Effect of propionate on pyruvate metabolism in adipose tissue

L. RESHEF, J. NIV, and B. SHAPIRO

Department of Biochemistry, The Hebrew University–Hadassah Medical School, Jerusalem, Israel

ABSTRACT Glyceride-glycerol formation in rat adipose tissue from pyruvate-2-^{14}C is increased by fasting, while fatty acid synthesis is markedly depressed. In tissues of fasted animals glyceride-glycerol formation is maximal with concentrations of pyruvate exceeding 2.5 mM. With 0.25 mM pyruvate-2-^{14}C, glyceride-glycerol formation is increased severalfold by the addition of 0.25 mM propionate. No further increase in synthesis is caused by propionate when pyruvate is supplied in optimal amounts. Addition of equimolar concentrations of acetate or pyruvate does not replace propionate.

The effect of propionate on glyceride-glycerol synthesis from pyruvate is also given by a series of even-chain fatty acids. However, only propionate promotes fatty acid synthesis in tissues of fasted and fed animals. Fixation of $^{14}CO_2$ in glyceride-glycerol is dependent on the presence of propionate and is maximal in tissues of fasted rats and when pyruvate is also added. Succinate has no significant effect.

Actinomycin treatment blocks glyceride-glycerol synthesis in tissues of fed and fasted animals, in the presence and absence of propionate. At the same time, fatty acid synthesis in tissues of fasted rats is markedly increased.

KEY WORDS propionate · pyruvate metabolism · rat · adipose tissue · glyceride-glycerol · actinomycin D · succinate

PROPIONATE PROMOTES LIPOGENESIS from the acetate in adipose tissue of fasted rats but not in that of fed ones (1). The effect was abolished by ethionine and actinomycin D treatment. It could be partly attributed to glycerophosphate formation from propionate. Glyceride-glycerol formation from propionate-1-^{14}C was enhanced by fasting. However, even after almost complete blockage of glyceride-glycerol formation by the addition of malonate, part of the activating effect of propionate persisted.

Since propionate has also been shown to promote lipogenesis from pyruvate (1), we thought that part of the activating effect of propionate might possibly be due to the induction of glycerophosphate formation from pyruvate. This effect may not require the conversion of propionate to succinate and thus may be resistant to malonate inhibition.

The effect of propionate on pyruvate metabolism of adipose tissue is investigated in the present communication.

MATERIALS AND METHODS

Preparation of the tissues, experimental procedure, and analysis of the lipids were as described previously (1). For $^{14}CO_2$ incorporation into glyceride-glycerol, epididymal fat pad slices were incubated in stoppered Warburg flasks with 0.1 μmole of $KH^{14}CO_3$ (specific activity 1 μc/mole) in 2 ml of Krebs-Ringer phosphate buffer, pH 7.4, at 37°C. The buffer was flushed with CO_2-free air for 1 hr before the experiment to remove most of the CO_2. After 2 hr of incubation the reaction was stopped by the injection of 0.3 ml of 1.5 N H_2SO_4 into the incubation medium.

The tissues were removed, washed several times with 0.9% NaCl, dried, and weighed, and analysis of the tissue lipid was carried out as described previously (1).

RESULTS

Lipogenesis by adipose tissue from pyruvate-2-^{14}C as a function of pyruvate concentration and the nutritional state of the donor animals is presented in Fig. 1. In tissues of fed animals most of the pyruvate carbons were found in the fatty acid moiety of the fat and very few in the glycerol part. Incorporation into fatty acid increased with increasing pyruvate concentration in the range tested.

Fasting changed the fate of pyruvate. Glycerol formation became dominant and considerably exceeded

