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SELECTIVE ANTILIPOLYTIC EFFECT OF BACITRACIN IN THE ISOLATED FAT CELL

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SUMMARY: Bacitracin, an antibiotic which decreases extracellular degradation, has been used to study peptide hormone degradation in vitro. The biologic effectiveness of these hormones in the presence of bacitracin has received minimal attention. This study demonstrates inhibition of lipolysis induced by both epinephrine and glucagon in the isolated fat cell (IFC). IFC from epididymal tissue were incubated with 0.5 μM epinephrine and increasing concentrations of bacitracin. Lipolysis was inhibited in a dose-dependent fashion, with a concentration of 5.7×10^{-4} M bacitracin suppressing lipolysis 50%. Increasing the concentration of epinephrine in the presence of a constant dose of bacitracin overcame the antilipolytic effect. Bacitracin did not increase oxidation of glucose-U-C^{14} over basal. In the perifusion system, acute exposure to 5.7×10^{-4} M bacitracin plus 5×10^{-9} M glucagon suppressed lipolysis below unstimulated basal levels. Constant bacitracin perifusion produced no change in basal lipolysis but blunted the response to glucagon. ^{125}I-glucagon degradation was decreased in the presence of bacitracin. Additional studies with dibutyryl cyclic AMP demonstrated that the antilipolytic effect of bacitracin is exerted at a step beyond the second messenger. Bacitracin exerts a direct antilipolytic effect in isolated fat cells without stimulating glucose uptake and may afford a means of studying antilipolysis in the absence of other insulin-like effects.

INTRODUCTION

Studies of the interactions of polypeptide hormones with their receptors have been complicated by degradation unassociated with receptor binding (1-4). Bacitracin has been used in incubation studies to prevent this degradation (5-9). Although bacitracin has been noted to increase the biologic effect of β-endorphin (6), possible biologic effects of bacitracin itself have not been noted. This may be explained in part by the fact that it has been included in incubation systems in which the hormone being studied did not exert a biologic effect (9). Another explanation might be that the biological effect of bacitracin replicated that of the hormone being studied and was therefore not noted. In the present communication we report on the antilipolytic effect of bacitracin in the isolated fat cell.

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METHODS AND MATERIALS

The following were purchased: bacitracin (zinc free) from Sigma, St. Louis, MO and Upjohn, Kalamazoo, MI; bovine serum albumin from Reheis Chemical Co., Kankakee, IL; ^{125}I-glucagon from New England Nuclear, Boston, MA; and crystalline glucagon was obtained as a gift from Dr. Ron Chance of Lilly Research Laboratories, Indianapolis, IN. Dibutyryl cAMP was obtained from Sigma, St. Louis, MO.

Perifusion. Isolated fat cells were prepared according to the method of Rodbell (10). The IFC were perifused as previously described (11,12) with KRH-1.5% BSA at 37°C. After 20 minutes of buffer perifusion to establish baseline levels of lipolysis, glucagon or dibutyryl cAMP (with or without bacitracin) was added to the buffer at the indicated concentrations. Degradation studies were conducted with a mixture of unlabeled and ^{125}I-labeled glucagon. One ml samples of perifusate were collected and placed on ice. Glucagon degradation was assessed by collecting alternate fractions into tubes containing 1 ml of 10% TCA. The tubes were centrifuged at 2000 rpm for 12 min. at 4°C. Pellets and supernatants were counted on a Packard Autogamma Spectrometer. In all experiments the ^{125}I-glucagon was more than 90% TCA precipitable prior to perifusion over IFC. Glycerol was measured by the method of Chernick (13) to monitor lipolysis.

Incubation. IFC were incubated at 37°C for 1 hour with varying concentrations of glucagon, epinephrine and bacitracin using modifications of the methods of Rodbell (10) and Gliemann (14) as outlined by Solomon, King, and Hashimoto (15). Glucose conversion to CO_2 and lipids was conducted in the presence of insulin or bacitracin (alone or combined) by the method of Kitabchi, Solomon, and Brush (16).

Kinetic Analysis of Data. Values for the percentage of undegraded glucagon were corrected for less than 100% TCA precipitability by dividing the experimental value by the fraction precipitated in the control experiments in which the glucagon was not exposed to degradation.

