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Antiketogenic Effect of Gluconeogenic Substrates. II. Effect of Pyruvate

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The effect of pyruvate on the fate of free fatty acids in isolated hepatocytes from starved rats has been studied. 5 mM pyruvate inhibited ketone bodies production variously from endogenous subtrates, acetate, octanoate and oleate. However, the incorporation of radioactivity into ketone bodies from acetate, octanoate or oleate was not affected by the presence of pyruvate. The oxidation of radioactive fatty acids to CO_2 was increased by the presence of pyruvate. These results suggest that pyruvate inhibited ketogenesis by increasing the rate of the tricarboxylic acid cycle.

The antiketogenic effect of lactate was studied by McGARRY and FOSTER (13) who showed that the effect of lactate was suppressed by ethanol. This fact suggests that the antiketogenic effect of lactate depends on its conversion to pyruvate. The mode of action of lactate and pyruvate on ketogenesis have been related to the competition between these compounds and free fatty acids for oxidation (12, 16); the modification of the redox state of the NAD couple in the cytoplasm and/or mitochondria (11); and the increase in the rate of the tricarboxylic acid cycle as a consequence of the enhancement of oxaloacetate concentration caused by lactate or pyruvate (8, 10, 17). However, none of these hypotheses has been proved beyond doubt. In addition, we have found (15) that another gluconeogenic substrate such as glycerol inhibited production of ketone bodies by increasing the rate of lipogenesis *de novo* without affecting the rate of the tricarboxylic acid cycle. The enhancement of lipogenesis *de novo* may be caused by small changes in glycerol phosphate concentrations (7, 15, 16); we therefore decided to investigate whether pyruvate inhibited ketogenesis through its conversion to glycerol phosphate or by another unknown mechanism.

Consequently, the present investigation was designed to study the effect of pyruvate on the fate of free fatty acids in isolated hepatocytes from starved rats. The effect oy pyruvate on ketone bodies production and on radioactivity incorporation in liver lipids, CO_2 and ketone

bodies from acetate, octanoate and oleate has been studied.

Materials and Methods

Reagents. Substrates, enzymes and coenzymes were obtained from Sigma, Boehringer, or Serva Feinbichemical. Standard analytical-grade laboratory reagents were purchased from Sigma or Merck (Darmstad, Germany). Bovine serum albumin was obtained from Armour Pharmaceutical Co. (Chicago, Illinois). Sodium pentobarbital was a gift from Abbot Laboratories (Madrid, Spain). 1-14C-acetate, 1-14C-octanoate and 1-14C-oleate were obtained from The Radiochemical Centre.

Animals. Female albino Wistar rats, fed on a stock laboratory diet, were used for experiments between 9 and 10 a.m. Animals were deprived of food 48 h before the experiments but were allowed to take water *ad libitum.*

Isolation of hepatocytes. Hepatocytes were prepared by the method of BERRY and FRIEND (3) with the modifications described by CORNELL *et al.* (4). Rat livers were perfused with calcium-free Krebs-Henseleit physiological saline containing colagenase and hyaluronidase (0.03 % w/v of each). After 20 min the liver was excised and incubated in the same medium for 10 min. The preparation was filtered through a nylon mesh (0.44 × 0.44 mm) and washed twice with Krebs-Henseleit physiological saline (9) containing dialysed bovine serum albumin (2.5 % w/v). 75-80 mg (wt.w.) of isolated hepatocytes were incubated in 4 ml of Krebs-Henseleit physiological saline containing dialysed bovine serum albumin (2.5 % w/v). The incubations were carried out at 37° C with carbogen (95 % O_2 : 5 % CO_2) as the gas phase. Hepatocytes coming from two rats were pooled and each pool considered as one observation. All experiments were carried out in duplicate.

Analytical methods. Acetoacetate and 3-hydroxybutyrate were assayed as described by WILLIAMSON *et al.* (18) and long chain acyl-CoA according to MICHAL and BERMEYER (14) Labeled ketone bodies were isolated as an acetone — mercurial complex as described by BATES *et al.* (2). Radioactivity found in the carbonylic carbon of acetoacetate was considered as one half of the total. Radioactivity in 3-hydroxybutyrate was calculated according to the 3-hydroxybutyrate/acetoacetate ratio measured spectrophotometrically. Radioactivity in CO_2 was measured by trapping in 1 N NaOH the CO_2 evolved after acidification of the incubation medium with 60 % (w/v) perchloric acid. Radioactivity in liver lipids was measured in the washed chloroform-methanol (2:1) liver cell extracts (5). These fractions are considered «liver lipids» which include triacylglycerols, phospholipids, cholesterol and cholesterol-esters.