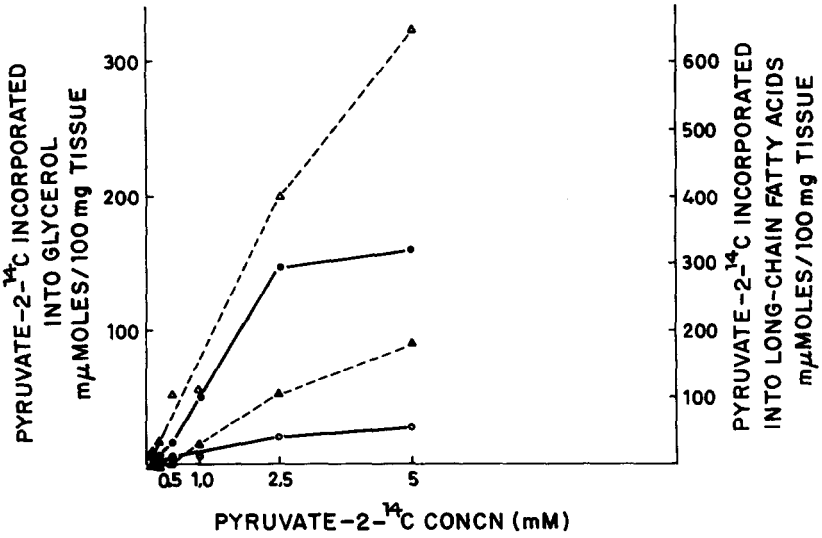

FIG. 1. Incorporation of pyruvate-2-^{14}C into glyceride-glycerol and fatty acids by adipose tissue of fed and fasted rats. Epididymal fat pads were incubated for 2 hr in 2 ml of Krebs-Ringer phosphate buffer, pH 7.4, with various concentrations of pyruvate-2-^{14}C. Incorporation into glyceride-glycerol and fatty acids was estimated as described in ref. 1. △, Incorporation into fatty acids by tissue of fed rats; ▲, the same in tissue of fasted rats; ○, incorporation into glyceride-glycerol by tissue of fed rats; ●, the same by tissue of fasted rats.

that in tissues of fed animals. The increase in the synthesis of the glycerol moiety was severalfold when concentrations of pyruvate between 1 and 2.5 mM were used. At the same time, fatty acid formation from pyruvate-2-^{14}C was markedly depressed.

When 0.25 mM propionate was present during the incubation of tissues of fasted animals with low concentration of pyruvate-2-^{14}C (0.25 mM), a marked stimulation of glyceride-glycerol formation was found. Little or no stimulation was obtained with tissues of fed animals. Fatty acid synthesis was increased in both types of tissue, while $^{14}CO_2$ production remained practically unchanged by the addition of propionate (Table 1). With higher concentrations of pyruvate-2-^{14}C (2.5 mM) propionate caused no further increase in the tissues of fasted animals (Table 2). Under these conditions both $^{14}CO_2$ and fatty acid-^{14}C formation were elevated.

Treating fasted animals with actinomycin D inhibited the incorporation of pyruvate into glyceride-glycerol in the absence and presence of propionate. On the other hand, the incorporation of pyruvate into long-chain fatty acid in tissues from fasted animals was enhanced by actinomycin D treatment (Tables 1 and 2). Replacing propionate with an equivalent amount of pyruvate or acetate (Table 3) did not yield similar activating effects.

As short-chain fatty acids have been shown to promote gluconeogenesis in kidney slices (2) as well as in the mammary gland of lactating cows (3), the effect of

TABLE 1 EFFECT OF PROPIONIC ACID (0.25 mM) ON THE METABOLISM OF PYRUVATE-2-^{14}C (0.25mM)

Treatment of Rats	No. of Expts.	Propionate (0.25mM)	Pyruvate Incorporated into		
			CO_2	Fatty Acid	Glyceride-Glycerol
			mμmoles/100 mg tissue		
Fed	10	−	36 ± 7.9	21 ± 4.7	7 ± 0.5
	10	+	45 ± 10.1	45 ± 9.7*	9 ± 0.6
Fed, actinomycin D-treated	3	−	66 ± 6.4	9 ± 2.9	0.5 ± 0.13
	3	+	57 ± 2.0	16 ± 4.1†	2.3 ± 0.5*
Fasted	15	−	33 ± 6.2	0.9 ± 0.16	7.3 ± 1.03
	15	+	43 ± 9.0	6.0 ± 1.5†	26.0 ± 1.80‡
Fasted, actinomycin D-treated	18	−	46 ± 3.8	7 ± 0.9	1.1 ± 0.12
	18	+	46 ± 3.3	12 ± 0.7‡	3.1 ± 0.54

Epididymal fat pads were incubated in the presence or absence of 0.25 mM pyruvic acid-2-^{14}C (S.A. 1μc/2 μmoles) in 2 ml of Krebs-Ringer phosphate buffer (pH 7.4). After 2 hr the incubation was stopped by the addition of 0.3 ml of 1.5 N H_2SO_4. Lipid was extracted and analyzed (1). Results are expressed as means ±SEM.

