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EFFECTS OF HYDROXYBRAZILIN ON GLYCOGEN SYNTHESIS IN PRIMARY CULTURED RAT HEPATOCYTES

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ABSTRACT: *Hydroxybrazilin was examined for its effects on glycogen synthesis in primary cultured rat hepatocytes. At 10^{-6} M hydroxybrazilin, glycogen synthesis was increased in basal state, but not in insulin stimulated state. However, any significant changes were not observed at 10^{-5}* M hydroxybrazilin in both states. The glycogen synthesis was rather suppressed at 10^{-5}*M hydroxybrazilin. It was also observed that hydroxybrazilin increased insulin sensitivity but not insulin responsiveness at 10^{-5}M concentration. These results suggest that hydroxybrazilin might exert hypoglycemic action through its effects on insulin receptor and post receptor events.*

Key Words: *Hydroxybrazilin, Hepatocyte, Glycogen Synthesis, Insulin Sensitivity*

INTRODUCTION

The liver is the main storage organ for excessive blood glucose. If blood glucose is elevated excessively, glucose is enzymatically polymerized to form glycogen. This process takes place mainly in the liver and is called glycogenesis. When the blood glucose begins to drop, the glycogen is converted to glucose by a different set of enzymes in a process known as glycogenolysis. Hormones, such as insulin, growth hormone, epinephrine, cortisol, glucagon and tyroxine, play importmant roles in the regulation of the plasma glucose concentration (Levine and Haft, *et al.*, 1970). In diabetic subjects, an absolute or relative lack of insulin occurs, which is a characteristic of type I- or type II-diabetes respectively. Many antidiabetic drugs used in type I and II diabetes, have been studied on their effects on insulin action (McCormick *et. al.*, 1986; Fleig *et. al.*, 1984; Rinninger *et. al.*, 1984; Colwell, 1964; Simonson *et. al.*, 1984 and Boshell *et. al.*, 1960). In our previous study (Kim, 1988) some of γ-pyranoid natural dyes were found to have stimulating effects on insulin action in experimental diabetes, which resulted in hypoglycemic

response. As an attempt to elucidate its hypoglycemic mechanism, we have investigated the effect of hydroxybrazilin, an active principle of *Haematoxylon campechianum*, on the glycogen synthesis in cultured rat hepatocytes.

MATERIALS AND METHODS

Isolation of Rat Hepatocytes

SD male rats weighing about 220g were supplied from Animal Breeding Center of Seoul National Unviersity. The rat was anesthetized with urethane (1 g/kg body weight, i.p.) between 9 a.m. and 10 a.m. Hepatocytes were isolated by the modification of a collagenase perfusion technique as described by Dickines and Peterson (1980).

The perfusion buffer (Ca^{++}, Mg^{++} free HBSS) was maintained at 37°C and gassed continuously with 95% O_2 and 5% CO_2. After 5 min. perfusion, collagenase was added to recirculating perfusion buffer (final concentration of 0.05%) and perfusion was continued for 15 min. Following digestion, the cell suspension was filtered through a 250 μm nylon mesh into a beaker. The filtrate was transferred to sterile 25 m*l* centrifuge tubes and the hepatocytes were centrifuged at 50×g for 4 minutes. The pellet was washed twice and the final sediment was resuspended in WO/BA-M_2 medium containing 10% FBS (Kletzien *et. al.*, 1976).

Hepatocyte Culture and Treatment of Hydroxybrazilin

The cell suspension was diluted to 1.0×10^6 cells/m*l* in the WO/BA-M_2 medium containing 10% FBS and 3 m*l* was pipetted into a collagen coated dish. Cell viability was above 90%.

Hepatocytes were inoculated and allowed to be incubated at 37°C in a humidified CO_2 incubator (5% CO_2, 95% air) for 4 hours. The medium was then replaced with serum free Dulbecco's MEM containing 11 mM glucose. After 24 hrs. incubation the medium was aspirated and fresh medium (serum free Dulbecco's MEM containing 11 mM glucose) with or without hydroxybrazilin ($10^{-4}-10^{-6}$M) was added and incubated for 22 hours.