Results

Table I shows the effects of pyruvate on ketone bodies production by isolated hepatocytes from starved rats. 5 mM pyruvate inhibited ketone bodies production from endogenous substrates, acetate, octanoate and oleate. The effect was especially significant in the case of endogenous substrates or acetate. 5 mM pyruvate inhibited acetoacetate synthesis from endogenous substrates and acetate but not from octanoate or oleate. 3-hydroxibutyrate production from endogenous substrates, octanoate and oleate, but not from acetate, was decreased by pyruvate. These results are not altogether surprising since acetate *per se* decreased 3-hydroxybutyrate synthesis. It is noteworthy that pyruvate inhibited the production of both species of ketone bodies only in the absence of exogenous substrates.

Table I. *Effect of pyruvate on ketone bodies production from endogenous substrates, acetate, octanoate and oleate.*
Hepatocytes from starved (48 h) rats were incubated for 1 h. Results are expressed in μmoles/h per g wet cells and are means ± S.E.M. (n = 6-8) * $p < 0.1$; ** $p < 0.01$ as compared with the same experiment in the absence of pyruvate.

Additions to the incubation medium	Acetoacetate	3-Hydroxybutyrate	Ketone bodies	3-Hydroxybutyrate/ acetoacetate
none	39 ± 1	20 ± 1	59 ± 1	0.47
5 mM pyruvate	10 ± 1 **	4 ± 1 **	14 ± 1 **	0.40
5 mM acetate	37 ± 2	7 ± 1	44 ± 2	0.16
5 mM acetate + 5 mM pyruvate	15 ± 1 **	4 ± 2	19 ± 2 **	0.26
2 mM octanoate	36 ± 3	58 ± 3	94 ± 6	1.81
2 mM octanoate + 5 mM pyruvate	32 ± 4	32 ± 3 **	64 ± 9 *	1.00
2 mM oleate	38 ± 1	50 ± 2	88 ± 3	1.33
2 mM oleate + 5 mM pyruvate	35 ± 2	36 ± 2 **	71 ± 4 *	1.03

Table II. *Effect of pyruvate on the fate of free fatty acids in isolated rat hepatocytes.*
Hepatocytes from starved rats were incubated for 1 h in the presence of 10 million d.p.m. per gram of wet cells of the radioactive fatty acid. Results are expressed in d.p.m. per g of wet cells × 10^{-3} and are means ± S.E.M. (n = 6-8). * $p < 0.1$; ** $p < 0.01$ as compared with the same experiment in the absence of pyruvate.

Additions to the incubation medium	Liver lipids	CO_2	Ketone bodies
1-^{14}C-acetate	131 ± 11	1.021 ± 68	115 ± 15
1-^{14}C-acetate + 5 mM pyruvate	200 ± 30 **	1.055 ± 38	194 ± 8 **
1-^{14}C-acetate + 5 mM acetate	42 ± 9	653 ± 72	273 ± 10
1-^{14}C-acetate + 5 mM acetate + 5 mM pyruvate	41 ± 1	2.646 ± 400 **	258 ± 20
1-^{14}C-octanoate + 2 mM octanoate	195 ± 33	1.423 ± 120	263 ± 20
1-^{14}C-octanoate + 2 mM octanoate + 5 mM pyruvate	360 ± 50 *	3.488 ± 600 **	247 ± 10
1-^{14}C-oleate + 2 mM oleate	1.393 ± 161	608 ± 90	214 ± 30
1-^{14}C-oleate + 2 mM oleate + 5 mM pyruvate	1.517 ± 200	1.024 ± 100 *	229 ± 30

Table II shows the effect of pyruvate on the fate of radioactivity from labeled acetate, octanoate and oleate. 5 mM pyruvate increased the incorporation of radioactivity into the liver lipids from octanoate but not from acetate or oleate. The incorporation of radioactivity into liver lipids from endogenous substrates was also increased by pyruvate. Actually, the addition of labeled acetate in the absence of non-labeled acetate was used to label only the acetyl-CoA pool from β-oxidation of endogenous fatty acids. 5 mM pyruvate increased the incorporation of radioactivity into CO_2 from acetate, octanoate and oleate. However, the incorporation of radioactivity into ketone bodies was not inhibited by pyruvate. This result may support the hypothesis that the ω-four carbon portion of fatty acid does not contribute to the acetyl-CoA pool disposable for acetoacetyl-CoA thiolase (11).

