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Effects of Extracellular Proteases on Incorporation of Amino Acids into Protein by Isolated Rat Tissues[1] (37960)

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Modification of cell surfaces with extracellular proteases has been found to simulate some of the metabolic effects of insulin *in vitro* (1–3). We have compared the effects of proteases on incorporation of amino acids into protein with their actions on glucose metabolism and lipolysis, in isolated fat cells and hemidiaphragms from rats. Four enzymes were employed: trypsin, α-chymotrypsin, alkaline protease (subtilisin BPN′, "Nagarse") (4), and thermolysin (5).

Materials and Methods. Isolated fat cells. Twice-crystallized trypsin and α-chymotrypsin were obtained from Nutritional Biochemicals or Sigma. *B. subtilis* alkaline protease (Nagase Sangyo K. K., Osaka, Japan) and *B. thermoproteolyticus* thermolysin (Daiwa Kasei K. K., Osaka, Japan) were kindly given by Dr. K. Titani. Other materials, procedures for preparation and incubation of isolated epididymal fat cells, and methods for determination of incorporation of ^{3}H-leucine into protein were as described elsewhere (6). Fat cells were incubated with 0.56 m*M* uniformly labeled ^{14}C-glucose for measurement of glucose oxidation, and with 0.55 μ*M* epinephrine for measurement of glycerol release, by methods previously described (7).

Isolated hemidiaphragms. Crystalline bovine insulin was from Boots, Nottingham, England, and trypsin and α-chymotrypsin were from Worthington. Individual rat hemidiaphragms (8) were incubated with 16.7 m*M* glucose for determinations of glucose uptake as previously described (8). Two hemidiaphragms per flask were incubated with 3.7×10^{-5} *M* ^{14}C-methionine (uniformly labeled, New England Nuclear) and 2.5 mg/ml of human serum albumin (Fraction V, Pentex), without added carbohydrate, for measurement of incorporation of the label into protein (9).

Results. Isolated fat cells. Insulin, trypsin, α-chymotrypsin, and alkaline protease stimulated glucose oxidation and inhibited the lipolytic effects of epinephrine, while thermolysin had no effect on these metabolic indices (Tables I and II). Table III shows that only insulin and chymotrypsin stimulated incorporation of leucine into protein. Alkaline protease and trypsin were ineffective, while thermolysin was clearly inhibitory. Inactivation of the catalytic site of chymotrypsin with diisopropyl fluorophosphate (Sigma) or L-1-tosylamido-2-phenylethyl chloromethyl ketone (Sigma) abolished the ability of the enzyme to stimulate leucine incorporation (data not shown). The chymotrypsin contained no detectable insulin, by radioimmunoassay (10).

Isolated hemidiaphragms. Insulin, trypsin, and α-chymotrypsin stimulated uptake of glucose from the medium (Table IV). As shown in Table V, incorporation of ^{14}C-methionine into protein was approximately doubled by insulin, but only weakly stimulated by trypsin, at a single concentration. Chymotrypsin was ineffective or inhibitory.

[1] A preliminary report of this work was presented at the 32nd annual meeting of the American Diabetes Association in Washington, D. C., in June 1972, and has been published in abstract form (*Diabetes* 21, Suppl. 1, 336 (1972)).

TABLE I. Incorporation of Glucose into CO_2 by Fat Cells.

Additions	Glucose incorporation (nanoatoms/10^5 cells/1 hr) [a]			
	Experiment 1			Experiment 2
None[b]	28 ± 1			37 ± 2
Insulin, 10 ng/ml	112 ± 9***			85 ± 7**
Protease	Trypsin	α-Chymotrypsin	Alkaline protease	Thermolysin
2 μg/ml			49 ± 4**	37 ± 2
5 μg/ml	38 ± 2**			
10 μg/ml		65 ± 3***	61 ± 2***	35 ± 1
25 μg/ml	39 ± 2**			
40 μg/ml				40 ± 2

[a] Incubations as described under *Methods*. Results of representative single experiments are shown; each protease was tested in at least two separate experiments with similar results. Between-experiment variability in absolute values reflects principally the variability of estimation of the number of cells.

[b] Results shown as mean incorporation ± SE for triplicate incubations. Significance of differences from basal incorporation calculated by two-tailed *t* test (18). Symbols: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

Discussion. Alkaline protease and chymotrypsin have nearly identical active centers (11, 12), and both cleave peptide chains at the carboxy side of hydrophobic residues (13). Trypsin and chymotrypsin display extensive homologies of primary structure and similar catalytic sites (14). Although, as previously reported by other investigators (1–3), these three serine proteases all promoted glucose metabolism and inhibited lipolysis of fat cells, we found that only chymotrypsin stimulated incorporation of leucine into protein. Conversely, thermolysin, which is structurally quite unrelated to the other proteases (5, 15–17), inhibited leucine incorporation without affecting the other metabolic indices. With hemidiaphragms also, the effects of proteases on glucose metabolism and methionine incorporation were dissociated.

TABLE II. Glycerol Release by Fat Cells.