Determination of Glycogen Synthesis

Hepatocyte monolayers treated with or without hematoxylin were incubated with [^{14}C]-glucose (sp. activity 20 μCi/mmol) and the monolayers were scraped off. The suspension was transferred to microcentrifuge tube and was centrifuged (1 minute, 12,000×g). The obtained cell pellet was dissolved in 30% KOH. Carrier glycogen (5 mg) was added to cell homogenate and was left standing at 90°C of 20 minutes. Glycogen was precipitated by the addition of 2 volumes cold ethanol, followed by centrifugation at 12,000×g for 8 minutes in 4°C cold room. The precipitate was washed twice with cold ethanol. Glycogen precipitate was finally resuspended in 500 μ*l* of water and radioactivity was counted in 10 m*l* of Bray's solution (Cooper, 1971). Protein content was determined by the method of Lowry *et al.* (1951).

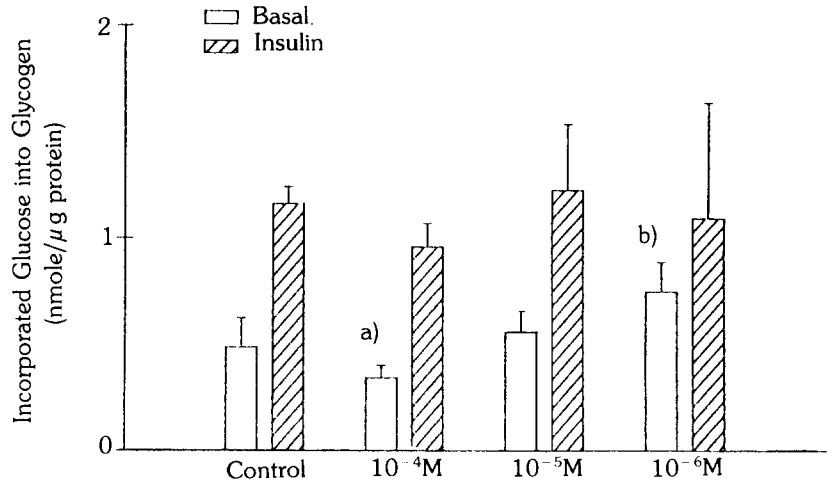

Figure 1. Effect of hydroxybrazilin ($10^{-4}-10^{-6}$M) on glycogen synthesis in primary cultured rat hepatocytes.
a), b): significantly different from control ($p<0.05$).

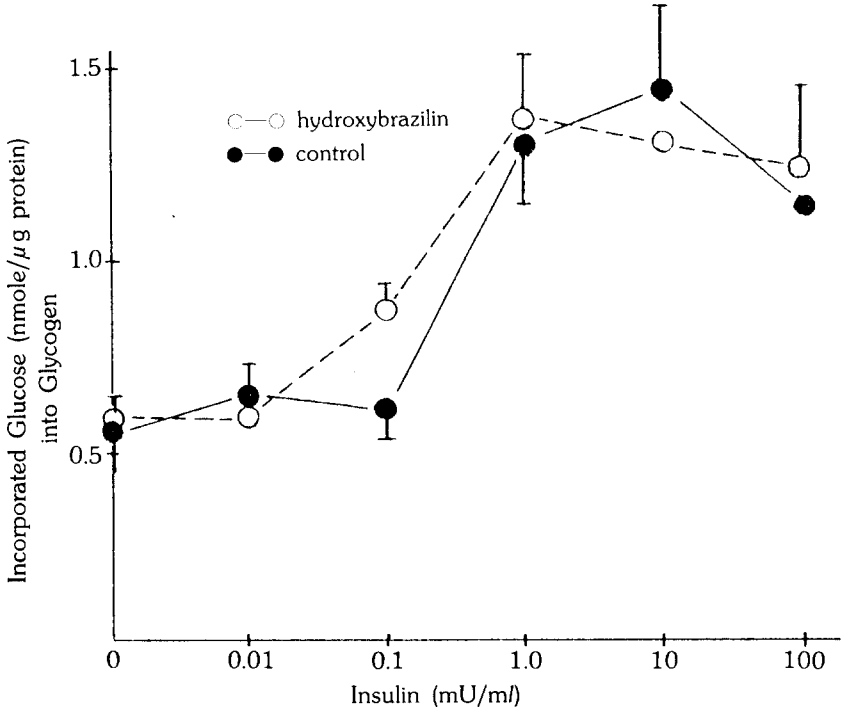

Figure 2. Effect of hydroxybrazilin (10^{-5}M) on the insulin-stimulated glycogen synthesis in primary cultured rat hepatocytes.

