INHIBITION OF GLUCOSE OXIDATION IN ADIPOSE TISSUE BY DIBUTYRYLADENOSINE-3',5'-PHOSPHATE

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Theophylline and caffeine increase the level of adenosine-3',5'-phosphate (cyclic AMP) by inhibiting phosphodiesterase (Butcher and Sutherland, 1962), and this fact has been used as a tool for studying the role of cyclic AMP in various tissues (Weiss et al., 1966; Turtle et al., 1967; Fisher and Ball, 1967). The observation that theophylline and caffeins both stimulate lipolysis in adipose tissue (Dole, 1961; Rizack, 1964; Raben and Matsuzaki, 1966) supports the concept that cyclic AMP is an intermediary in lipolysis. Lipolytic hormones, such as epinephrine, also increase the level of cyclic AMP in adipose tissue (Butcher et al., 1965) and stimulate the oxidation of glucose (Cahill et al., 1960). It was of great interest, therefore, to find that incubation of adipose tissue in a medium containing theophylline and glucose-^{14}C showed the expected stimulation of lipolysis but an unexpected inhibition of the incorporation of radioactivity into CO_2 (Bray, 1966; Blecher, 1967). Further studies with theophylline showed that it inhibited the incorporation of radioactivity from glucose-1-^{14}C into CO_2, but had no effect on glucose-6-^{14}C or fructose-^{14}C (Bray, 1966, and unpublished observations).

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To determine whether the inhibition of incorporation of label from glucose-1-^{14}C into CO_2 by theophylline was due to an increase in cyclic AMP or to some other effect of theophylline, the following experiments were carried out using N^6-2'-O-dibutyryladenosine-3', 5'-phosphate (dibutyryl cyclic AMP or DBC), a derivative of cyclic AMP which is not hydrolyzed by phosphodiesterase (Posternak et al., 1962). It was found that dibutyryl cyclic AMP, like theophylline, inhibited the conversion of radioactivity from glucose-1-^{14}C to CO_2, and that dibutyryl cyclic AMP competitively inhibited 6-phosphogluconate dehydrogenase.

Methods and Materials:

The adipose tissue used in these experiments was obtained from the epididymal fat of 200 to 300 gram male Holtzman rats which had been allowed free access to Purina Chow until killed by a blow on the head. Isolated adipose cells were prepared by incubating adipose tissue with collagenase as described by Rodbell (1964). Adipose cells were incubated in siliconized vials in 1 ml of Krebs-Ringer bicarbonate buffer containing 2 mM glucose labelled in either the 1 or 6 position. N^6-2'-O-dibutyryl adenosine-3', 5'-phosphate, purchased from Calbiochem, was added to separate vials in a final concentration of 0.1, 0.3, 1.0 or 3.0 mM. After incubating the vials for 1 hour at 37° C under an atmosphere of 95% O_2-5% CO_2, 0.5 ml of Hyamine® was added to a polyethylene cup suspended from the cap and 0.5 ml of 0.5 N sulfuric acid was added to the cells and the vials incubated for another hour to collect the radioactive CO_2, which was counted by placing the polyethylene cup in a liquid scintillator (Bray, 1960). The free fatty acids in the medium and cells were determined by the method of Dole (1956).

Homogenates of fat were prepared by homogenizing 1 gram of fat in

3 ml of cold 0.25 M sucrose and centrifuging at 1000 xG for 10 to 20 minutes in the cold. The liquid phase between the fat cake and the pellet was centrifuged at 15,000 xG for 20 minutes in a refrigerated centrifuge and the supernatant used as the enzyme. Glucose-6-phosphate dehydrogenase was assayed as described by Lohr and Waller (1963), and 6-phosphogluconate dehydrogenase was assayed by slight modifications of the method of Huggins and Yao (1959). The reciprocal of the initial velocity was plotted against the reciprocal of the square of the substrate concentration.

