ANTILIPOLYTIC ACTION OF VALINOMYCIN AND NONACTIN IN ISOLATED ADIPOSE CELLS THROUGH INHIBITION OF ADENYL CYCLASE

J. F. Kuo and I. K. Dill

Department of Metabolic Chemotherapy, Lederle Laboratories,

American Cyanamid Company, Pearl River, N. Y. 10965

Received June 21, 1968

Recent evidence indicated that several hormones elevate the cellular concentration of adenosine 3′,5′-monophosphate (cyclic AMP) in the hormone sensitive tissues through an activation of adenyl cyclase (Sutherland et al., 1965). The lipolysis induced by the hormones in adipose tissue (cells) is due to an activated lipase mediated by the cyclic nucleotide (Sutherland et al., 1965; Weiss et al., 1966; Butcher, 1966; Rizack, 1961, 1964). The effects of valinomycin and several other "ionophorous" antibiotics on natural and artificial membranes, and consequently their effects on ion transport, are well documented (Pressman et al., 1967; Moore and Pressman, 1964; Pressman, 1965; Mueller and Rudin, 1967). Since adenyl cyclase occurs in the plasma membrane of adipose cells (Sutherland et al., 1965:, Taunton et al., 1967; Rodbell, 1967 ; Kuo, 1968), as in liver (Sutherland et al., 1965; Jungas and Ball, 1963), erythrocytes (Sutherland et al., 1965; Davoren and Sutherland, 1963), and adrenal cells (Taunton et al., 1967), it seemed that a possible interaction of the antibiotics with the adipose cells may affect adenyl cyclase, and thus inhibit lipolysis.

By a direct measurement of the formation of cyclic AMP-8-^{14}C in adipose cells prelabeled with adenine-8-^{14}C, it is shown that the antilipolytic action of valinomycin and nonactin was at least in part due to the inhibition of adenyl cyclase. Phosphodiesterase was not affected.

METHODS

Preparation of isolated adipose cells from rat epididymal fat pads and determination of free fatty acid released by incubated cells were performed according to the method of Rodbell (1964), with some modifications (Kuo et al., 1966, 1967).

Adenyl cyclase was assayed by measuring the formation of cyclic AMP-8-^{14}C in free adipocytes that were preincubated with adenine-8-^{14}C and glucose, as described below: Pooled fat pads from 15 male Sprague-Dawley rats weighing 150 g, were incubated for 1.5 h, at 37°, with shaking, in 4 ml Krebs-Ringer bicarbonate buffer, pH 7.4, containing 160 mg dialyzed bovine serum albumin, 40 µmoles glucose, and 20 µC adenine-8-^{14}C (55.5 mC per mmole, Schwarz). Another 40 µmoles glucose and 10 µC adenine-8-^{14}C were then added to the incubation mixture together with 7 mg collagenase (Worthington). The digestion was completed in 40 min. The dispersed adipocytes were filtered through a nylon cloth, and were washed free of excess adenine-8-^{14}C with buffer, and finally suspended in 100 ml bicarbonate-albumin medium made to 10 mM theophylline. One ml aliquots of the adipocyte suspension were incubated for 15 min at 37°, with shaking. The raction was terminated by the addition of 0.5 ml of 5% trichloroacetic acid (TCA). The cells were ruptured by mixing with a Vortex Jr. mixer. Cyclic AMP-8-^{14}C in the TCA- supernatant was isolated, without passage through Dowex resin, by the $BaSO_4$ method of Krishna, as described by Rodbell (1967). About 8% of the starting radioactivity (30×10^6 cpm) was found in the TCA-supernatant, of which about 10% was recovered in the cyclic nucleotide fraction. The cyclic nucleotide represented exclusively the radioactivity presented in the $BaSO_4$-supernatant, as verified by paper chromatography (Ho et al., 1967). Phosphodiesterase was assayed by the method of Ho et al.(1967).