* $P < 0.05$ when compared with corresponding incubation without propionate.
† $P < 0.1$ " "
‡ $P < 0.01$ " "

TABLE 2 EFFECT OF PROPIONIC ACID (2.5 mM) ON THE METABOLISM OF PYRUVATE-2-^{14}C (2.5 mM)

Treatment of Rats	No. of Expts.	Propionate (2.5mM)	Pyruvate Incorporated into		
			CO_2	Fatty Acids	Glyceride-Glycerol
			mμmoles/100 mg tissue		
Fed	8	−	148 ± 15.2	132 ± 20	23 ± 2.4
	5	+	170 ± 23	261 ± 28.5	28 ± 3.4
Fed, actinomycin D-treated	3	−	303 ± 53	117 ± 23	2.3 ± 1.4
	3	+	283 ± 36	159 ± 5.8	4.6 ± 1.4
Fasted	10	−	105 ± 11.4	48 ± 10.1	75 ± 8.2*
	5	+	178 ± 16.1	88 ± 15.1	84 ± 13.4*
Fasted, actinomycin D-treated	8	−		127 ± 27	10 ± 1.4
	8	+		173 ± 33	11.5 ± 1.3

Condit ions as in Table 1, except that concentrations of pyruvate and propionate were 2.5 mM.
*$P < 0.01$ when compared with fed animals.

TABLE 3 EFFECT OF ACETATE, PROPIONATE, AND CARRIER PYRUVATE ON THE METABOLISM OF PYRUVATE-2-^{14}C IN ADIPOSE TISSUE FROM FASTED RATS

Addition	Pyruvate Incorporated into		
	CO_2	Long-Chain Fatty Acids	Glyceride-Glycerol
None	26	2	6
Sodium propionate (0.25 mM)	35	16	24
Sodium acetate (0.25 mM)	25	2	8
Sodium pyruvate (0.25 mM)	21	4	9

Conditions as in Table 1. One representative experiment out of eight is given.

TABLE 4 EFFECT OF SHORT-CHAIN FATTY ACIDS ON THE METABOLISM OF PYRUVATE-2-^{14}C IN ADIPOSE TISSUE FROM FASTED RATS

Addition	No. of Expts.	Pyruvate Incorporated into		
		CO_2	Fatty Acids	Glyceride-Glycerol
None	9	41 ± 4.1	1.0 ± 0.1	11 ± 1.0
Acetate	3	38 ± 7.5	0.7 ± 0.3	29 ± 9.0*
Propionate	9	46 ± 2.6	4.6 ± 1.1†	31 ± 3.6†
Butyrate	9	37 ± 5.1	1.0 ± 0.1	45 ± 5.5†
Caprylate	9	35 ± 4.6	1.0 ± 0.2	54 ± 7.4†

Conditions as in Table 1. All acids were added in a concentration of 0.25 mM except for acetic acid (2.5 mM).
* $P < 0.01$ compared to control.
† $P < 0.05$ "

several other acids was compared with that of propionate (Table 4). All the acids tested, from acetate up to caprylate, promoted the conversion of pyruvate-2-^{14}C to glyceride-glycerol in adipose tissue of fasted rats. Acetate was used in 10-fold concentration, as equimolar concentrations (0.25 mM) gave no effect (Table 3). Propionate was, however, exceptional in promoting the formation of fatty acids as well as glyceride-glycerol (Table 4). No effect was found on $^{14}CO_2$ production from pyruvate.

$^{14}CO_2$ *Fixation into Glyceride-Glycerol*

Since one of the first steps in propionate metabolism involves the fixation of CO_2 (4), the conversion of $^{14}CO_2$ into glyceride-glycerol was investigated in the presence of propionate, pyruvate, and succinate (Table 5). $^{14}CO_2$ fixation into glyceride-glycerol in tissues from fed or fasted animals was very low. The addition of sodium propionate increased this activity markedly. No $^{14}CO_2$ was found in the fatty acid moiety of the lipid. Sodium pyruvate itself had some activating effect and also enhanced the effect of propionate. No significant effect was obtained with succinate. The fixation of $^{14}CO_2$ into glyceride-glycerol was enhanced with tissues from fasted animals.

