AN IN VITRO SYSTEM FOR STUDYING HORMONAL INDUCTION OF HEPATIC GLUCONEOGENIC ENZYMES

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In a series of studies on the regulation of enzyme activities by hormones and on the role of enzymes in homeostasis it was shown that adrenocorticoid hormones were capable of inducing increases in activities of liver enzymes involved in gluconeogenesis. Hepatic glucose-6-phosphatase (G-6-Pase) activity which represents the final common path of gluconeogenesis and glycogenolysis increased after administration of cortisone (Weber et al., 1955, Ashmore et al., 1956). Subsequently, it was demonstrated that the activity of another rate-limiting enzyme of gluconeogenesis, fructose-1,6-diphosphatase (FDPase) and also phosphohexose isomerase, aldolase and lactic dehydrogenase were increased by cortisone injection in normal, adrenalectomized and hypophysectomized rats (Weber et al., 1961a, b, Weber, 1963). Injection of ethionine (Kvam and Parks, 1960, Weber et al., 1961a), puromycin (Weber and Singhal, 1964a) or actinomycin (Weber et al., 1963, Weber and Singhal, 1964a) inhibited these increases, suggesting that the corticoid hormones acted at the molecular level by inducing de novo synthesis of the enzymes involved in gluconeogenesis. Similar data were also reported for phosphoenolpyruvate carboxykinase after cortisone injection (Shrago et al., 1963, Lardy et al., 1964).

Most of these studies involved injection of the inducing hormones over a period of days. However, recently it was found that

triamcinolone (9-fluoro-16-hydroxyprednisolone diacetate, Lederle) was capable of causing in a few hours an increase in the activities of the key gluconeogenic enzymes, G-6-Pase and FDPase (Weber et al., 1964). The triamcinolone-induced rapid rise was also blocked by actinomycin (Weber, Singhal, and Srivastava, to be published) and thus it was considered that de novo synthesis of these enzymes occurs as part of an early response to gluconeogenic hormones which involves new RNA synthesis.

Since triamcinolone made it possible to study the induction of carbohydrate enzymes in a short period, it has become feasible to investigate the mechanism of enzyme increases in vitro. The present report describes a system which is suitable for analyzing the behavior of gluconeogenic enzymes in liver slices taken from animals treated with hormones and/or inhibitors of protein synthesis. The results indicate that the enzyme synthetic processes induced by triamcinolone and blocked by actinomycin can be followed in liver slices.

MATERIALS AND METHODS

Male Wistar rats weighing 90 to 100 g were kept in separate cages and maintained on Purina laboratory chow and water ad libitum. The animals were divided into 4 experimental groups: (1) normal rats, (2) rats treated with triamcinolone, (3) rats injected with triamcinolone and actinomycin, (4) rats injected with actinomycin alone. In group (2) enzyme induction was carried out by injecting triamcinolone (i.p., 1 mg/100 g rat) daily for 3 days. The rats were killed on the 4th day. In the 3rd group, for prevention of enzyme synthesis, triamcinolone-treated animals were also injected with actinomycin (i.p., 10 ug/100 g rat) daily for 3 days. Actinomycin was administered one-half hour earlier than triamcinolone. The animals were killed on the 4th day.

Animals were killed by stunning and decapitation and were exsanguinated. The livers were rapidly excised and kept in a Petri dish on ice. Liver slices of approximately uniform thickness (30-50 microns) were cut with a Stadie Riggs microtome and rapidly weighed on a torsion balance. One or two slices amounting to approximately 200-250 mg of wet weight were incubated in 6 ml of isotonic KCl with bicarbonate buffering at pH = 7.4 and shaken at 85 oscillations per minute in a water bath at 37°C. In order to follow the sequence of events, tissues were harvested at 0 minute (initial), and after 45 and 90 minutes incubation. Slices and incubation media were quantitatively transferred into an all-glass homogenizer tube, and were homogenized with a Teflon pestle turning at the rate of 600 r.p.m. for 20 seconds. The homogenates were made up to 10 ml with the isotonic KCl solution and a sample was taken for direct counting of nuclei as described previously (Weber and Cantero, 1957). Aliquots amounting to 1 to 2 ml were stored in flat bottom vials and frozen in a deep-freeze at -20°C. The activities of G-6-Pase and FDPase were assayed in the homogenate as referred to previously (Weber et al., 1961b). Enzyme activities were expressed in umoles of substrate metabolized per counted nucleus (per average cell) in 1 hour at 37°C.

It was found that carrying out the incubations in air, oxygen or nitrogen did not appreciably affect the results. It was also observed that unusual thinness or thickness of the slices resulted in poor repeatability. However, under carefully standardized conditions similar data were obtained by different members of the team over a period of a year.