RESULTS AND DISCUSSION

As shown in Fig. 1, valinomycin, a cyclodepsipeptide, and nonactin, a cyclic macrotetrolide, inhibited about 85% of the norepinephrine-induced

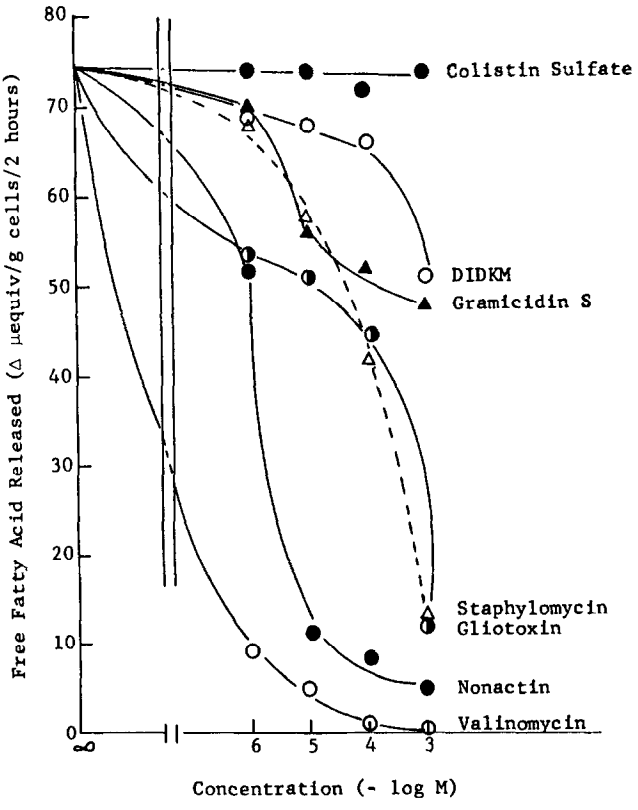

Fig. 1. Concentration-dependent inhibition of the norepinephrine-induced lipolysis by some antibiotics in isolated adipose cells. Adipocytes were incubated for 2 h in 1 ml bicarbonate-albumin medium, in the presence of 0.2 μg norepinephrine, and varying concentrations of antibiotics, as indicated. Each treatment was performed in triplicate and the means are presented.

---

lipolysis at 1 μM and 10 μM, respectively. Staphylomycin, another cyclodepsipeptide antibiotic, and some cyclic polypeptide antibiotics, such as gramicidin S, and collistin sulfate, were also tested, and showed little or no effect up to 10 μM. Gliotoxin, a cyclic dipeptide derivative, and 3,6-diisopropyl-2,5-diketomorpholine (DIDKM), the smallest cyclodepsipeptide possible, were also less effective. Valinomycin and nonactin also blocked lipolysis mediated by corticotropin, or by caffeine and theophylline (Table 1).

TABLE 1

INHIBITORY EFFECTS OF VALINOMYCIN AND NONACTIN ON LIPOLYSIS INDUCED BY NOREPINEPHRINE, CORTICOTROPIN, CAFFEINE, OR THEOPHYLLINE

Antibiotics	Free fatty acid released (△μequiv/g cells/2 h)			
	Norepinephrine (0.1 μg/ml)	Corticotropin (0.1 μg/ml)	Caffeine (1 mM)	Theophylline (0.5 mM)
None	55.5 ± 1.8	57.3 ± 2.4	54.6 ± 2.4	52.6 ± 1.1
Valinomycin, 1 μM	13.0 ± 1.5	10.2 ± 1.4	9.1 ± 0.6	10.5 ± 0.8
Nonactin, 10 μM	14.2 ± 0.5	11.2 ± 1.5	14.0 ± 0.8	15.2 ± 0.5

Experimental conditions were the same as for Fig. 1. Each treatment was performed in triplicate and the means (± S. E.) are presented.

