Effect of Chlorophenoxyisobutyrate on Free Fatty Acid Utilization by Mammalian Cells[1] (35701)

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Ethyl-α-chlorophenoxyisobutyrate (Clofibrate) is used widely in the treatment of hyperlipidemia (1). The ethyl ester is hydrolyzed rapidly, and the acid, chlorophenoxyisobutyrate (CPIB), is transported in the plasma as a complex with albumin (2). Because of its wide clinical usage, the metabolic effects of CPIB are of considerable interest and have been investigated extensively. Some actions attributed to this drug include inhibition of triglyceride release from the liver (3–5), interference with lipoprotein synthesis (5), inhibition of fatty acid synthesis (6), stimulation of lipoprotein lipase activity in adipose tissue (7), and enhancement of triglyceride uptake by adipose tissue (8).

In addition to these actions, Thorp (9) has suggested that CPIB displaces organic anions from serum albumin, thereby increasing their uptake by the tissues. Barrett (10) extended this hypothesis to include free fatty acid (FFA) release from adipose tissue. The present work represents a further extension of this concept to FFA utilization. Our studies were performed *in vitro* with the Ehrlich ascites cell model system. FFA utilization was investigated thoroughly in this experimental model (11), and we thought that the use of this model would allow for more definitive basic interpretations of the data.

Methods. Ehrlich ascites cells were transplanted, harvested, and washed as described previously (12). Radioactive fatty acids were purified by partition with alkaline ethanol (12). Human plasma albumin was incubated with activated charcoal to remove inherent FFA (13), dialyzed (14), and combined with fatty acids by the Celite method (15). Protein concentration was measured by the biuret method (16), FFA by titration (17) and radioactivity by liquid scintillation counting (12). The sodium salt of CPIB was a gift from Dr. Jerome Noble, Ayerst Laboratories, New York, N.Y. All solutions and incubation media contained 132 m*M* NaCl, 4.9 m*M* KCl, 1.2 m*M* $MgSO_4$ and 16 m*M* Na_2HPO_4, and they were adjusted to pH 7.4 with 0.1 *N* HCl.

Suspensions of washed Ehrlich cells were incubated with albumin-bound FFA in a 37° water bath with shaking at 80 oscillations/min (12). Air served as the gas phase. After incubation, the cells were isolated by centrifugation, washed with a protein-free buffered salt solution of the composition described above, and extracted with a chloroform–methanol (7:3, v/v) solution (12). The chloroform phase was isolated in a separatory funnel, and the radioactivity contained in one aliquot of it was measured following evaporation of the solvent and addition of a toluene–methanol scintillator solution (12). Additional aliquots of the chloroform phase were subjected to thin-layer chromatography on Adsorbosil-5 (12), and the radioactivity contained in the separated lipids was measured after the segments of the gel were collected and added to a dioxane-containing scintillator solution (18). Quenching was monitored with an external standard. Production of $^{14}CO_2$ by the cells was measured using flasks that were sealed with rubber serum stoppers and that contained removable glass center wells for addition of KOH (12).

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TABLE I. Effect of CPIB on Palmitate Uptake.[a]

CPIB (m*M*)	Palmitate-1-^{14}C uptake as FFA (neq/10^6 cells)[b]	
	$\bar{\nu} = 2$[c]	$\bar{\nu} = 4$
0	11.0 ± 0.58	38.4 ± 2.1
0.63	12.6 ± 0.25[d]	45.5 ± 1.4[d]
0.84	14.1 ± 0.36[e]	48.7 ± 1.8[d]
1.27	14.9 ± 0.60[e]	52.1 ± 4.1[d]
1.68	20.0 ± 0.70[e]	55.1 ± 1.5[e]

[a] Incubation was for 2 min in a medium containing 0.5 m*M* albumin.

[b] Each value is the mean of 4 determination ± SE.

[c] Molar ratio of palmitate-1-^{14}C to albumin present in the incubation medium.

[d] $0.01 < p < 0.5$.

[e] $p < 0.01$.

Results. The effect of CPIB on the quantity of palmitate-1-^{14}C taken up by the cells is shown in Table I. In these experiments, uptake refers only to the amount of radioactivity associated with the cells in unesterified form, *i.e.*, as cell FFA. A significant increase in palmitate uptake occurred when the CPIB concentration was 0.63 m*M* or greater. Similar results were observed at both of the palmitate–albumin molar ratios that were tested.

