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EFFECTS OF PIERICIDIN A ON THE METABOLISM OF ISOLATED ADIPOSE CELLS

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Abstract—The effects of piericidin A, a structural analog of coenzyme Q, on the metabolism of isolated adipose cells were studied. Piericidin A inhibited both the basal and the insulin-stimulated glucose and fructose utilization (oxidation and lipogenesis). Piericidin A completely abolished the stimulatory effects of proteases (*Bacillus subtilis* protease, *Streptomyces griseus* protease and α-chymotrypsin) on glucose utilization. The utilization of palmitic acid was inhibited by piericidin A to a greater extent than amino acids. Piericidin A accelerated glycogenolysis, but exerted no significant effects on the level of lipid content or the oxidation of the cellular components. The lipolysis induced by lipolytic hormones (corticotropin and norepinephrine) or phosphodiesterase inhibitors (caffeine and theophylline) or by both was also blocked by piericidin A. The effects of peiricidin A on the isolated adipose cells were interpreted as probably being due to plasma membrane effects and possible effects on the adenyl cyclase system.

PIERICIDIN A, a structural analog of coenzyme Q, has been reported to inhibit the NADH oxidase and succinic oxidase system in beef heart mitochondria.[1] In our work, the effects of piericidin A on the metabolism of isolated adipose cells were studied, particularly with regard to the processes of lipolysis, glycogenolysis and oxidation of cellular materials, and the utilization of glucose, fructose, palmitic acid and amino acids.

METHODS AND MATERIALS

The epididymal fat pads used in the preparation of adipose cells for this study were obtained from male Sprague–Dawley rats (110–150 g) which had been fed Purina laboratory chow. The procedures for preparing and incubating the isolated adipose cells, for determining the incorporation of ^{14}C from uniform lylabeled glucose-^{14}C (glucose-U-^{14}C), fructose-^{14}C (fructose-U-^{14}C) or a mixture of uniformly labeled amino acid-^{14}C (amino acid-U-^{14}C) into CO_2, total lipid, fatty acid and glyceride-glycerol, and for measuring free fatty acid release due to lipolysis, were essentially the same as described by Rodbell,[2] with some modifications.[3] Incorporation of a radioactive amino acid mixture into adipose cell protein was determined as follows: after incubation, the cell suspension was extracted of lipid, and the aqueous phase was placed in a vacuum oven at room temperature to remove excess residual solvent. After the addition of 1 vol. of 10% trichloroacetic acid (TCA), the precipitated protein was collected by centrifugation, and was washed once with 10% TCA. The protein was then dissolved in 0·2 ml of 97% formic acid, and the radioactivity was counted in a scintillation liquid composed of 1500 ml toluene, 1500 ml methylcellosolve and 15 g

Scintillator Butyl-PBD (Ciba). The esterification of radioactive palmitic acid into cell total lipid was determined by the method described previously.[4] ^{14}C-labeled glycogen was determined by the method of Gutman *et al.*[5] The gas phase was 95% O_2–5% CO_2 (v/v).

Collagenase was purchased from Worthington; norepinephrine (DL-arterenol HCl), α-chymotrypsin (crystalline), palmitic acid-1-^{14}C (29·6 mc/m-mole) and coenzyme Q_{10} (ubiquinone-50) were from Calbiochem; insulin (24 i.u./mg), corticotropin (121 units/mg), protease Type VI (repurified, from *Streptomyces griseus*), protease Type VIII (Subtilopeptidase A, crystalline, from *Bacillus subtilis*, special strain) and caffeine were from Sigma Chemical Co.; glucose-U-^{14}C (0·5 mc/7·8 mg), fructose-U-^{14}C (0·5 mc/0·5 mg), and a mixture of amino acid-U-^{14}C (1 mc/0·67 mg) were from New England Nuclear Corp.; bovine serum albumin (Fraction V) was from Armour; and theophylline was from Mann Research Labs.

