Modification of intracellular glucose metabolism in human skin fibroblast preincubated with *p*-chlorophenoxyisobutyrate

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- 1 The effect of *p*-chlorophenoxyisobutyrate (CPIB) on glucose metabolism human skin fibroblasts was examined.
- 2 CPIB increased the incorporation of 2-deoxy-D-[U-¹⁴C]glucose into skin fibroblasts.
- 3 CPIB decreased [¹⁴C]CO₂ production from D-[U-¹⁴C]glucose but did not affect pyruvate dehydrogenase activity.
- 4 CPIB reduced fatty acid oxidation activity and cholesterol synthesis but increased triglyceride synthesis.
- 5 These effects of CPIB were observed both in the presence and in the absence of insulin.
- 6 One possible mechanism of CPIB on reducing plasma glucose may be due to the increase of glucose incorporation into the cells and triglyceride synthesis in the cells.

Keywords *p*-chlorophenoxyisobutyrate skin fibroblasts glucose

Introduction

p-Chlorophenoxyisobutyrate (CPIB) is known to reduce the elevated levels of serum triglyceride in man, especially those seen in type IV hyperlipidaemic patients (Best & Duncan, 1965). On the other hand, CPIB is known to reduce the elevated levels of plasma glucose in diabetes mellitus (Fenderson *et al.*, 1974) and its effect is independent of current treatment with oral hypoglycaemic drugs (Barnett *et al.*, 1977). Ip *et al.* (1976) report that CPIB increases insulin sensitivity and lipogenic action in adipose tissue. In this work, we investigated the effect of CPIB on glucose metabolism in human skin fibroblasts to clarify the effect on intracellular glucose metabolism in man.

Methods

Chemicals

Insulin (from porcine pancreas) was purchased from Sigma Chemical Company. CPIB (sodium salt) was obtained from Nissan Kagaku (Tokyo, Japan). [¹⁻¹⁴C]pyruvic acid (specific activity 20 mCi mmol⁻¹), [¹⁻¹⁴C]palmitic acid (specific

activity 53 mCi mmol⁻¹) were purchased from New England Nuclear Corporation. D-[U-¹⁴C]-glucose (specific activity 281 mCi mmol⁻¹), 2 deoxy-D-[U-¹⁴C]glucose (specific activity 310 mCi mmol⁻¹) were purchased from Amersham Co. NCS was obtained from Amersham Co. (Arlington Height, IL, USA).

Culturing of human skin fibroblasts

Human skin fibroblasts were cultured by the method of Oram *et al.* (1981). Seven to 10 passages were cultured with Dulbecco's modified Eagle (DME) medium containing 10% fetal calf serum (FCS). Human skins fibroblasts were sown into 25 mm petri dishes and confluent cells (approximately 2×10^5 cells/dish) were used for the experiments.

Measurement of fatty acid oxidation activity

Fatty acid oxidation activity was measured according to the method of Morisaki *et al.* (1982). The incubation mixture consisted of 1 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 3% BSA, 10 mM ATP, 1 mM

[1-¹⁴C]palmitic acid (1 µCi), fibroblasts (2 × 10⁵ cells approximately) and indicated amount of insulin and CPIB. Incubation was carried out for 3 h at 37° C in sealed flasks filled with 5% CO₂ in O₂. Filter paper dipped with Protosol (New England Nuclear) for trapping [¹⁴C]CO₂ was stuffed in the flasks. The reaction was stopped by adding 0.5 ml of 6N H₂SO₄ and incubating the mixture for a further 30 min. Radioactivities in filter papers were counted in a liquid scintillation counter.

Measurement of glucose oxidation activity

Glucose oxidation activity was determined by measuring [¹⁴C]CO₂ production from D-[U-¹⁴C]-glucose in the same conditions as those with fatty acid oxidation, in place of [1-¹⁴C]palmitic acid.

Measurement of incorporation of glucose into skin fibroblast

2-deoxy-D-[U-¹⁴C]glucose (0.1 µCi) was added into the medium containing 10% FCS in the presence or absence of insulin (100 µu ml⁻¹ medium) and incubated with skin fibroblasts for 24 h. Then the cells were washed with phosphate buffered saline (PBS) five times and the incorporated radioactivities were counted.

Measurement of pyruvate dehydrogenase activity

Pyruvate dehydrogenase activity was measured using the method of Saltiel *et al.* (1982). The activity was assayed by measuring the conversion of [1-¹⁴C]pyruvic acid to [¹⁴C]CO₂.

Cholesterol and triglyceride synthesis from glucose

D-[U-¹⁴C]glucose (0.1 µCi) was added in the

medium and incubated with skin fibroblasts for 24 h. Then the cells were washed with PBS for five times and the lipids were extracted with Folch's method (Folch *et al.*, 1957) and applied to thin layer chromatography (Skipiski *et al.*, 1965). The radioactivities in cholesterol and triglyceride fraction were counted.