Table III shows the long-chain acyl-CoA concentration in the experimental conditions depicted in table I and II. The long-chain acyl-Coa concentrations were not affected by 5 mM pyruvate. This result contrasts with the effect of glycerol in the same circumstances (15). Thus, glycerol decreased the long-chaing acyl-CoA concentrations in the presence of acetate or octanoate.

Table III. *Effect of pyruvate on long-chain acyl-CoA concentration in isolated rat liver hepatocytes.*

Hepatocytes from starved rats were incubated for 30 min. Results are expressed in μmoles/g wet cells and are means ± S.E.M. (n = 6 — 8).

Additions to the incubation medium	Long-chain acyl-CoA
none	4.9±0.5
5 mM pyruvate	5.6±0.5
5 mM acetate	5.9±0.4
5 mM pyruvate+5 mM acetate	6.5±0.4
2 mM octanoate	4.9±0.4
5 mM pyruvate+2 mM octanoate	4.8±1.2
2 mM oleate	7.5+0.9
5 mM pyruvate+2 mM oleate	7.1±1.1

Discussion

We have previously reported (15) that glycerol may inhibit ketogenesis by the activation of lipogenesis *de novo*. Thus, glycerol inhibited ketogenesis from oleate, octanoate and acetate, a fact consistent with the idea that glycerol changes the fate of acetyl-CoA. In addition, glycerol increased the incorporation of free fatty acid into liver lipids and decreased long-chain acyl-CoA concentrations (15). However, pyruvate inhibited ketogenesis from oleate, octanoate and acetate (table I) with minimal changes in the rates of lipogenesis (table II) and long-chain acyl-CoA concentrations (table III). These results clearly suggest that pyruvate inhibits ketogenesis by a different mechanism from that of glycerol.

In the presence of exogenous substrates, pyruvate inhibited 3-hydroxybutyrate synthesis without affecting acetoacetate synthesis (table I). This may suggest that the effect of pyruvate on ketone bodies production from exogenous substrates was brought about by a change in the cellular redox state. However, this was not the case of ketogenesis from endogenous substrates where the acetoacetate synthesis was also inhibited. This suggests that ketogenesis from endogenous substrates was inhibited at some step other than the 3-hydroxybutyrate dehydrogenase catalyzed reaction.

On the other hand, the substantial increase of CO_2 production from fatty acids caused by the presence of pyruvate (table II) suggests that pyruvate increased the rate of the tricarboxylic acid cycle. Actually, an increase in the rate of the tricarboxylic acid cycle may inhibit ketogenesis by diverting the acetyl-CoA available for ketone bodies production (8, 10, 17). Therefore, pyruvate may increase oxaloacetate concentrations through the

pyruvate carboxylase catalyzed reaction, a fact that may account for the increase of the rate of tricarboxylic acid cycle (8).

In conclusion, our results suggest that pyruvate inhibits ketogenesis by, the increase of the rate of trycarboxylic acid cycle. Pyruvate may increase the oxaloacetate concentrations which enhances the rate of tricarboxylic acid cycle resulting in the diversion of the acetyl-CoA available for ketone bodies production.

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Resumen

Se estudia el efecto del piruvato sobre el destino de los ácidos grasos en hepatocitos aislados procedentes de ratas en ayunas. A concentración 5 mM inhibe la producción de cuerpos cetónicos a partir de substratos endógenos, acetato, octanoato u oleato. Sin embargo, la incorporación de la radioactividad a cuerpos cetónicos procedente de acetato, octanoato u oleato no está afectada por la presencia del piruvato. El piruvato aumenta la oxidación de los ácidos grasos a CO_2, lo que sugiere que el piruvato inhibe la estogénesis a través de la activación del ciclo tricarboxílico.

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