RESULTS

As shown in Figure 1, bacitracin inhibited epinephrine-stimulated lipolysis in a dose dependent fashion. The molar amount of bacitracin required to repress lipolysis by one-half was 10^7 times more than the amount of insulin needed for the same effect. The antilipolytic effect of a given dose of bacitracin was overcome by increasing the amount of epinephrine (Figure 2), indicating that the isolated fat cells were still viable and able to respond to a lipolytic stimulus. Cell viability was further demonstrated by measurement of conversion of glucose-U-^{14}C uptake and conversion to CO_2. In the presence of insulin alone glucose-U-^{14}C uptake increased in a dose-dependent fashion, plateauing at an insulin concentration of 1000 μU/ml (Table I). The addition of a constant concentration of bacitracin (5.7×10^{-4} M) shifted the curve to the left - increasing glucose-U-^{14}C uptake at lower insulin concentrations (Table IA). Incubation with increasing concentrations of bacitracin alone also increased glucose-U-^{14}C uptake above baseline levels, although the effect was minimal when compared to insulin (Table IB).

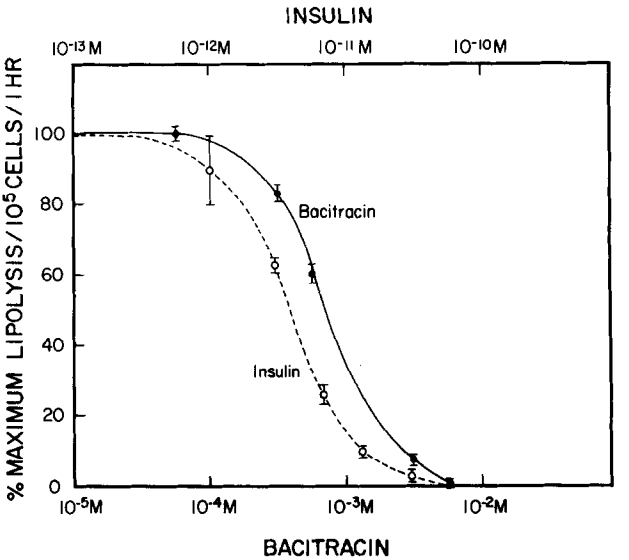

Figure 1. Inhibition of epinephrine-induced lipolysis in the isolated fat cell. Isolated fat cells were incubated for 1 hour at 37°C with 0.5 M epinephrine in the presence of varying concentrations of either insulin or bacitracin. Note that the concentrations of insulin on the upper abscissa are 10^{-8} M less than the concentrations of bacitracin on the lower abscissa. (N=3).

Perifusion of the isolated fat cells with 5 x 10^{-9} M glucagon over a 5 minute period causes prompt initiation of glycerol output (17). Termination of lipolysis was accomplished promptly by removal of the hormonal stimulus.

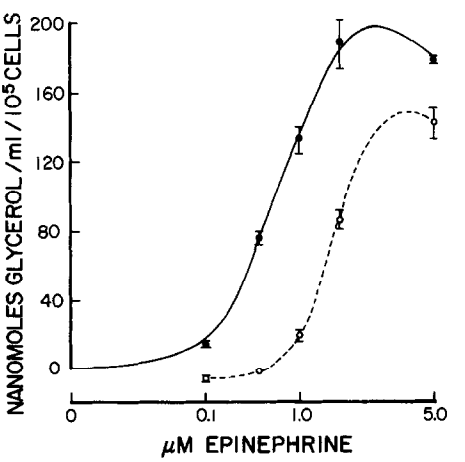

Figure 2. The effect of increasing concentrations of epinephrine on bacitracin-induced suppression of lipolysis. Isolated fat cells were incubated for 1 hour at 37°C with 1.4 x 10^{-3} M bacitracin in the presence of varying concentrations of epinephrine. Note that in the presence of bacitracin alone lipolysis was suppressed below baseline levels (broken ordinate). Lipolysis in the presence of bacitracin (o) was consistently suppressed below levels obtained with equivalent doses of epinephrine alone (●). (N=3).