As shown in Table 2, norepinephrine elevated cyclic AMP levels in adipose cells, and such elevation was reduced by valinomycin and nonactin. Gramicidin S, which showed little inhibition of the norepinephrine-induced lipolysis (Fig. 1), exerted no significant effect on the elevated cyclic AMP levels. Similar results were also obtained when corticotropin was employed instead of norepinephrine. Even though the quantity of cyclic AMP isolated was not

TABLE 2

COMPARISON OF THE EFFECTS OF VALINOMYCIN, NONACTIN AND GRAMICIDIN S ON CYCLIC AMP LEVELS IN ISOLATED ADIPOSE CELLS

Antibiotics	Accumulation of cyclic AMP-8-^{14}C (cpm/g cells)		
	Control	Norepinephrine (0.1 μg/ml)	Norepinephrine (1.0 μg/ml)
None	25,800 ± 1,160	82,040 ± 5,400	142,280 ± 3,080
Valinomycin, 1 μM	25,300 ± 1,310	40,010 ± 2,100	68,610 ± 3,600
Nonactin, 10 μM	25,550 ± 1,190	48,510 ± 4,200	76,500 ± 1,800
Gramicidin S, 1 mM	25,400 ± 1,170	80,890 ± 1,250	140,950 ± 1,890

Isolated adipose cells were incubated for 15 min with 10 mM theophylline in the presence or absence of norepinephrine. The radioactivity found in the cyclic nucleotide fraction at zero time was 9,360 ± 100 cpm per g cells. Each treatment was performed in triplicate and the means (± S. E.) are presented.

determined, the cyclic AMP-8-^{14}C formed by isolated adipose cells that were preincubated with adenine-8-^{14}C, permitted a simple and sensitive assay for adenyl cyclase directly in the cells.

The possibility that the antilipolytic action of valinomycin and nonactin is due to an activation of phosphodiesterase is excluded, since the antibiotics were without effect on the disappearance of 0.01 μC uniformly labeled cyclic AMP-^{14}C (53.8 mC per mmole, New England Nuclear) incubated for 30 min with tissue or cell homogenate in 1 ml reaction mixture.

Whether the inhibitory effect of valinomycin and nonactin on adenyl cyclase is due to direct antibiotic-membrane interactions, or changes in the ion environment as a result of such interaction, is still not clear.

Acknowledgement----- We thank Mr. W. W. Andres of these laboratories for the pure preparations of valinomycin, nonactin, and 3,6-diisopropyl-2,5-diketomorpholine, and Dr. P. W. Trown for the sample of gliotoxin.

REFERENCES

Butcher, R. W., Pharmacol. Rev., 18, 237 (1966).
Davoren, P. R. and Sutherland, E. W., J. Biol. Chem., 238, 3016 (1963).
Ho, R. J., Jeanrenaud, B., Posternak, TH, and Renold, A. E., Biochim. Biophys. Acta, 144, 74 (1967).
Jungas, R. L. and Ball, E. G., Biochemistry, 2, 383 (1963).
Kuo, J. F., Holmlund, C. E., Dill, I. K. and Bohonos, N., Arch. Biochem. Biophys., 117, 269 (1966).
Kuo, J. F., Dill, I. K. and Holmlund, C. E., J. Biol. Chem., 242, 3659 (1967).
Kuo, J. F., J. Biol. Chem., 243, 211 (1968).
Moore, C. and Pressman, B. C., Biochem. Biophys. Res. Commun., 15, 562 (1964).
Mueller, P. and Rudin, D. O., Biochem. Biophys. Res. Commun., 26, 398 (1967).
Pressman, B. C., Natl. Acad. Sci. U. S., 53, 1076 (1965).
Pressman, B. C., Harris, E. J., Jagger, W. S. and Johnson, J. H., Proc. Natl. Acad. Sci. U. S., 58, 1949 (1967).
Rizack, M. A., J. Biol. Chem., 236, 657 (1961).
Rizack, M. A., J. Biol. Chem., 239, 392 (1964).
Rodbell, M., J. Biol. Chem., 239, 375 (1964).
Rodbell, M., J. Biol. Chem., 242, 5744 (1967).
Sutherland, E. W., Oye, I. and Butcher, R. W., Recent Progr. Hormone Res., 21, 623 (1965).
Taunton, O. D., Roth, J. and Pastan, J., Biochem. Biophys. Res. Commun., 29, 1 (1967).
Weiss, B., Davies, J. I. and Brodie, B. B., Biochem. Pharmacol., 15, 1533 (1966).