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EFFECTS OF AVENACIOLIDE ON LIPOLYSIS AND ON GLUCOSE, AMINO ACID AND PALMITIC ACID METABOLISM IN ISOLATED ADIPOSE CELLS

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Abstract—Avenaciolide, an antifungal lactone, inhibited the lipolysis in adipose cells mediated by lipolytic hormones (adrenocorticotropin and norepinephrine) and phosphodiesterase inhibitors (caffeine and theophylline). Although without effect on the basal oxidation of glucose, avenaciolide abolished the stimulation of glucose oxidation caused by insulin or proteolytic enzymes. It also eliminated the stimulatory effects of these substances on lipogenesis, but inhibited this process to some extent *per se* as well. Oxidation and esterification of palmitic acid were inhibited, but little effect on amino acid metabolism was noted.

AVENACIOLIDE, $C_{15}H_{22}O_4$, a neutral metabolite of *Aspergillus avenaceus* G. Smith, has been reported to possess antifungal activity.[1] In a study of the action of selected compounds on the metabolism of isolated adipose cells it was found that avenaciolide exerted unexpected effects on certain hormone-catalyzed reactions. Presentation of these findings constitutes the subject matter of this report.

METHODS AND MATERIALS

Epididymal fat pads from male Sprague–Dawley rats that weighed 110–150 g have been used in this study. The procedures for preparing and incubating isolated adipose cells, for determining the amount of ^{14}C incorporated into CO_2 and into cellular lipids, and for measuring the free fatty acid (FFA) release due to lipolysis were essentially the same as described by Rodbell,[2] with some modification.[3] The following procedure was used to determine the incorporation of radioactive amino acids into cell protein: the incubation medium, after extraction for the determination of total lipid, was placed in a vacuum oven at room temperature to remove residual solvent; protein was precipitated by the addition of one volume of 10% trichloroacetic acid (TCA), was collected by centrifugation, was washed once with 5% TCA, and was then dissolved in 0·2 ml 97% formic acid. 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 palmitic acid into cellular lipids was determined by the method previously described.[4]

Unless otherwise specified, the incubation mixture consisted of 1 ml Krebs–Ringer bicarbonate buffer, pH 7·4, containing 4% dialyzed bovine serum albumin (BSA), and free adipocytes ranging from 31 to 40 mg, and a specified amount of either

glucose-U-^{14}C, palmitic acid-1-^{14}C or amino acid-U-^{14}C mixture as indicated in Fig. 1 and Table 1. The gas phase was 95% O_2–5% CO_2. Each treatment was incubated in triplicate or quadruplicate in each experiment, and each experiment was carried out at least two times to insure its reproducibility.

Theophylline was purchased from Mann Research Laboratories; norepinephrine DL-arterenol HCl), α-chymotrypsin (crystalline, 11,800 ATEE units/mg), trypsin