RESULTS

Piericidin A at a concentration as low as 5 μg/ml was found to inhibit the basal utilization of glucose (oxidation and lipogenesis) by isolated adipose cells (Fig. 1). The stimulatory effects of α-chymotrypsin,[6] *S. griseus* protease[3] and *B. subtilis* protease[4] on the utilization of glucose were completely abolished by piericidin A. Insulin, however, still exhibited about 2-fold stimulation of glucose utilization in the presence of piericidin A (up to a concentration of 40 μg/ml). The inhibitory effects of piericidin A on lipogenesis from glucose, in the presence or absence of insulin and proteases

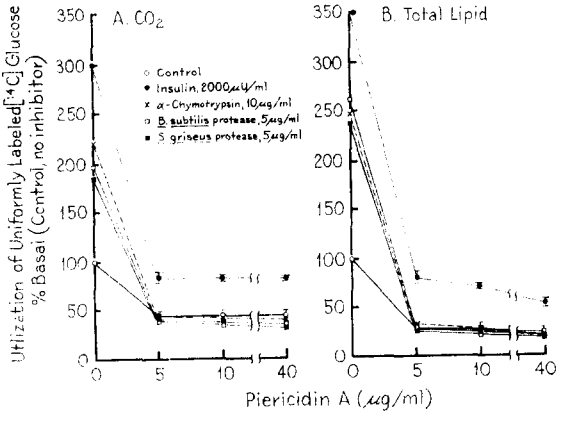

FIG. 1. Inhibitory effects of piericidin A on the basal and elevated glucose utilization induced by insulin and proteolytic enzymes in isolated adipose cells. Adipose cells were incubated at 37° for 2 hr in 1 ml Krebs–Ringer bicarbonate buffer, pH 7·4, containing 4% dialyzed bovine serum albumin and 0·2 μc (1 μmole) glucose-U-^{14}C. The basal rates (control, no piericidin A) of the conversion of glucose to CO_2 (A) and total lipid (B) are 0·39 and 0·76 μmole/g cells/2 hr respectively. Each treatment was performed in triplicate and the mean values (± S.E.) are presented. S.E. of the means is less than ± 4 per cent for those that are not indicated.

involved both the synthesis of fatty acid and glyceride-glycerol moieties (Table 1). Incorporation of the isotope into fatty acid as stimulated by insulin and proteases was somewhat more depressed than incorporation into glyceride-glycerol. Piericidin A also inhibited fructose utilization by adipose cells (Table 2). As with glucose

(Fig. 1), the insulin-stimulated conversion of fructose to CO_2 and total lipid was affected to a greater extent than the basal process.

Piericidin A at 5 μg/ml also inhibited the oxidation and esterification of palmitic acid-1-^{14}C to a greater extent (90 and 60 per cent respectively) than it inhibited the utilization of a mixture of amino acid-U-^{14}C (oxidation, 15 per cent; lipogenesis, 30 per cent; protein synthesis, 20 per cent); see Figs. 2 and 3.

TABLE 1. EFFECTS OF PIERICIDIN A ON THE SYNTHESIS OF FATTY ACID AND GLYCERIDE-GLYCEROL FROM GLUCOSE BY ISOLATED ADIPOSE CELLS*

Additives	Conversion of glucose-U-^{14}C to			
	Fatty acid		Glyceride-glycerol	
	Control	Piericidin A	Control	Piericidin A
	(mμmole/g cells/2 hr)		(mμmole/g cells/2 hr)	
None	232 ± 12	38 ± 8 (87)	357 ± 18	116 ± 12 (68)
Insulin, 2000 μunits/ml	1141 ± 2	92 ± 5 (92)	512 8	301 ± 15 (41)
B. subtilis protease, 5 μg/ml	850 ± 6	65 ± 6 (92)	593 ± 5	100 ± 5 (83)
S. griseus protease, 5 μg/ml	871 ± 15	51 ± 5 (94)	651 ± 12	111 ± 3 (83)
α-Chymotrypsin, 10 μg/ml	1060 ± 18	54 ± 7 (96)	654 ± 16	132 ± 9 (80)

* A portion of the total lipid extract from Fig. 1 was subjected to saponification. The value after subtracting fatty acid from total lipid is assumed to represent glyceride-glycerol. Piericidin A, 10 μg/ml, was used. Each treatment was performed in triplicate and the mean values (± S.E.) are presented. The values in the parentheses refer to per cent inhibition by piericidin A.