DISCUSSION

Lipogenesis from acetate in tissues of fasted animals is promoted by propionate partly by a malonate-sensitive and partly by an malonate-resistant mechanism (1). Propionate was shown to increase glyceride-glycerol formation in tissues of fasted rats in two ways. In one, propionate itself served as source of glycerol (1). In the second, it promoted the conversion of pyruvate to glycerol.

The conditions required for obtaining an effect of propionate on glyceride-glycerol formation from pyruvate-2-^{14}C resemble those which induce increased lipogenesis from acetate (1). The increase is most marked with tissues of fasted animals. It can be shown only with low concentrations of pyruvate and is almost completely abolished by actinomycin D treatment. This second effect of propionate may be the source of the malonate-resistant promotion of fatty acid formation from acetate.

The promotion of glyceride-glycerol formation from pyruvate-2-^{14}C is not specific for propionate but is also given by several even-chain fatty acids. The mechanism of this promotion may be similar to that described for the gluconeogenic effect of butyrate and other acids in

TABLE 5 EFFECT OF SUCCINIC ACID, PYRUVIC ACID, AND PROPIONIC ACID ON INCORPORATION OF $NaH^{14}CO_3$ INTO GLYCERIDE-GLYCEROL BY RAT EPIDIDYMAL FAT PAD

Addition	No. of Expts.	Propionate (0.25mM)	$NaH^{14}CO_3$ Incorporated into Glyceride-Glycerol	
			Fed	Fasted
			mμmoles/100 mg tissue	
None	8	−	0.24 ± 0.04	0.065 ± 0.030
	8	+	1.3 ± 0.37*	2.8 ± 0.42*
Pyruvate (0.25 mM)	5	−	0.4 ± 0.06†	0.49 ± 0.21†
	7	+	2.9 ± 0.75†	4.0 ± 0.56*†
Succinate (0.25 mM)	2	−	0.25 ± 0.02‡	0.18 ± 0.18‡
	5	+	2.5 ± 0.76‡	3.0 ± 0.72‡

Tissues from fed and fasted animals were incubated with 0.05 mM $KH^{14}CO_3$ (S.A. 1μc/1μmole) in Warburg flasks. After 2 hr of incubation, the lipid was extracted and hydrolyzed, and glycerol was analyzed (1).
* $P < 0.01$ when compared with results without propionate.
† $P = 0.05$–0.1 when compared with results without pyruvate.
‡ Not significant when compared with results without succinate.

kidney slices (2) and in the lactating cow (3). The promoting effect may be attributable to the acyl CoA formed from these substances. Acyl CoA activates pyruvate carboxylase (5) and thus diverts pyruvate to oxalacetate and to gluconeogenesis. In the case of adipose tissue this would lead mainly to glyceride-glycerol formation.

However, propionate is outstanding among all the acids tried in causing increased conversion of pyruvate into long-chain fatty acids as well. The lack of a concomitant rise in fatty acid-^{14}C synthesis with the even-chain acids may be due to the dilution of the ^{14}C-acetyl CoA formed from pyruvate-2-^{14}C with ^{12}C-acetyl CoA produced from the even-chain acids. Such a dilution will not be caused with propionate alone, so that the rise in fatty acid synthesis is revealed. In addition, inhibition of acetyl CoA formation from pyruvate by even-chain acids (6) has to be considered as a cause for the lack of promoting effect these acids have on fatty acid formation from pyruvate. However, all these mechanisms are difficult to reconcile with the lack of effect of these acids on $^{14}CO_2$ production from pyruvate. These considerations leave open the possibility of a specific effect of propionate.

The adaptive nature of the propionate effect still has to be resolved. One of the enzymes in the conversion of propionate or pyruvate to glyceride-glycerol, phosphoenol pyruvate carboxylase, has been shown (7) to increase in activity during fasting. Although this enzyme has little activity in adipose tissue, its presence there has to be postulated in face of the demonstrated conversion of pyruvate and propionate to glyceride-glycerol, unless a different and as yet unknown pathway is assumed.

$^{14}CO_2$ incorporation into glyceride-glycerol was markedly increased in the presence of propionate and much less with pyruvate. Apparently the two acids have slight synergistic effect on $^{14}CO_2$ conversion to glycerol. Since succinate had no effect, propionate, propionyl CoA, or one of its carboxylation products may thus be considered as the activator of glycerol formation from pyruvate.

The increased fatty acid synthesis casused by actinomycin D treatment of fasted rats, shown previously with acetate (1), has now also been shown with pyruvate. This effect cannot at present be explained.

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