RESULTS AND DISCUSSION

The gluconeogenic enzyme activities and their behavior in normal, in triamcinolone-injected, in triamcinolone- and actinomycin-

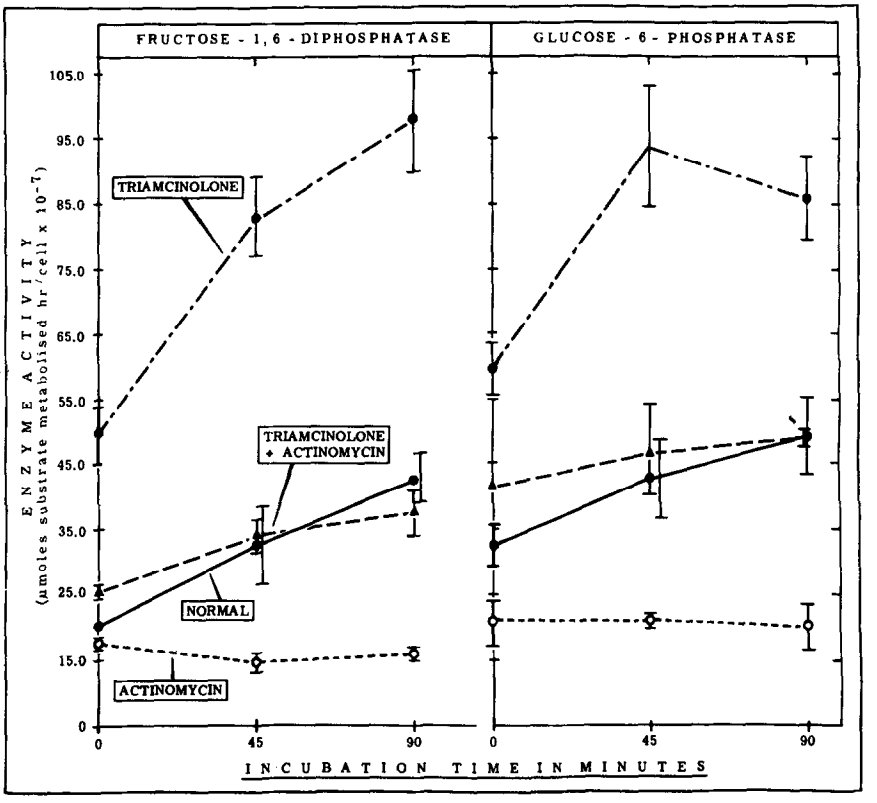

Figure 1. Behavior of gluconeogenic enzymes in slices of liver from animals pretreated with triamcinolone, triamcinolone and actinomycin, actinomycin alone or untreated (normal). The experimental conditions are given in the paper.

treated rats and in rats injected with actinomycin alone are shown in Figure 1. In slices taken from normal, uninjected rats the enzyme activities during the 90-minute incubation period showed a steady rise which was more marked for FDPase than for G-6-Pase. In animals injected with triamcinolone the initial (0 minute) enzyme activities were markedly increased, which confirms previous findings from this laboratory (Weber et al., 1964). In slices taken from the triamcinolone-injected rats the rise in these enzyme activities over the 90-minute incubation period was markedly higher than that found in normal rats. In actinomycin-treated, triamcinolone-injected rats the G-6-Pase and FDPase activities in the initial samples were in the

activity range found in normal animals which is in line with our previous report (Weber et al., 1964). In slices taken from these animals the enzyme increases during incubation were in the range observed in normal rats. In animals which received only actinomycin the enzyme activities in the initial samples were in the same range or somewhat lower than in slices taken from normal, untreated rats. This is in agreement with observations that injection of actinomycin in normal animals did not affect enzyme activities appreciably (Gelboin and Blackburn, 1964, Greengard, 1963, 1964). It is interesting that in these incubation studies evidence was found indicating interference with the synthetic processes in slices taken from actinomycin-treated animals, because both enzyme activities remained at the initial level and failed to show the rise regularly occurring in slices from normal, untreated rats. Addition of actinomycin (1 ug) or puromycin (2 mg) to the incubation medium also blocked the rise of both enzyme activities in slices from normal rats, suggesting that the rise under these conditions was due to new enzyme synthesis.

Preliminary results also showed a very marked increase in gluconeogenic enzyme activities under these experimental conditions in slices from acutely alloxan diabetic rats. The increases were blocked in slices from animals injected with actinomycin or ethionine which is in line with results obtained in vivo previously (Weber and Singhal, 1964b).

The presented results indicate that in the slice system described it is possible to follow increases in liver G-6-Pase and FDPase activities during the 90-minute incubation. Since the slices were taken from rats which were shown to carry out de novo synthesis of these gluconeogenic enzymes as a result of induction by corticosteroid hormones, (Weber et al., 1961a, Weber, 1963, Kvam and Clark, 1960, Weber and Singhal, 1964a, Weber et al., 1963, Weber et al., 1964) the data are compatible with the suggestion that the enzyme synthetic processes continued to operate under in vitro conditions.

In conclusion, the presented results suggest that a slice system is available for investigation of a corticosteroid induced increase in hepatic gluconeogenic enzymes and for the analysis of inhibition of these events by actinomycin.

SUMMARY

The behavior of activities of key gluconeogenic enzymes, hepatic glucose-6-phosphatase and fructose-1,6-diphosphatase, was followed in a slice system during a 90-minute incubation period.

1. In slices from normal, untreated rats the enzyme activities showed an easily measurable increase.

2. In slices from animals injected with the fluorinated adrenocorticosteroid hormone, triamcinolone, the enzyme activity increase was 2 to 3 fold of that found in slices from normal rats.

3. Actinomycin treatment of triamcinolone-injected rats blocked the effect of triamcinolone in increasing enzyme activities during incubation; the rise found was similar to that occurring in normal, untreated rats.

4. In slices obtained from rats injected with actinomycin alone, the activities of both gluconeogenic enzymes failed to rise during the incubation.

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