PREFERENTIAL OXIDATION OF FATTY ACIDS BY RAT SMALL INTESTINE

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In a previous study from this laboratory it was shown that mitochondria can be harvested from rat small intestinal epithelial cells and that these mitochondria show intact oxidative phosphorylation [1]. Further studies [2] showed that the isolated mitochondria are able to oxidize octanoate and palmitate completely to CO_2 and H_2O, whereas earlier studies [3] showed that 3-hydroxybutyrate is also a substrate for these mitochondria. It is the purpose of the present paper to extend these studies with observations carried out with isolated rat small intestine, perfused *in vitro* with glucose and/or long-chain fatty acids, octanoate or 3-hydroxybutyrate.

The present experiments are carried out with small intestines isolated from fed male white rats (250–300 g). The animals were operated under Nembutal narcosis. The operation was carried out in principle as described by Gerber [4]. The rectum was cut between two ligatures and mobilized, followed by a complete coecum and large bowel resection. The duodenum was opened about 5 cm from the stomach and a cannula inserted so that the small intestine could be rinsed with 50 ml warm saline. After the passage of air the duodenum and the terminal ileum were tied. The abdominal aorta was prepared free from the A. coeliaca until the bifurcatio iliaca. Loose ligatures were placed around the aorta, distal and proximal from the A. mesenterica superior, whereas the A. renalis dextra was tied. Loose ligatures were also placed under the V. porta and the V. mesenterica superior, after tying the V. gastricuduodenalis and V. lienalis. Then the aorta was cannulated with a needle (Braunüle, size 0.5 R; Braun, Melsungen, Germany) a little above the bifurcatio iliaca and the needle tip moved upwards until the A. mesenterica superior was reached. The cannula was fixed by tying the loose distal ligature. The ligature around the aorta proximal of the A. mesenterica superior was tied and the perfusion was started at a rate of 4 ml per min. The portal vein was opened as close to the liver as possible with another needle (Braunüle, size 1 R) and the tip of the cannula pushed away from the liver and fixed with the ligatures. The whole preparation was removed from the rat and placed on a perspex disc of the apparatus designed for liver perfusion by Scholz [5]. The perfusion rate was increased to 8 ml/min after a "zero time" sample of the perfusion medium was collected. During the cannulation, the rat's own blood was removed from the preparation by perfusion with about 20 ml "artificial" perfusion medium prior to recirculation was started. The perfusion volume amounted to 67 ± 14 ml and the temperature was 33 ± 2°. The perfusion medium was essentially according to Forth [6], consisting, in addition to the substrate to be specified, of Krebs-Henseleit [7] bicarbonate buffer (pH 7.4) with 5.6% polyvinyl pyrrolidone (M.W. 25,000–30,000) 3.4% fatty acid-poor bovine serum albumin (Pentex), 2 X washed human erythrocytes (hematocrit finally about 20%), 10 mg% papaverin, 4 mg % heparin and 4 mg % promethazin. Perfusion was terminated after 1 hr, the perfusion volume measured and a sample taken for analysis.

It can be seen from table 1 that glucose, long-chain

Table 1
Substrates for rat small intestinal metabolism during (vascular) in vitro perfusion.

Substrate added	Substrate used (μmole/hr)	Theoretical maximal rate of ATP formation[a] (μmole/hr)
5.2 mM glucose	110[b]	1480
0.36 mM 1-^{14}C-palmitate (1 μM = 0.015 μCi)	3.8	490
1.0 mM 1-^{14}C-palmitate (1 μM = 0.01 μCi)	12.1	1561
2.0 mM 1-^{14}C-octanoate (1 μM = 0.004 μCi)	20.6	1257
5.4 mM D(−)3-hydroxybutyrate	42.6[c]	912

[a] Assuming a P:O ratio of 3 for NADH oxidation.
[b] 75 μmole converted into lactate, since 150 μmole of lactate accumulated.
[c] In this experiment the rate of acetoacetate accumulation was 8.5 μmole/hr.