Results:

The effect of DBC on the incorporation of radioactivity into CO_2 and on lipolysis was examined in five experiments, and a typical result is shown in Figure 1. No lipolysis was detectable when the concentration of dibutyryl cyclic AMP was less than 1.0 mM, but at 1.0 and 3.0 mM there was a sharp increase in the release of free fatty acids. With each increment in DBC, there was a further decrease in the incorporation of radioactivity from glucose-1-^{14}C into CO_2, until at 3.0 mM DBC, similar quantities of radioactive ^{14}carbon were incorporated into CO_2 by cells incubated with glucose-1-^{14}C or glucose-6-^{14}C. The conversion of radioactivity from glucose-6-^{14}C to CO_2 in control cells was about one-sixth that of glucose-1-^{14}C and was increased at concentrations of DBC which caused lipolysis.

To test whether the decreased incorporation of radioactivity from glucose-1-^{14}C into CO_2 observed when adipose cells were incubated with dibutyryl cyclic AMP was the result of a direct effect on the enzymes of the pentose cycle, both glucose-6-phosphate dehydrogenase and 6-phosphogluconate were examined at several concentrations of substrate, in the presence and absence of DBC. There was no inhibitory effect of DBC on the activity of glucose-6-phosphate dehydrogenase, but there was a consistent inhibitory effect on the

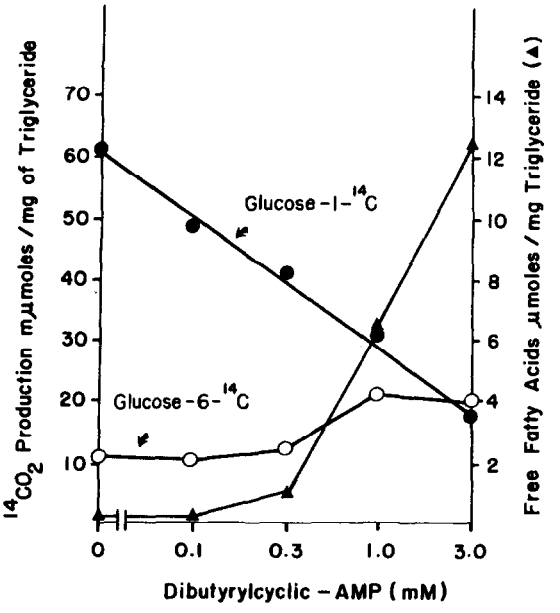

Figure 1. Effect of dibutyryl cyclic AMP on lipolysis and glucose oxidation in adipose cells.

Approximately 0.3 ml of adipose cells were incubated for 1 hr at 37° in 1 ml of Krebs-Ringer bicarbonate buffer containing 40 mg of albumin and 2 μmoles of glucose labelled in the 1 or 6 position.

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activity of 6-phosphogluconate dehydrogenase in six experiments. The effect of DBC on the activity of 6-phosphogluconate dehydrogenase from one experiment is shown in Figure 2 where the reciprocal of the initial velocity of the reaction is plotted against the reciprocal of the square of the substrate concentration. DBC was a competitive inhibitor of 6-phosphogluconate dehydrogenase since there was no effect on enzyme activity at high substrate concentrations and a progressively greater effect at low substrate concentrations.

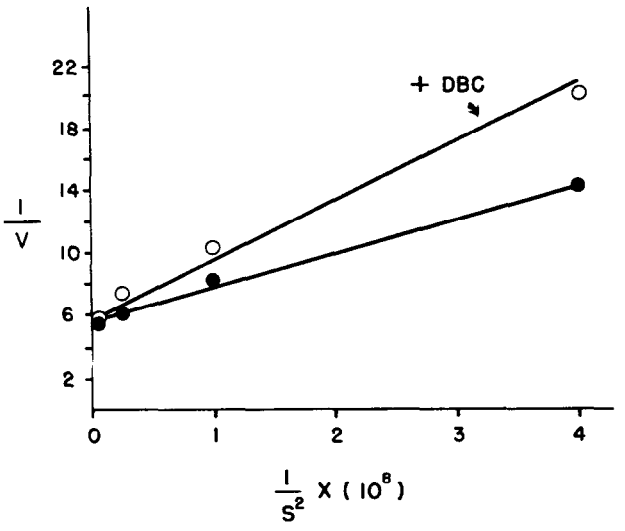

Figure 2. Effect of dibutyryl cyclic AMP on the activity of 6-phosphogluconate dehydrogenase in an homogenate of fat.