TABLE 2. COMPARISON OF INHIBITION OF BASAL AND INSULIN-STIMULATED UTILIZATION OF GLUCOSE AND FRUCTOSE BY PIERICIDIN A IN ADIPOSE CELLS*

Additives	Parameters	Glucose-U-^{14}C		Fructose-U-^{14}C	
		Control	Piericidin A	Control	Piericidin A
		(μmoles converted/g cells/2 hr)			
None	CO_2	0·41 ± 0·01	0·18 ± 0·01 (56)	1·03 ± 0·02 (56)	0·42 ± 0·03 (59)
	Total lipid	0·58 ± 0·01	0·25 ± 0·02 (57)	1·44 ± 0·03	0·72 ± 0·01 (50)
Insulin, 2000 μunits/ml	CO_2	2·15 ± 0·03	0·30 ± 0·01 (86)	2·30 ± 0·01	0·45 ± 0·03 (80)
	Total lipid	3·57 ± 0·05	0·65 ± 0·02 (82)	3·49 ± 0·01	0·78 ± 0·03 (78)

* Isolated adipose cells were incubated for 2 hr with glucose-U-^{14}C (0·2 μc or 1 μmole/ml) or fructose-U-^{14}C (0·5 μc or 20 μmole/ml), with or without additives as indicated. The amount of piericidin A used was 20 μg/ml. Each treatment was performed in triplicate and the mean values (± S.E.) are presented. The values in the parentheses represent per cent inhibition of hexose utilization by piericidin A.

The effects of piericidin A on the metabolism of cellular material of ^{14}C-prelabeled adipose cells were studied. Piericidin A at 5 μg/ml exerted no effect on the total lipid content of radioactive cells during a 2-hr incubation period. The rate of glycogenolysis

was, however, greatly accelerated, even though oxidation of ^{14}C-labeled cellular materials to $^{14}CO_2$ was slightly inhibited (Fig. 4). Similar observations on the acceleration of glycogenolysis in adipose cells by puromycin have been reported by Gutman *et al.*[5]

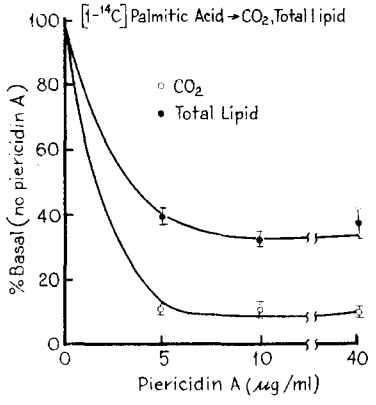

FIG. 2. Inhibitory effects of piericidin A on palmitic acid utilization by isolated adipose cells. Conditions as for Fig. 1, except that glucose was replaced by palmitic acid-1-^{14}C, 0·4 μc (13·6 mμmole) per 1 ml of incubation medium. The basal rates (control, no piericidin A) of the oxidation and the esterification of palmitic acid by the cells are 2·1 and 240·1 mμmole/g cells/2 hr. Each treatment was performed in triplicate and the mean values (± S.E.) are presented.

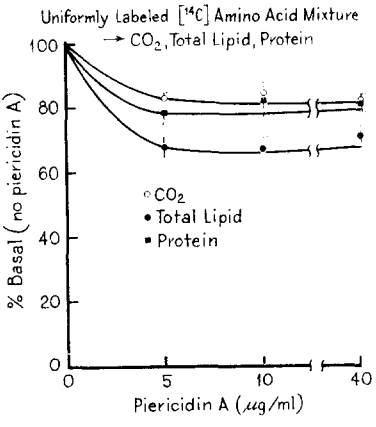

FIG. 3. Inhibitory effects of piericidin A on amino acid utilization by isolated adipose cells. Conditions as for Fig. 1, except that glucose was replaced by a mixture of amino acid-U-^{14}C, 0·4 μc (0·27 μg) per 1 ml of incubation medium. The basal rates (control, no piericidin A) of oxidation, lipogenesis and protein synthesis by adipose cells are 0·69, 0·12 and 0·25 μg/g cells/2 hr respectively. Each treatment was performed in triplicate and the mean values (± S.E.) are presented.

It was desirable to know whether piericidin A can also inhibit lipolysis. The results shown in Table 3 indicate that piericidin A inhibited the lipolysis induced by either lipolytic hormones (corticotropin and norepinephrine) or phosphodiesterase inhibitors (caffeine and theophylline), used singly or in combination.

Although the inhibition by piericidin A of NADH oxidase and succinic oxidase activities of beef heart mitochondria in a cell-free system can be partially restored by the addition of coenzyme Q_2 or Q_{10},[1] no significant recovery from the inhibition of metabolism in adipose cells was observed by the addition of coenzyme Q_{10} (up to 40 μg/ml). The metabolic parameters examined included utilization of glucose, palmitic acid oxidation and esterification, and amino acid metabolism, and the processes of lipolysis, glycogenolysis and the oxidation of cellular components.