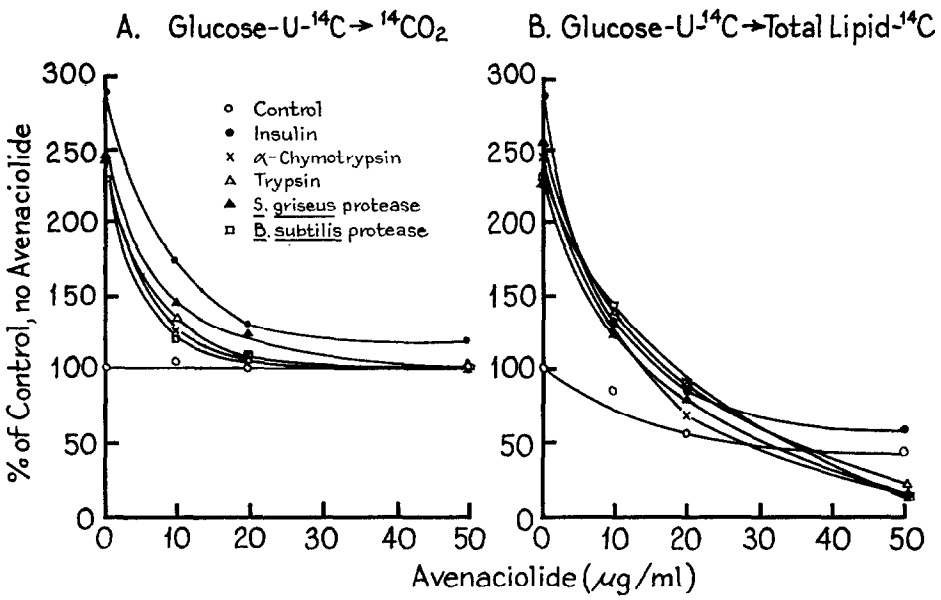

FIG. 1. Effects of avenaciolide on the conversion of glucose-U-^{14}C to CO_2 (A) and total lipid (B) by isolated adipose cells. Free adipocytes were incubated in 1 ml Krebs–Ringer bicarbonate buffer, pH 7·4, containing 4% dialyzed bovine serum albumin (BAS) and 0·2 μc glucose-U-^{14}C and sufficient unlabeled glucose to make a concentration of 1 mM. The cells were incubated for 2 hr, with shaking, at 37° in a gas phase of 95% O_2–5% CO_2. The basal rates (control without avenaciolide) of conversion of glucose to CO_2 and total lipid were 0·42 and 0·87 μmole/g cells/2 hr respectively. Each treatment was performed in triplicate and mean values are presented in the figure. If present, the concentration of insulin was 2000 μunits/ml; α-chymotrypsin, 10 μg/ml; trypsin, 10 μg/ml; *S. griseus* protease, 5 μg/ml; *B. subtilis* protease, 5 μg/ml. For further experimental details, see text.

(crystalline, 12,000 BAEE units/mg), and palmitic acid-1-^{14}C (29·6 mc/m-mole) were from Calbiochem; insulin (24 i.u./mg, lot 14B-0600), bovine serum albumin (BSA, Fraction V, lot 15B-1520), protease Type VI (from *Streptomyces griseus*, repurified, 1 mg will liberate approximately 3·8 μmole tyrosin/min from casein at pH 7·5 at 37°), protease Type VIII (Subtilopeptidase A, crystalline, from *Bacillus subtilis*, 1 mg will liberate approximately 11 μmole tyrosin/min from casein at pH 7·5 at 37°), and caffein were from Sigma. D-Glucose-U-^{14}C (14·3 mc/m-mole) and amino acid-U-^{14}C mixture (100 μc/67 μg, see catalog No. NEC-445 for amino acid composition) were products of New England Nuclear Corp. Avenaciolide was a kind gift of Dr. F. H. Stodola of Northern Utilization Research and Development Division, U.S. Department of Agriculture.

RESULTS

The effects of avenaciolide on the conversion of glucose-U-^{14}C to CO_2 (A) and total lipid (B) are presented in Fig. 1. The most striking observation is that avenaciolide at concentration up to 50 μg/ml (0·188 mM) exerted no effect on the basal rate of glucose oxidation, whereas it dramatically inhibited the oxidation stimulated by insulin,[2, 3] α-chymotrypsin and trypsin,[5] *S. griseus* protease[3] and *B. subtilis* protease.[4, 6]

TABLE 1. EFFECTS OF AVENACIOLIDE ON LIPOLYSIS BY ISOLATED ADIPOSE CELLS INCUBATED WITH ACTH, NOREPINEPHRINE, CAFFEINE, AND THEOPHYLLINE SINGLY OR IN COMBINATION*

Lipolytic agents	Avenaciolide (50 μg/ml)	
	−	+
	FFA release ($\Delta\mu$Equiv/g cells hr)	
ACTH (1 μg/ml)	28·8 ± 2·1	10·8 ± 1·2
Norepinephrine (0·2 μg/ml)	32·4 ± 0·0	10·8 ± 0·0
Caffeine (3 mM)	58·8 ± 2·4	6·6 ± 0·6
Theophylline (0·5 mM)	43·2 ± 1·4	12·4 ± 2·4
ACTH (1 μg/ml) + caffeine (1 mM)	55·2 ± 1·2	10·2 ± 1·8
ACTH (1 μg/ml) + theophylline (0·5 mM)	55·2 ± 1·2	6·6 ± 0·6
Norepinephrine (0·2 μg/ml) + caffeine (1 mM)	54·0 ± 2·4	9·6 ± 1·2
Norepinephrine (0·2 μg/ml) + theophylline (0·5 mM)	57·6 ± 2·4	11·4 ± 0·6

