EVIDENCE FOR THE INVOLVEMENT OF CYCLIC-3',5'-ADENOSINE MONOPHOSPHATE IN GLUCOSE UTILIZATION BY ISOLATED RAT EPIDIDYMAL ADIPOSE CELLS*

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The effects of insulin upon triglyceride lipase, glycogen synthetase and glycogen phosphorylase activities in adipose tissue appear to be mediated by the inhibitory influence of this hormone upon the production of cyclic-3',5'-adenosine monophosphate (cAMP) by the adenyl cyclase reaction (Butcher, 1966; Jungas, 1966). As also pointed out by Jungas (1966), several observations lend credence to a hypothesis that the stimulatory effects of insulin on the glucose entry mechanism might also be related to tissue levels of cAMP. Nicotinic acid, an activator of cAMP phosphodiesterase (Krishna, 1966), caused hypoglycemia *in vivo* (Cherkes, 1941; Root, 1964) and exhibited insulin-like effects on glucose utilization by epididymal fat pads (Lee, 1961; Krahl, 1964). The lipolytic agent caffeine, which, like theophylline, inhibits cAMP phosphodiesterase (Butcher, 1962; Hynie, 1966), caused small decreases in glucose uptake and substantial inhibitions of glucose utilization in fat pads (Vaughan, 1961; Anderson, 1966); caffeine also produced hyperglycemia in intact animals (Kuftinec, 1964).

We have tested the aforementioned hypothesis by parallel measurements of glucose utilization and lipolysis in the absence of glucose by

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free adipose cells in the presence of various agents known to affect tissue levels of cAMP. Dibutyryl cAMP and theophylline inhibited glucose utilization when stimulating lipolysis. Conversely, inhibition of the synthesis of cAMP by insulin or stimulation of the catabolism of cAMP by nicotinic acid stimulated glucose utilization while inhibiting lipolysis.

Experimental

Preparation of free rat epididymal adipose cells, assays of glucose utilization, and determinations of cell protein were performed as described previously (Blecher, 1967). Incubation mixtures in glucose utilization studies contained no serum albumin; lipolysis was assayed in the absence of glucose, but with 4% serum albumin. In the following tables, glucose uptake is expressed as μmoles per mg cell protein in 2 hr, while glucose utilization is given in terms of μmoles of U-^{14}C-glucose carbon converted to $^{14}CO_2$ and ^{14}C-total lipids per mg cell protein in 2 hr. Free fatty acids (FFA) were extracted from total incubation mixtures according to Rodbell (1964), and quantified by microtitration with aqueous NaOH to a Nile Blue A end point. Glycerol in incubation mediums was assayed by the coupled enzymatic system of Weiland (1965). Production of FFA and glycerol are expressed as μeq and μmoles, respectively, produced per mg cell protein in 2 hr. Cell protein ranged between 0.3 and 0.5 mg per vessel. Values in the following tables are the means ± standard errors from at least three experimental replications. cAMP was obtained from Sigma and N^6, 2'-O-dibutyryl cAMP (DBcAMP) was a product of Calbiochem.; the latter contained butyric acid as a contaminant.

Results and Discussion

Theophylline, which increases levels of cAMP in adipose tissue apparently by inhibiting the catabolic cAMP phosphodiesterase reaction (Hynie, 1966), markedly inhibited glucose utilization by adipose cells while greatly stimulating lipolysis; the effects of theophylline were proportional to concentration (Table 1). Insulin, an

TABLE 1. Antagonistic Effects of Insulin and Theophylline on Lipolysis and Glucose Utilization by Free Adipose Cells*

Additions	Free fatty acid production	U-^{14}C-Glucose ------>	
		CO_2	Total lipids
Basal	0.35±0.03	2.02±0.10	2.02±0.09
Theophylline, mM			
0.1	3.87±0.52	1.40±0.12	1.59±0.05
2.5	12.10±0.84	0.28±0.01	0.40±0.06
Insulin, 1 mU/ml	0.25±0.06	4.73±0.03	5.23±0.36
Insulin (1 mU/ml) + Theophylline, mM			
0.1	0.35±0.06	4.32±0.15	5.10±0.10
2.5	10.49±1.14	3.92±0.15	5.07±0.22

* Methodology and manner of data presentation for this and subsequent tables are described in Experimental.

anti-lipolytic hormone reportedly by virtue of its inhibitory effect upon the synthesis of cAMP by the adenyl cyclase reaction (Butcher, 1966; Jungas, 1966), not only antagonized the lipolytic effect of concentrations of theophylline up to 2.5 mM, but also reversed the inhibitory effects of theophylline on glucose utilization (Table 1).