TABLE IA

Effect of Insulin With and Without Bacitracin on Glucose Oxidation in the Isolated Fat Cell

Insulin Concentration (uU/ml)	Nanoatoms $^{14}CO_2$/ 10^5 cells/hour	Bacitracin 5.7×10^{-4}M	Nanoatoms $^{14}CO_2$/ 10^5 cells/hour
0	0	+	0
10	0	+	15.8 ± 1.0
20	11.4 ± 2.1	+	61.1 ± 17.3
35	38.8 ± 6.6	+	70.4 ± 18.4
50	47.1 ± 1.4	+	68.4 ± 1.4
100	64.0 ± 7.5	+	100.6 ± 3.2
1000	104.3 ± 10.8	+	104.8 ± 12.2

TABLE IB

Effect of Bacitracin on Glucose Oxidation on the Isolated Fat Cells

Bacitracin Concentration	Nanoatoms $^{14}CO_2/10^5$ cells/hour
0	0
5.4×10^{-5}M	3.8
2.5×10^{-4}M	6.4
1.8×10^{-3}M	9.5
3.1×10^{-3}M	18.2
5.4×10^{-3}M	21.8

Incubation Mixture: Each incubation vessel contained 130×10^3 fat cells, 100×10^3 cpm as glucose-U-^{14}C with 0.55 mM glucose in total volume of 2 ml containing Krebs-Ringer-Hepes, pH 7.4 with 4% bovine serum albumin for 2 hours. Results are reported as nanoatoms of glucose - ^{14}C carbon converted into $^{14}CO_2$ per 100,000 cells in 2 hours above baseline. The baseline value of 15.8 ± 5.6 has been subtracted. Each value represents a mean of 3 observations ± SEM. (The fourth column in Table IA shows $^{14}CO_2$ production at the same insulin concentration with bacitracin added.)

This was accompanied by degradation of the glucagon. Bacitracin significantly decreased glucagon degradation in the perifusion system throughout the time period studied. (Analysis by two-tailed *t* test showed $p<0.01$). As shown in Figure 3, the addition of bacitracin (5.7×10^{-4}M) to the hormone-buffer mixture caused glycerol to be suppressed below baseline levels. After bacitracin had been cleared from the system by further perifusion with buffer alone, glycerol output returned to baseline levels (as seen in Figure 3). After a washout period of 15 minutes, restimulation of the cells with glucagon alone produced a glycerol peak, consistent with the period of contact with hormone. These data also strongly support the concept that bacitracin exposure does not permanently damage the cell.

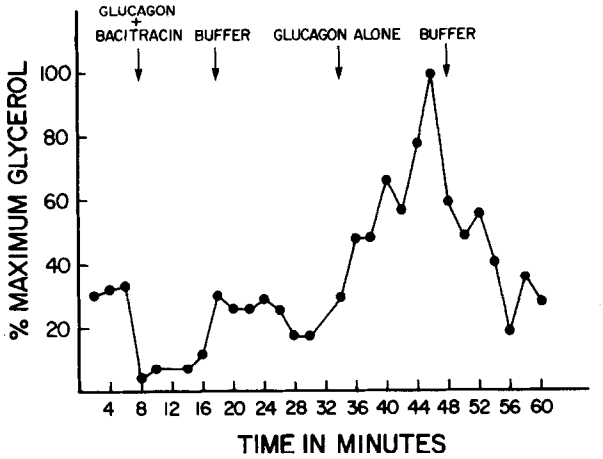

Figure 3. Effect of bacitracin on glucagon-induced lipolysis. Isolated fat cells were perifused at 37°C with 1.5% BSA-KRH containing 5×10^{-9} M glucagon with or without bacitracin (5.7×10^{-4} M). (N=4).

DISCUSSION

In order to partially characterize the site of the antilipolytic action of bacitracin, isolated fat cells were perifused with 5×10^{-5} M dibutyryl cAMP in the presence and absence of bacitracin (5.7×10^{-4} M). Glycerol output increased as expected in response to perifusion with dibutyryl cAMP alone. When bacitracin was added to the dibutyryl cAMP buffer mixture qlycerol output either remained at or was suppressed below baseline levels (Table II).