The assay mixture contained: 0.5 ml of 0.5 M Tris pH 7.5; 0.1 ml of 0.5 M $MgCl_2$; 1.5 ml distilled water; 0.1 ml 0.1 M cysteine pH 7.5 (freshly prepared); 0.5 ml enzyme; 0.05 ml 3×10^{-2} M TPN. The reaction was started by adding 0.1 ml of 6-phosphogluconic acid at one of several concentrations. DBC, when present, was added as 0.1 ml of 3×10^{-3} M DBC in place of 0.1 ml of water.

Discussion:

Sutherland and Rall (1960) originally demonstrated the role of cyclic AMP as an activator of phosphorylase, and Posternak, Sutherland and Henion (1962) showed that the synthetic analogue, dibutyryl cyclic AMP, possessed similar properties. Cyclic AMP has subsequently been found to regulate the activity of other enzymes involved in glucose metabolism besides phosphorylase. Specifically, cyclic AMP activates phosphofructokinase (Mansour, 1963; Denton and Randle, 1966), inhibits glycogen synthetase (Rosell-Perez and Larner, 1964) and activates lipolysis (Rizack, 1964). The net effect of these various interactions is to mobilize stored glycogen and fat when the intracellular concentration of cyclic AMP is elevated and to produce storage of glycogen

and fat when the concentration of cyclic AMP is low (Atkinson, 1966).

The present experiments extend the hypothesis that the intracellular regulation of glucose metabolism is partially controlled by the levels of adenylate by showing that cyclic AMP regulates the activity of the pentose cycle by competitively inhibiting 6-phosphogluconate dehydrogenase. Our studies also suggest that changes in the intracellular concentration of cyclic AMP may explain variations in the contribution of the pentose cycle during the oxidation of glucose when adipose tissue is incubated with epinephrine or insulin (Landau and Katz, 1965). Elevated levels of cyclic AMP produced by epinephrine (Butcher et al., 1965) or theophylline (Butcher and Sutherland, 1962) could inhibit 6-phosphogluconate dehydrogenase and reduce the supply of NADPH available for fat synthesis. Conversely, reduced concentrations of cyclic AMP produced by removal of epinephrine, or by adding insulin (Butcher et al., 1966; Jungas, 1966) could remove the inhibition of 6-phosphogluconate and thus increase the synthesis of reduced pyridine nucleotides.

Such a model for the regulation of the pentose cycle by a competitive inhibition between 6-phosphogluconate dehydrogenase and cyclic AMP, however, does not entirely explain the role of epinephrine in glucose metabolism by adipose tissue, since theophylline (Vaughan, 1961) and dibutyryl cyclic AMP both decrease whereas epinephrine increases glucose oxidation. Experiments by Cahill et al. (1960) and Blecher (1967) suggest that the increase in free fatty acids released in the presence of epinephrine may be responsible for the stimulation of glucose oxidation. However, epinephrine can increase the oxidation of glucose, even when lipolysis is inhibited (Bray, 1967). In recent experiments (Bray and Goodman, unpublished observations) we have shown that epinephrine, like insulin, increases the intracellular concentration of a non-metabolizable sugar, arabinose, suggesting that epinephrine may increase

the entry of glucose into fat cells. The implications of this observation and its relation to the fact that epinephrine increases cyclic AMP within the adipocyte (Butcher et al., 1965), are currently under investigation.

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