Nicotinic acid, an anti-lipolytic agent by virtue of its stimulatory effect on cAMP phosphodiesterase (Krishna, 1966), was without significant effect upon basal lipolysis, but markedly inhibited theophylline-induced lipolysis (Table 2); similar observations in fat

TABLE 2. Control of Lipolysis and Glucose Uptake and Utilization in Free Adipose Cells by Theophylline and Nicotinic Acid

Additions	Free fatty acids	Glucose uptake	U-^{14}C-Glucose --->	
			CO_2	Total lipids
Basal	0.18±0.06	2.21±0.29	3.14±0.12	2.19±0.31
Theophylline, 0.5 mM	6.82±0.07	1.50±0.11	1.46±0.01	1.02±0.05
Nicotinic acid, 1.0 mM	0.15±0.05	3.17±0.31	3.42±0.03	2.96±0.05
Theophylline + Nicotinic acid	0.39±0.07	2.02±0.06	2.22±0.11	1.75±0.12

pads have been reported recently (Peterson, 1967). Furthermore, nicotinic acid alone caused small but significant stimulations of glucose uptake and utilization, and partially reversed the inhibitory effects of theophylline upon these parameters of glucose metabolism.

In agreement with the reports of Vaughan (1960) and Butcher et al (1965), but in contrast to the observations of Weiss et al (1966), exogenous cAMP at concentrations up to 10 mM failed to stimulate lipolysis in adipose cells. However, a more lipid-soluble derivative, DBcAMP, stimulated lipolysis; the effect was proportional to DBcAMP concentration (Table 3). This derivative has been reported to be more resistant than cAMP to the action of cAMP phosphodiesterase (Posternak, 1962), and has been previously shown to be a potent lipolytic agent in adipose tissue (Butcher, 1965). Furthermore, concentrations of DBcAMP which stimulated lipolysis also markedly in-

TABLE 3. Effects of Dibutyryl Cyclic 3',5'-AMP and Theophylline on Lipolysis and Glucose Utilization by Free Adipose Cells

Additions	Glycerol production	U-^{14}C-Glucose ---->	
		CO_2	Total lipids
Basal	0.58±0.07	2.87±0.11	3.10±0.18
DBcAMP, mM			
3.0	3.97±0.11	0.69±0.02	0.80±0.04
1.5	3.64±0.03	0.84±0.03	0.98±0.03
0.75	2.46±0.06	1.18±0.01	1.21±0.02
0.30	0.77±0.17	6.10±0.23	6.52±0.41
0.15	0.44±0.08	5.45±0.06	5.39±0.06
0.075	0.28±0.03	4.32±0.06	4.01±0.06
0.03	0.12±0.03	2.94±0.18	2.80±0.15
Theophylline, 0.5 mM	2.64±0.21	1.40±0.19	1.51±0.12
DBcAMP (3 mM) + Theophylline (0.5 mM)	3.86±0.09	0.45±0.01	0.63±0.02

hibited the conversion of glucose to CO_2 and lipids (Table 3). Effects of theophylline could not be superimposed upon those of high concentrations of DBcAMP.

Curiously, 0.075 - 0.3 mM DBcAMP, concentrations too low to stimulate lipolysis, actually stimulated glucose utilization (Table 3). This stimulatory effect on glucose utilization was also noted with high (5 - 10 mM) concentrations of cAMP, but it should be re-emphasized that no concentration of exogenous cAMP stimulated lipolysis. Pastan (1966) has previously reported a stimulatory effect of 0.25 mM DBcAMP on the oxidation of glucose to CO_2 by thyroid slices. In an

TABLE 4. Effect of Palmitic Acid on Glucose Utilization by Free Adipose Cells

Additions	U-^{14}C-Glucose ------>	
	CO_2	Total lipids
Basal	2.82	2.86
Palmitate, mM		
4.0	0.19	0.24
3.0	0.72	1.01
2.0	3.81	5.37
1.0	4.05	4.84
0.5	3.34	3.39
0.1	2.71	2.84
0.05	2.91	2.82

effort to explore this biphasic effect of DBcAMP on glucose utilization, we observed that butyric acid, an appreciable contaminant of our commercial preparation of DBcAMP, did not influence lipolysis (glycerol production) or glucose utilization by adipose cells when tested over a wide range of concentrations.

Since the inhibitory effects of DBcAMP and theophylline on glucose utilization could have been secondary to excessive production of free fatty acids, the effects of exogenous palmitic acid on glucose utilization were examined; the results suggested that this was not the case. As seen in the experiment described in Table 4, 3 - 4 mM palmitate, similar to the amounts of free fatty acids produced by 2.5 mM theophylline (Table 1), and which might have had a detergent effect upon adipose cells, did indeed inhibit glucose utiliza-

tion markedly. However, 0.5 - 2 mM palmitate, concentrations similar to the amounts of free fatty acids produced by strongly lipolytic concentrations of theophylline (0.1 - 0.5 mM, Tables 1 - 3), actually increased glucose utilization. This latter unexpected finding requires further investigation.

The above evidence, although indirect and perhaps only circumstantial, certainly suggests that cAMP may play a role in glucose transport and utilization. It would be desirable, of course, to be able to correlate these variations in glucose utilizations with changes in tissue levels of cAMP; however, because of technical limitations no attempt was made to assay for the extremely low levels of cAMP anticipated to be present in the numbers of adipose cells used in these experiments.

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