Perfusiontime 1 hr. Glucose, lactate, D(−)3-hydroxybutyrate and acetoacetate were determined in $HClO_4$-deproteinized and subsequently neutralized samples enzymatically, as shown in [8–11], respectively. Palmitate and octanoate disappearance was calculated from the radioactivity disappeared during perfusion. For these radioactivity measurements samples of the perfusionmedium, after centrifugation, were measured by liquid scintillation counting as described by Patterson and Greene [12].

and medium-chain fatty acids may be good substrates for metabolism. Low concentrations of palmitate are not well extracted, whereas D(−)3-hydroxybutyrate takes an intermediate position. In experiments with 3-^{14}C-DL-3-hydroxybutyrate (not shown) it was found that the percentage of radioactivity removed during perfusion was about twice as high as the percentage of D(−)3-hydroxybutyrate disappeared, indicating the utilization of L(+)3-hydroxybutyrate. This compound could be activated in the medium-chain fatty acyl-CoA synthetase reaction [13], prior to complete oxidation.

From table 2 it can be seen that, at least under the conditions of the test, glucose catabolism is not clearly influenced by continuous insulin addition. The animals were pretreated with mannoheptulose to ensure a low endogenous insulin concentration prior to operation. From these 4 experiments it appears that as an average, 71.2% of the glucose removed was converted to lactate and that as much as 28.8% was probably completely oxidized. This situation changes when together with glucose, oleate or octanoate are added to the perfusion medium (table 2). Now 95.8% (average) of the glucose is converted to lactate and only 4.2% is completely oxidized. From the measurement of the oleate or octanoate disappearance (corrected for the small amount accumulated in the intestine after perfusion, as measured in the homogenized tissue) it must be concluded that oleate or octanoate oxidation yield the bulk of the energy. It appears then that in small intestine during fatty acid oxidation, pyruvate oxidation is inhibited. For the possible mechanism of this inhibition, we refer to the work of Garland et al. [14], who concluded from their studies on pyruvate metabolism in muscle that inhibition of pyruvate dehydrogenase may be caused by high acetyl-CoA/CoA and NADH/NAD^+ ratios, and to a possible inactivation of pyruvate dehydrogenase as described by Linn et al. [15] and Wieland and Jagow-Westermann [16] in other tissues than intestine.

These present data are in agreement with other tissues in which fatty acids are also a preferred substrate when compared with glucose. However, under the present conditions glycolysis is not inhibited while fatty acids are oxidized (table 2). Maybe that here hexokinase is the rate-limiting step and not the phosphofructokinase or pyruvate kinase reaction (although pyruvate kinase has been found to be of the Liver-type as judged by the allosteric stimulation by fructose-1,6-P_2; results not shown). Srivastava and Hübscher [17] indeed observed that hexokinase in homogenates of small intestine is the enzyme which limits the rate of glycolysis.

Although the rate glycolysis is increased in diabetes [18], insulin is probably not directly influencing this process. In the first place we find no effect of insulin (table 2) during *in vitro* perfusion and in the second place diabetes and not fasting (both conditions with low insulin levels in the blood) causes an increased rate of glycolysis [18]. It is possible that not only glucose-feeding, after a period of fasting, increases soluble intestinal hexokinase [19, 20], by a release from particle-bound hexokinase

Table 2
Influence of insulin and fatty acids on glucose metabolism of in vitro perfused rat small intestine.

Pretreatment of rat	Addition to perfusion medium	Glucose used	Lactate formed	Fatty acid removed
			(μmole/hr)	
Mannoheptulose[a]	5.2 mM glucose	96	140	–
Mannoheptulose[a]	5.2 mM glucose	98	141	–
Mannoheptulose[a]	10 U insulin + 5.2 mM glucose	111	158.5	–
Mannoheptulose[a]	8.7 U insulin + 5.2 mM glucose	93	128	–
None	1.3 mM 1-^{14}C-oleate[b] + 5.2 mM glucose	93.5	179	7.4
None	1.2 mM 1-^{14}C-oleate[b] + 5.2 mM glucose	115	225	7.7
None	2 mM 1-^{14}C-octanoate[c] + 8.3 mM glucose	170	330	28.1
None	2 mM 1-^{14}C-octanoate[c] + 8.3 mM glucose	149	277	23.9

[a] 200 mg mannoheptulose (a gift from Schering A.G. Berlin, which is gratefully acknowledged) was injected intraperitoneally 2 hr prior to operation.
[b] 1 μM = 0.006 μCi.
[c] 1 μM = 0.004 μCi.

[21] mainly bound in the brush-border region of the epithelial cells [22], but also hyperglycemia per se. That hexokinase activity in intestinal mucosa indeed rises in diabetes has been shown earlier [23]. Anderson and Zakim [24] recently described that alloxan-diabetes, in contrast to fasting, in rats causes no reduction of jejunal hexokinase and pyruvate kinase.

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