Additions: Epinephrine, 0.55 μM	Glycerol release (nmoles/10^5 cells/1 hr) [a]			
	Experiment 1			Experiment 2
+ No other agent[b]	81 ± 2			163 ± 19
+ Insulin, 4 ng/ml	21 ± 3***			31 ± 10**
+ Protease	Trypsin	α-Chymotrypsin	Alkaline protease	Thermolysin
2 μg/ml				170 ± 5
5 μg/ml	64 ± 3**			
10 μg/ml		49 ± 7*	55 ± 5**	162 ± 8
50 μg/ml				131 ± 12

[a, b] Same as for Table I.

TABLE III. Incorporation of ^{3}H-Leucine into Protein by Fat Cells.

	Leucine incorporation (nmoles/10^6 cells/1 hr) [a]			
Additions	Experiment 1	Experiment 2	Experiment 3	Experiment 4
None[b]	2.50 ± 0.14	1.72 ± 0.09	2.16 ± 0.10	3.63 ± 0.09
Insulin, 40 ng/ml	3.31 ± 0.10***	2.47 ± 0.18**	2.68 ± 0.09**	5.55 ± 0.03***
Protease	Trypsin	α-Chymotrypsin	Alkaline protease	Thermolysin
0.2 μg/ml	2.59 ± 0.09		2.12 ± 0.16	
0.3 μg/ml		1.92 ± 0.06*		
0.4 μg/ml			2.08 ± 0.13	3.35 ± 0.27
1 μg/ml	2.56 ± 0.08	2.01 ± 0.11*		
2 μg/ml			2.14 ± 0.06	3.20 ± 0.04**
5 μg/ml	2.36 ± 0.08	2.17 ± 0.18*		
10 μg/ml			1.98 ± 0.18	2.85 ± 0.03***
15 μg/ml	2.33 ± 0.15	2.12 ± 0.14*		
45 μg/ml	2.22 ± 0.11*	1.82 ± 0.07		
50 μg/ml				2.60 ± 0.04***

[a] Same as for Table I.

[b] Results shown as mean incorporation ± SD for triplicate or quadruplicate incubations. Calculations and symbols same as for Table I.

TABLE IV. Glucose Uptake by Hemidiaphragms.

Additions	Glucose uptake[a] (mg/g dry wt)	P
None[b]	21.1 ± 0.3	
Insulin		
1×10^{-4} U/ml	28.1 ± 1.7	<0.02
4×10^{-4} U/ml	43.0 ± 2.0	<0.001
8×10^{-4} U/ml	50.6 ± 5.1	<0.01
α-Chymotrypsin		
7.8 μg/ml	28.7 ± 1.7	<0.02
15 μg/ml	36.5 ± 2.2	<0.01
30 μg/ml	39.3 ± 0.6	<0.001
78 μg/ml	37.8 ± 2.8	<0.01
Trypsin		
7 μg/ml	32.1 ± 3.4	<0.05
15 μg/ml	32.1 ± 1.7	<0.01
72 μg/ml	40.3 ± 0.2	<0.001
150 μg/ml	43.8 ± 1.1	<0.001

[a] Incubations as described under *Methods*.

[b] Results shown as mean uptake ± SE for triplicate incubation. Significance of differences from basal incorporation calculated by two-tailed *t* test (18).

Our results indicate that there is no simple correspondence between the kinds of perturbations of cell surfaces which stimulate glucose metabolism and those which affect incorporation of amino acids into protein. No protease could mimic all the effects of insulin in both tissues studied. Therefore, glucose metabolism and lipolysis alone are not adequate indices of the diversity of actions of insulin.

Summary. With isolated fat cells, trypsin, α-chymotrypsin, and *B. subtilis* alkaline protease stimulated glucose oxidation and inhibited lipolysis, as did insulin. Only insulin and α-chymotrypsin stimulated incorporation of ^{3}H-leucine into protein. *B. thermoproteolyticus* thermolysin had no effect on glucose metabolism or lipolysis, but inhibited leucine incorporation.

With isolated hemidiaphragms, insulin, trypsin, and α-chymotrypsin stimulated uptake of glucose, but only insulin promoted incorporation of ^{14}C-methionine into protein.

We conclude that the actions of proteases on isolated tissues are not genuinely "in-

TABLE V. Incorporation of ^{14}C-Methionine into Protein by Hemidiaphragms.

Additions	Methionine incorporation[a]			
	Experiment 1		Experiment 2	
	cpm/mg protein	*P*	cpm/mg protein	*P*
None[b]	1264 ± 54		1828 ± 72	
Insulin				
5×10^{-4} U/ml			2616 ± 133	<0.01
1×10^{-3} U/ml			2404 ± 52	<0.02
1×10^{-2} U/ml	2462 ± 134	<0.001	2748 ± 186	<0.01
α-Chymotrypsin				
1.75 μg/ml			1589 ± 102	NS
79 μg/ml	1047 ± 23	<0.05		
158 μg/ml	1257 ± 41	NS		
Trypsin				
72 μg/ml	1475 ± 44	<0.05		
145 μg/ml	1272 ± 93	NS		

[a, b] Same as for Table IV.

sulin-like," because they do not entail the full spectrum of effects of the hormone.

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