* Free adipocytes were incubated in 1 ml glucose-free bicarbonate medium (made to 4% BSA) for 1 hr in the absence and presence of avenaciolide and lipolytic agents at the concentrations indicated. Each treatment was performed in triplicate and mean values (±S. E.) are presented in the table. Little or no fatty acids were released by the adipocytes in the absence of lipolytic agents.

Moreover, although the oxidation stimulated by the proteolytic enzymes was nearly completely depressed by avenaciolide at a concentration of 20 μg/ml (0·075 mM), higher concentrations failed to depress glucose oxidation below the control value. On the other hand, the basal and stimulated lipogenesis were progressively inhibited by avenaciolide.

Avenaciolide was also found to be without effect on the oxidation of cellular materials of adipose cells that were prelabeled by exposure to glucose-U-^{14}C (unreported data). The identity of the endogeneous ^{14}C-labeled substances is unknown. However, the lack of effect by avenaciolide on the oxidation of these substances parallels the observation made on the basal oxidation of extracellular glucose (see Fig. 1A).

The oxidation of palmitic acid-1-^{14}C by adipose cells was inhibited by avenaciolide to a greater extent (66 per cent at 50 μg/ml) than its esterification, 28 per cent (see Fig. 2). The metabolism of an amino acid mixture to CO_2, protein, and total lipid was measured. No effect was noted on CO_2 production, whereas at 50 μg/ml, avenaciolide inhibited the metabolism of amino acid to protein and total lipid by 15 and 30 per cent respectively (Fig. 3). It seems that palmitic acid metabolism was affected to a greater extent by avenaciolide than was amino acid metabolism, and in the latter case, conversion to lipid was inhibited to the greatest extent.

It is desirable to know whether avenaciolide can also inhibit lipolysis. The results shown in Table 1 clearly indicate that this is the case. Avenaciolide at 50 μg/ml, inhibited up to 89 per cent of the lipolysis induced by either lipolytic hormones

(ACTH and norepinephrine) or phosphodiesterase inhibitors (caffeine and theophylline), whether employed singly or in combination.

DISCUSSION

Although avenaciolide, up to 50 μg/ml, was without effect on the basal oxidation of glucose, it abolished or sharply reduced the stimulatory effects on this parameter caused by insulin and proteolytic enzymes. In this respect its action is unlike that of

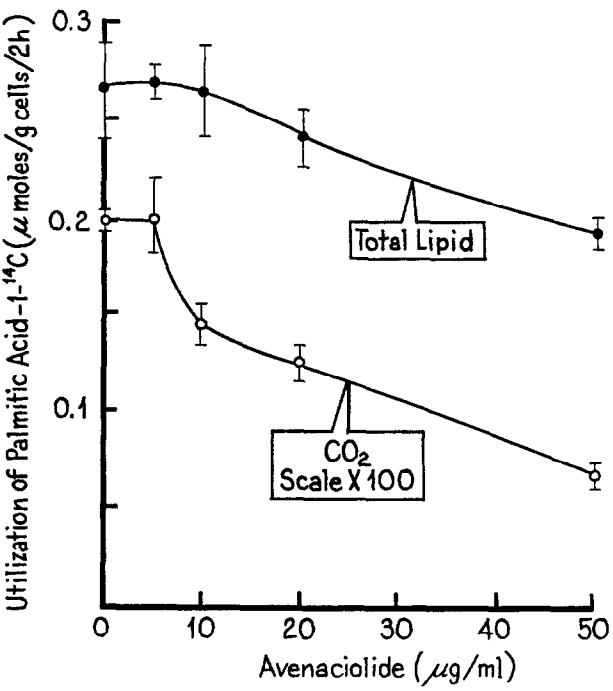

FIG. 2. Effects of avenaciolide on the oxidation and esterification of palmitic acid-1-^{14}C by isolated adipose cells. Experimental conditions were essentially the same as in Fig. 1. Free adipocytes were incubated for 2 hr in 1 ml bicarbonate-BSA medium containing 0·4 μc palmitic acid-1-^{14}C (0·013 μmole). No glucose was present in the incubation medium. Each treatment was performed in quadruplicate and mean values (±S. E.) are presented in the figure.