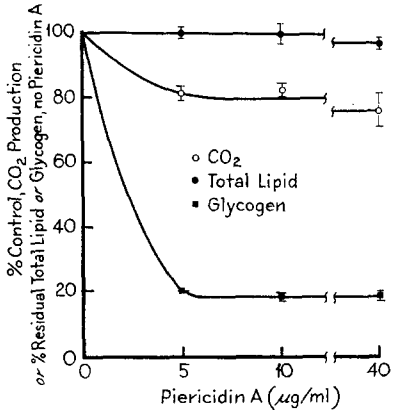

FIG. 4. Effects of piericidin A on the oxidation of cellular materials, total lipid content and the rate of glycogenolysis in ^{14}C-prelabeled adipose cells. About 3 g adipose tissue was digested for 1 hr with 4 mg collagenase in 4 ml albumin–bicarbonate medium containing 10 μc (1 μmole) glucose-U-^{14}C ($1{\cdot}8 \times 10^7$ cpm). The ^{14}C-prelabeled cells thus obtained were washed 3 times with 10 vol. of glucose-free medium, and incubated for 2 hr in the same medium with varying concentrations of piericidin A as indicated. The radioactivity in CO_2, total lipid and glycogen of controls after a 2-hr incubation was 6310 ± 51, 56506 ± 1209 and 570 ± 19 cpm respectively. Each treatment was performed in quadruplicate and the mean values (± S.E.) are presented.

TABLE 3. ANTILIPOLYTIC ACTION OF PIERICIDIN A ON ISOLATED ADIPOSE CELLS*

Lipolytic agents	Piericidin A (μg/ml)		
	0	5	15
	Free fatty acid (Δ μEquiv/g cells/hr)		
Corticotropin (1 μg/ml)	36·8 ± 2·0	20·0 ± 2·5	7·2 ± 0·0
Norepinephrine (0·2 μg/ml)	22·8 ± 2·1	10·5 ± 4·6	3·2 ± 0·4
Caffeine (3 mM)	35·6 ± 4·5	11·6 ± 2·0	4·4 ± 1·0
Theophylline (1 mM)	47·2 ± 9·8	10·8 ± 3·0	1·2 ± 1·8
Corticotropin (1 μg/ml) + caffeine (1 mM)	78·0 ± 4·3	26·0 ± 1·7	9·6 ± 5·0
Corticotropin (1 μg/ml) + theophylline (0·5 mM)	77·6 ± 3·5	20·4 ± 6·0	3·6 ± 1·8
Norepinephrine (0·2 μg/ml) + caffeine (1 mM)	85·6 ± 7·1	16·8 ± 3·7	2·4 ± 0·7
Norepinephrine (0·2 μg/ml) + theophylline (0·5 mM)	75·2 ± 9·1	16·8 ± 5·6	2·0 ± 0·1

* Isolated adipose cells were incubated for 1 hr with lipolytic agents, singly or in combination, in the presence and absence of piericidin A as indicated. Glucose was omitted from the albumin–bicarbonate medium. Each treatment was performed in triplicate and the mean values (± S.E.) are presented. Release of free fatty acid is presented as the difference between the mean values obtained for the treated and control cell suspension. The amount of free fatty acid released in the absence of added lipolytic agents was 1·2 ± 0·5 μEquiv/g cells/1 hr.

DISCUSSION

The observation on the effect of piericidin A on the metabolism of several substrates by isolated adipose cells cannot be explained solely on the basis of its inhibition of the electron transport system. If a major action of piericidin A on adipose cells is to inhibit ATP formation, protein synthesis would be greatly inhibited. Glucose and palmitic acid metabolisms were blocked to a much greater degree by piericidin A than amino acid. Moreover, piericidin A blocked the augmented glucose utilization (elicited by insulin and proteases) to a greater extent than the basal process. Metabolism of glucose, palmitic acid and amino acid in adipose cells was affected in a similar manner by avenaciolide,[7] an antifungal lactone.

Several compounds that directly or indirectly inhibit ATP synthesis in the mitochondrial electron transport system, such as amytal,[8] oligomycin, rotenone and 2,4-dinitrophenol,[9] have recently been reported to inhibit lipolysis in adipose tissue. However, the antilipolytic action of piericidin A, and probably of the substances mentioned above, may not be explained satisfactorily on the basis of reduced ATP formation, since presumably only a very small fraction of ATP is used by the adenyl cyclase system. It is suggested that action of piericidin A on the plasma membrane of adipose cells could affect both sugar utilization and lipolysis. Inhibition of lipolysis by *B. subtilis* protease is considered due to its effects on the cell membrane, thus directly or indirectly affecting cyclase activity.[10]

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