wish to thank Mrs R. Kurpershoek-Davidov and Mr M. Machielse for technical assistance and Drs T.J.C. Ruigrok and H.R. de Jonge for advice.

TABLE III

GLUCOSE UPTAKE AND LACTATE PRODUCTION IN VASCULARLY PERFUSED RAT SMALL INTESTINE UNDER DIFFERENT CONDITIONS

The perfusions were carried out with Medium II (see Table I). Measurements were done over a perfusion time of 30 min. Glucose[9] and lactate[20] were determined in the recirculated medium at zero time and 30 min after centrifugation of the samples.

Perfusion conditions	*Glucose uptake (μmoles/h)*	*Lactate output (μmoles/h*	*Flow (ml/min)*
Aerobic	78	88	7.6
Aerobic	52	41	10.9
Anaerobic	310	545	10.9
Anaerobic	221	414	10.8
Aerobic + 0.5 mM dinitrophenol	228	277	10.7
Aerobic + 0.5 mM dinitrophenol	252	292	11.0

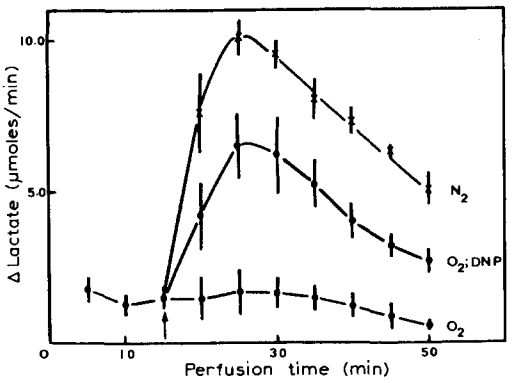

Fig. 1. Pasteur effect in vascularly perfused rat small intestine. The perfusions were carried out with Medium II (see Table I). Arterial and venous samples were taken from the recirculated medium at the times indicated. Mean flow was 10.7 ml/min. Where indicated dinitrophenol (DNP) was added to the incubation medium (final concn of 0.4 mM) or O_2–CO_2(95:5, v/v) was replaced by N_2–CO_2(95:5, v/v) Lactate was determined as described in Table III. The results are expressed as means ± S.E. (n = 4).

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