INFLUENCE OF INSULIN AND GLUCAGON ON KETOGENESIS BY ISOLATED RAT HEPATOCYTES

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INTRODUCTION

The quantitative roles of insulin and glucagon in the regulation of hepatic ketogenesis are still not defined adequately. Glucagon added in vitro has been reported by certain workers to stimulate ketogenesis in perfused livers from normal-fed rats (Heimberg, Weinstein, and Kohout, 1969; Weinstein, Klausner, and Heimberg, 1973). However, other workers did not observe a ketogenic effect of the hormone under similar conditions (McGarry and Foster, 1978; McGarry, Wright, and Foster, 1975).

Similar studies with insulin have also given conflicting results. Insulin added in vitro was reported to suppress ketogenesis in livers from normal-fed rats perfused with oleate but the quantitative changes were small (Heimberg, Van Harken, Weinstein, and Kohout, 1969). The hormone added in vitro also suppressed ketogenesis in perfused livers from rats pretreated with anti-insulin serum (AIS) for 3 h (Woodside and Heimberg, 1976). Insulin alone had no effect, however, on ketogenesis in perfused livers from rats pretreated with AIS for 10 h, although the hormone did suppress this process when glucose was present in the perfusate (Woodside and Heimberg, 1976). Other workers have reported that the hormone had no antiketogenic effect in perfused livers from fasted (McGarry and Foster, 1971), alloxan diabetic (Heimberg et

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Thus, it is clear that further study is necessary to define the quantitative roles of insulin and glucagon in the direct regulation of hepatic ketogenesis. We have studied this problem utilizing isolated rat hepatocytes from normal fed rats and are reporting our initial findings here.

EXPERIMENTAL METHODS AND RESULTS

Isolated hepatocytes were prepared from the livers of normal fed male rats weighing 200-300 gms by perfusion with collagenase to digest the connective tissue. The basic procedures used were similar to those described by Seglen (1973) with the exception that Krebs-Ringer bicarbonate (KRB) solution was used as the basic perfusion medium instead of the balanced salt solution buffered with HEPES. Isolated cells were suspended at a concentration of 25-35 mg/ml in KRB containing 4% bovine serum albumin (BSA) which had been defatted and dialyzed prior to use. The suspended cells were preincubated while being gassed constantly with 95% O₂/5% CO₂ for 30 min prior to use, for reasons described by Claus, Pilkis, and Park (1975). Then 50-70 mg cells (wet weight) were added to 25 ml flasks in a final volume of 4 ml of 4% BSA in KRB containing variable amounts of oleate and hormone as indicated. The flasks were gassed briefly with 95% O₂/5% CO₂, stoppered, and incubated for 1 h. The reaction mixture was then deproteinized by addition of Ba(OH)₂ and ZnSO₄. The concentrations of acetoacetate and β-hydroxybutyrate in the supernatant were analyzed by an enzymatic procedure (McGarry, Guest, and Foster, 1970).

The influence of varying doses of glucagon on ketogenesis was examined in isolated hepatocytes from normal-fed rats incubated with either no added fatty acid or with 0.75 mM oleate [the approximate concentration of FFA observed in plasma of fasted rats (Scow and Chernick, 1970)]. The results shown in Figure 1 are from a representative experiment, but have been verified in several additional experiments. It can be seen that glucagon in the doses added had no stimulatory effect on ketogenic rates obtained in hepatocytes incubated without added FFA. With 0.75 mM oleate, basal rates of ketogenesis were increased approximately two- to three-fold. Moreover, under these conditions, glucagon stimulated ketogenesis markedly. Maximal increases in rate were induced by glucagon at a concentration of 2-3 nM and were over four-fold greater than control rates. The concentration of hormone required for one-half maximal stimulation in rate was approximately 0.3 nM.

The effects of varying doses of insulin on the stimulation in ketogenic rates induced by 0.25 and 0.50 nM glucagon were examined,