Bacitracin is a cyclic polypeptide antibiotic which exerts its bactericidal action by preventing the dephosphorylation of undecanyl pyrophosphate, a necessary step in the formation of the bacterial cell wall

TABLE II

Effect of Bacitracin on Dibutyryl Cyclic AMP- Induced Lipolysis in the Perifused Isolated Fat Cell

Perfusion Medium	N	% Maximum glycerol release	
Buffer	15	18.2 ± 3.9	
			P<0.001
+ dibutyryl cAMP (5.0×10^{-5} M)	8	88.0 ± 3.3	
			P<0.001
dibutyryl cAMP + bacitracin (5.7×10^{-4} M)	14	22.8 ± 2.0	

Isolated fat cells were perifused with Krebs-Ringer Hepes, pH 7.4 with 1.5% bovine serum albumin plus the indicated concentrations of dibutyryl cAMP with or without bacitracin at 37°C for 20 min. intervals. Peak qlycerol output was then compared with the maximum output expected from dibutyryl cAMP alone. Each value represents a mean of the indication number of observations ± SEM.

(18). It has been used by other investigators to prevent extracellular degradation of various protein hormones in incubation systems (5-9,18). In most of these systems no biologic effect of bacitracin has been noted. As our data show, bacitracin exerts an antilipolytic effect at concentrations currently in use by other investigators.

The mechanism by which bacitracin exerts its antilipolytic effect is unclear. Desbuquois and Cuatrecasas (8) have shown that bacitracin selectively inhibits glucagon degradation without affecting specific binding to liver cell membranes in the incubation system. Along with ACTH, bacitracin was the most effective peptide in preventing glucagon degradation in their system. The effect on glucagon degradation occurs at much lower concentrations of bacitracin than those associated with antilipolysis. In the rat adipocyte glucagon degradation in artificial media is decreased by 50% at a bacitracin concentration of 3.0×10^{-5}M (9), whereas suppression of lipolysis to 50% of maximum requires 7×10^{-4}M. (This concentration does not inhibit peptide hormone degradation in serum (5)). Inhibition of hormone degradation should cause glucagon-induced lipolysis to be enhanced in the presence of bacitracin. Instead, it is suppressed below baseline levels. This might be due to complexing with the hormone in the medium, thus not allowing for hormone to bind with cell membrane receptors and initiate the lipolytic stimulus. However, glucagon internalization continued at concentrations 10 times higher than those needed to inhibit lipolysis by 50% (20). Furthermore, our experiments show that isolated fat cells are resistant to stimulation by two different classes of lipolytic stimuli (protein and catecholamine), which presumably have two different types of receptors. Therefore, we feel that the antilipolytic effect of bacitracin is probably exerted at the level of the cell membrane itself or beyond.

In our studies bacitracin appears to exert its antilipolytic effect at a site beyond the generation of the cAMP messenger. Dibutyryl cAMP is a form of cAMP which is more readily transported across the cell membrane and which initiates lipolysis without obligatory generation of endogenous cAMP (22,23).

However, in the presence of bacitracin glycerol output no longer increases in response to dibutyryl cAMP. Bacitracin is known to increase the transmembrane transport of divalent cations (24). Calcium was the only such cation included in our incubation buffer, and increased transport might account for the antilipolytic action of bacitracin in the presence of dibutyryl cAMP.

In contrast to its antilipolytic effect, bacitracin (unlike insulin) had relatively minor effects on C^{14}-U-glucose uptake and incorporation into lipid. That bacitracin did not disrupt the mechanism for glucose transport is evidenced by the fact that glucose uptake in the presence of insulin was actually increased - probably secondary to decreased insulin degradation by the incubation media. Furthermore, glucose-U-^{14}C uptake was mildly increased in the presence of increasing concentrations of bacitracin alone - an effect which we currently cannot explain.

In summary, we have demonstrated that bacitracin exerts a biologic effect in the isolated fat cell, both in the incubation and perifusion systems. Furthermore, this effect is evident at concentrations currently being used by other investigators to prevent hormone degradation in binding and other experimental studies.

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