Protein assay

Protein concentration was determined by the method of Lowry *et al.* (1951).

Results

Effect of preincubation with CPIB on the incorporation of glucose into skin fibroblasts

Skin fibroblasts were preincubated with DME containing 10% FCS for 3 or 6 days with CPIB. Then 2-deoxy-D-[U-¹⁴C]glucose was added into the medium and incubated for 24 h. The incorporated 2-deoxy-D-[U-¹⁴C]glucose increased with preincubation time in the presence of 0.5 mg ml⁻¹ or 1.0 mg ml⁻¹ of CPIB (Table 1). The enhanced incorporation was dependent on CPIB concentration.

Effect of CPIB on the incorporation of glucose into skin fibroblasts

The indicated amount of CPIB was added into the medium containing 2-deoxy-D-[U-¹⁴C]glucose in the presence and absence of insulin and incubated for 24 h. Then the incorporated radioactivities were counted. The incorporated 2-deoxy-D-[U-¹⁴C]glucose increased with CPIB concentration. CPIB-induced enhancement of glucose incorporation was observed even in the presence of insulin (Table 2).

Table 1 Effect of preincubation with CPIB on the incorporation of 2 deoxy-D-[U-¹⁴C]-glucose into skin fibroblasts

CPIB (mg ml ⁻¹)	Incorporated 2-deoxy-D-[U- ¹⁴ C]glucose (10 ³ d min ⁻¹ /2 × 10 ⁵ cells)		
	Preincubation time (days)		
	0	3	6
0	0.75 ± 0.1	0.82 ± 0.1	0.80 ± 0.21
0.5	0.82 ± 0.1	1.17 ± 0.1	1.95 ± 0.05
1.0	0.92 ± 0.1	2.15 ± 0.07	3.2 ± 0.25

Skin fibroblasts were preincubated with CPIB of 0.5 mg ml⁻¹ concentration or 1.0 mg ml⁻¹ concentration for indicated times. Then 2-deoxy-D-[U-¹⁴C]glucose was added to the dishes and incubated for 24 h. The incorporated radio-activities were measured as described in Methods. Values are means ± s.d. of 10 dishes. * *P* < 0.01, ** *P* < 0.005.

Table 2 Effect of CPIB on the incorporation of 2-deoxy-D-[U-¹⁴C]glucose into skin fibroblasts

CPIB (mg ml ⁻¹)	Incorporated 2-deoxy-D-[U- ¹⁴ C]glucose (10 ³ d min ⁻¹ 2 × 10 ⁵ cells) Insulin (100 μu ml ⁻¹)	
	(-)	(+)
0	1.05 ± 0.04	1.17 ± 0.03
0.5	1.32 ± 0.09	1.90 ± 0.08
1.0	1.40 ± 0.07	2.28 ± 0.06

Skin fibroblasts were preincubated for 6 days with indicated amount of CPIB. Then 2-deoxy-D-[U-¹⁴C]glucose was added to the medium in the presence or absence of insulin (100 μu ml⁻¹) and incubated for 24 h. The incorporated radioactivities are measured as described in Methods. Values are means ± s.d. of 10 dishes. * *P* < 0.005 compared with control.

Effect of CPIB on CO₂ production from glucose or FFA and pyruvate dehydrogenase activity

CPIB inhibited CO₂ production from glucose in the presence or absence of insulin (Table 3). But CPIB did not significantly affect fatty acid oxidation activity and pyruvate dehydrogenase activity in the presence or absence of insulin (Table 3).

Effect of CPIB on cholesterol and triglyceride synthesis from glucose

CPIB increased triglyceride synthesis from glucose but inhibited cholesterol synthesis from glucose in the presence or absence of insulin (Table 4).

Discussion

Addition of CPIB increased the incorporation of 2-deoxy-D-[U-¹⁴C]glucose into skin fibroblasts in the absence of insulin. The enhancement of 2-deoxy-D-[U-¹⁴C]glucose incorporation into skin fibroblasts depends on preincubation time of 3 to 6 days as shown in Table 1. Even in the presence of insulin, further enhancement of glucose incorporation was observed (Table 2). These results indicated that the mechanism of CPIB-induced glucose incorporation might be different from that of insulin or that CPIB might enhance the mechanism of insulin action. Ip *et al.* (1976) reported that CPIB changed the insulin sensitivity in adipose tissue. Clinical observations that CPIB improved glucose tolerance