inhibitors of glucose transport, such as phlorizin[7] and 3-*O*-methylglucose,[4, 8] which inhibit basal and insulin- or protease-stimulated glucose utilization. A possible implication of these observations is that the glucose transport system stimulated by insulin or proteolytic enzymes may differ from normal pathways.

Many types of compounds have been reported to inhibit hormone-induced lipolysis in adipose tissue. Fain *et al.*[9] studied the effects of compounds on the lipolysis induced in isolated adipose cells by catecholamines, ACTH or growth hormone, and dexamethasone. Actinomycin, puromycin, and phenoxybenzamine, an α-adrenergic blocking agent, inhibited the lipolytic effects caused by growth hormone and dexamethasone, but were without effect on the lipolysis stimulated by catecholamines or

ACTH. In contrast, β-adrenergic blocking agents inhibited only the lipolysis mediated by catecholamines and ACTH. Nicotinic acid was unique in that it inhibited the lipolytic effects by all the hormones tested. More recently, Fassina[10] has shown that

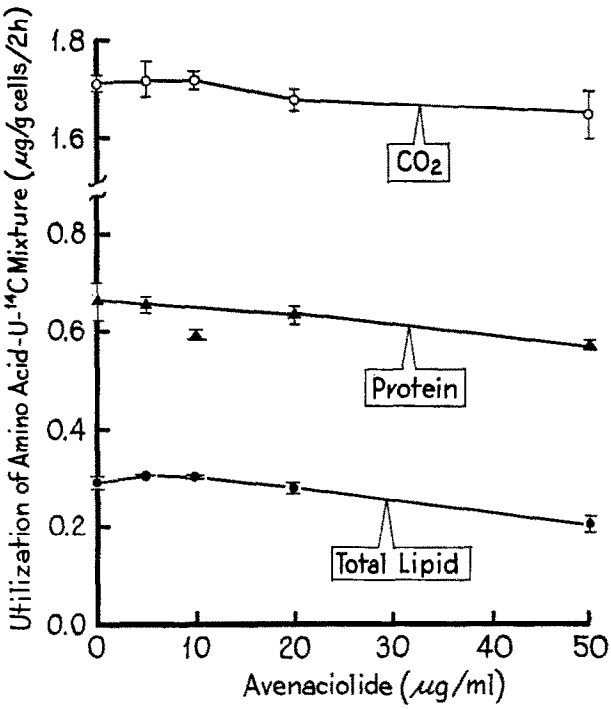

FIG. 3. Effects of avenaciolide on the metabolism of an amino acid-U-^{14}C mixture by isolated adipose cells. Experimental conditions were essentially the same as in Fig. 1. Free adipocytes were incubated for 2 hr in 1 ml bicarbonate-BSA medium containing 0·8 μc amino acid-U-^{14}C mixture (0·54 μg) in the presence of varying concentrations of avenaciolide as indicated. No glucose was present in the incubation medium. Each treatment was performed in quadruplicate and mean values (±S. E.) are presented in the figure.

oligomycin, rotenone, and 2,4-dinitrophenol, agents which inhibit oxidative phosphorylation at different stages, inhibit catecholamine-stimulated lipolysis. Avenaciolide, an antifungal antibiotic, blocked the lipolysis stimulated by ACTH, norepinephrine or by inhibitors of phosphodiesterase, and therefore can be distinguished from α-adrenergic blocking agents. However, from the existing data it is not possible to state whether its action resembles that of β-adrenergic blocking agents, nicotinic acid, or inhibitors of oxidative phosphorylation.

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