Table II shows the influence of albumin concentration on the effect produced by a given quantity of CPIB. Both the CPIB *concentration* and the palmitate-1-^{14}C–albumin *molar ratio* were the same in all of the experiments. However, in Exps. 3 and 4, the albumin and palmitate *concentrations* were 4 times larger than in Exps. 1 and 2. The CPIB–albumin molar ratio was 3.2 in the first two experiments and 0.84 in Exps. 3 and 4. A marked increase in palmitate uptake occurred when the CPIB–albumin ratio was 3.2; little change was noted when the ratio was 0.84. These data demonstrate that the important parameter influencing palmitate uptake in this model system was the CPIB–albumin molar ratio rather than the CPIB concentration *per se*.

Previous studies with the Ehrlich cell system indicated that the oxidation and esterification of FFA supplied in the incubation medium was dependent upon the magnitude of FFA uptake (11). Hence, it was of interest to determine whether increased FFA utilization also accompanied the CPIB-induced increase in FFA uptake. Results concerning oleate-1-^{14}C utilization during 30-min incubations in media containing glucose are shown in Table III. As noted with palmitate, oleate uptake as FFA was greater when the medium contained CPIB. Oleate incorporation into cell phospholipids and neutral lipid esters also was greater in the presence of CPIB. However, oleate oxidation to $^{14}CO_2$ was not increased by CPIB.

Discussion. FFA utilization by Ehrlich cells increased when CPIB was added to the incubation medium. This occurred at CPIB concentrations that are similar to those which depressed FFA release from adipose tissue and inhibited fatty acid synthesis *in vitro* (6, 10). Moreover, these CPIB concentrations also are similar to those present in the plasma of humans following oral administration of Clofibrate (2). The main factor regulating the effect of CPIB on FFA uptake in the Ehrlich cell system was the CPIB–albumin

TABLE II. Effect of CPIB on Palmitate Uptake Relative to Albumin Concentration.[a]

Expt. no.	Palmitate-1-^{14}C (m*M*)	Albumin (m*M*)	Palmitate-1-^{14}C uptake as FFA (neq/10^6 cells)[b]	
			Without CPIB	With CPIB (0.42 m*M*)
1	0.26	0.13	9.6	15.1
2	0.26	0.13	6.5	14.2
3	1.0	0.50	16.1	17.4
4	1.0	0.50	8.3	7.6

[a] Incubation was for 2 min. The total volume of the incubation mixture was 5 ml.

[b] Mean of 2 determinations.

TABLE III. Effect of CPIB on FFA Utilization.[a]

Cell fraction	Oleate-1-^{14}C incorporation (neq/10^8 cells)[b] Without CPIB	With CPIB (1.27 mM)
FFA	20 ± 0.3	25 ± 0.1[c]
Phospholipids	160 ± 2.1	219 ± 1.0[c]
Neutral lipid esters	114 ± 1.5	140 ± 0.6[c]
CO_2	43 ± 1.4	46 ± 1.4[d]

[a] Incubation for 30 min in media that contained 0.5 mM albumin, 1.0 mM oleate-1-^{14}C and 11 mM glucose. The total volume of the incubation medium was 5 ml.

[b] Each value is the mean of 4 determinations ± SE.

[c] $p < 0.01$.

[d] $p < 0.1$.

molar ratio. Therefore, it is reasonable to suggest that the increased uptake may be secondary to displacement of some FFA from strong to weaker albumin binding sites. A weakening of the FFA–albumin association would be expected to increase FFA availability to the cells and, hence, result in increased FFA uptake (11). As anticipated, the increased FFA uptake was accompanied by increased incorporation of fatty acid into cell lipid esters. However, it is unclear why this did not also produce an increase in FFA oxidation.

The results obtained with this model system indicate that one metabolic effect of CPIB may be to increase the transfer of albumin-bound fatty acid to the tissues. The studies of Walton *et al.* (19) in patients with hyperlipidemia demonstrated that CPIB increased the catabolic rate of plasma lipoproteins, perhaps by enhancing the peripheral utilization of lipoprotein-lipids. Fatty acids contained in triglycerides probably pass through an FFA intermediate during clearance from the plasma (20). Taken together, this information suggests the possibility that Clofibrate may act by stimulating the uptake of fatty acids that are released during hydrolysis of lipoprotein triglycerides.

Summary. The Ehrlich ascites cell system was employed as an experimental model to investigate the effects of CPIB on FFA utilization. Addition of CPIB to the incubation medium increased FFA uptake by the cells. Incorporation of FFA into cell lipid esters also was greater when CPIB was present. We suggest that the CPIB-induced enhancement in FFA utilization may result from displacement of some FFA to weaker albumin binding sites, thereby making the FFA more available to the cells.

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