Table 3 Effect of CPIB on [¹⁴C] CO₂ production from various precursors

Precursor		[¹⁴ C]CO ₂ production (10 ³ d min ⁻¹ /2 × 10 ⁵ cells) CPIB concentration (mg ml ⁻¹)		
		0	0.5	1.0
D[U- ¹⁴ C]glucose	Insulin (-)	0.80 ± 0.05	0.55 ± 0.03	0.22 ± 0.02
	Insulin (+)	1.60 ± 0.05	0.60 ± 0.03	0.30 ± 0.03
[1- ¹⁴ C]pyruvate	Insulin (-)	1.05 ± 0.1	0.95 ± 0.04	0.09 ± 0.06
	Insulin (+)	1.40 ± 0.25	1.20 ± 0.10	1.15 ± 0.15
[1- ¹⁴ C]palmitate	Insulin (-)	12.00 ± 2.5	9.50 ± 2.0	10.00 ± 1.00
	Insulin (+)	13.00 ± 2.00	12.00 ± 0.5	11.00 ± 1.00

Skin fibroblasts were preincubated for 6 days with indicated amount of CPIB. Skin fibroblasts were incubated in the presence or absence of insulin (100 μu ml⁻¹) with indicated concentration of CPIB and three precursors. Produced [¹⁴C]CO₂ was determined as described in Methods. Values are means ± s.d. of 5 experiments. * *P* < 0.01, ** *P* < 0.005.

Table 4 Effect of CPIB on cholesterol and triglyceride synthesis from D-[U-¹⁴C]-glucose

Synthesis		Incorporated [¹⁴ C]glucose ($10^3 \text{ d min}^{-1}/2 \times 10^5 \text{ cells}$) CPIB concentration (mg ml^{-1})		
		0	0.5	1.0
Cholesterol synthesis	Insulin (-)	0.90 ± 0.05	0.55 ± 0.10	0.25 ± 0.07
	Insulin (+)	1.50 ± 0.15	0.60 ± 0.20	0.25 ± 0.04
Triglyceride synthesis	Insulin (-)	0.45 ± 0.02	0.70 ± 0.05	0.75 ± 0.05
	Insulin (+)	0.60 ± 0.04	0.68 ± 0.04	0.80 ± 0.05

Skin fibroblasts were incubated with D-[U-¹⁴C]glucose in the presence or absence of insulin ($100 \mu\text{u ml}^{-1}$) with indicated concentration of CPIB. Formed [¹⁴C]cholesterol and triglyceride were determined as described in Methods. Values are means ± s.d. of 5 experiments. * $P < 0.01$, ** $P < 0.005$.

(Ferrari *et al.*, 1977) might be explained by the fact that CPIB enhanced glucose incorporation into the cells. But the mechanism by which CPIB increases glucose incorporation into the cells are obscure. Considering that CPIB effect was observed without insulin and that it required the preincubation time with CPIB, one possible mechanism of CPIB on the improvement of glucose metabolism might be induced by the modification of intracellular metabolism of glucose. Therefore, the effects of CPIB on the intracellular glucose metabolism were investigated. CPIB did not change CO₂ production from [1-¹⁴C]pyruvate or [1-¹⁴C]palmitate and decreased CO₂ production from D-[U-¹⁴C]-glucose (Table 3). These results suggested that CPIB did not act on citric acid cycle and free fatty acid (FFA) oxidation process including FFA CoA synthesis and the following β oxidation process. Therefore, the decrease of CO₂ production from glucose by CPIB might be due to the increase in other metabolic pathway. CPIB enhanced triglyceride synthesis but inhibited cholesterol synthesis (Table 4). Insulin is known to stimulate lipids synthesis simultaneously, such as triglyceride, cholesterol, FFA and phospholipids from glucose (Warren *et al.*,

1966). But it is reported by Ip *et al.* (1976) that the effects of CPIB are different from those of insulin when adipose tissue is incubated with glucose. CPIB enhanced triglyceride synthesis but not cholesterol synthesis. These results indicated that CPIB seemed to enhance triglyceride synthetic processes directly. But the precise relation between the intracellular action and enhancement of glucose incorporation of CPIB is still unclear. CPIB-induced triglyceride synthesis might be due to the increase in the α-glycerophosphate from glucose but not FFA. Ip *et al.* (1976) reported that CPIB was able to exert an antilipolytic effect by itself. Therefore released FFA to blood was inhibited by CPIB. FFA inhibits the incorporation of glucose into the cells (Barrett, 1970). Therefore, triglyceride synthesis increment might induce the increase in insulin action and the incorporation of glucose into the cells. The precise effect of CPIB on muscle or adipose tissue, in which glucose is supposed to be mainly utilized is still not known. Further investigations are required to clarify the relationship between intracellular glucose and lipid metabolism modified by CPIB and glucose incorporation into cells, including in muscle and adipose tissues.

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