Statistical Analysis

Statistical analysis was performed with Duncan's multiple range test.

RESULTS AND DISCUSSION

Insulin resistance may result from prereceptor, receptor, and/or postreceptor defects (Olefsky and Kolterman, 1981; Pizza *et. al.*, 1981 and Olefsky, 1981) and may be manifested to be an increase in the concentration of insulin necessary for a half-maximal effect (decreased sensitivity) or a decrease in the maximal response (decreased responsiveness), or both. In type II diabetes, insulin resistance is one of the major causes of hyperglycemia. To develop new antidiabetic agents, we have started to screen natural products. As a result of our trials, hydroxybrazilin has been already proved to have hypoglycemic action in experimental diabetic rodents. In the present study the effects of hydroxybrazilin on glycogen synthesis was investigated in cultured rat hepatocytes as a trial to elucidate its hypoglycemic mechanism.

The concentration of glucose and the incubation the period for the proper experimental condition were determined as follows. Dulbecco's MEM was supplemented with 11 mM glucose and this particular concentration was chosen to balance the known positive effect of glucose itself on glycogen synthesis against the ongoing glycogenolysis (Frank *et. al.*, 1981 and Hue *et. al.*, 1975). The glycogen synthesis was known to be sufficiently increased by insulin at this glucose concentration (Spence and Koudelka, 1985). In the preliminary experiments, 1.5 hour incubation did not show any significantly enhanced insulin actions (124±24% stimulation vs. basal), but with 3 hours' incubation, insulin sufficiently stimulated the glycogen synthesis (223±39% stimulation vs. basal). Based on this results, the

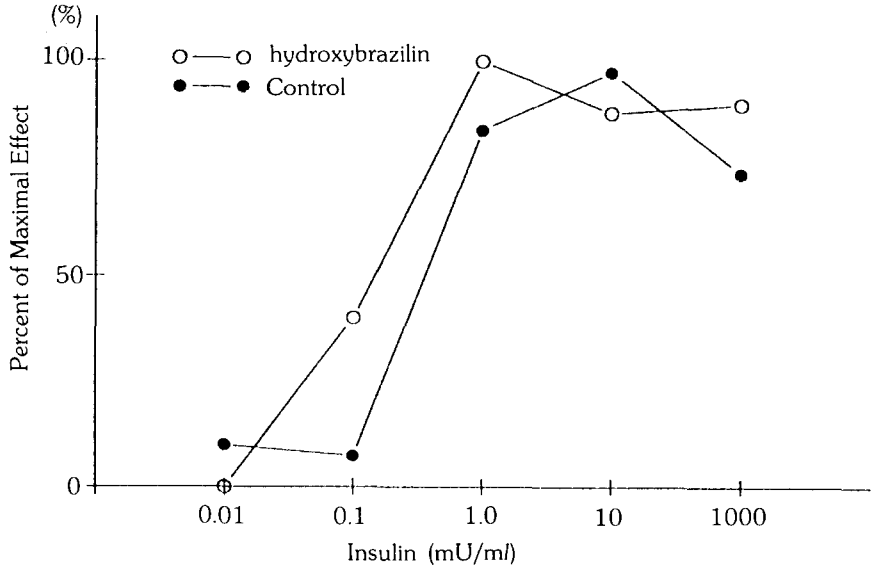

Figure 3. Percent stimulation of insulin action on glycogen synthesis by the treatment of hydroxybrazilin (10^{-5}M).

rate of glycogen synthesis was determined after 3 hours' incubation.

Hydroxybrazilin, a γ-pyranoidal natural dye, increased the glycogen synthesis under this experimental condition. (Fig. 1). At 10^{-6}M hydroxybrazilin in the glycogen synthesis was increased in basal state but not in insulin stimulated state.

However, any significant changes in synthetic rate of glycogen were not observed at 10^{-5}M hydroxybrazilin in both states. At 10^{-4}M hydroxybrazilin the glycogen synthesis was rather suppressed. It was not able to determine the rate of glycogen synthesis at 10^{-3}M hydroxybrazilin because of cell death. The decrease of glycogen synthesis at higher concentration of hydroxybrazilin might be due to its cytotoxicity. Bacteriostatic and antiviral action of hydroxybrazilin might be also based on its cytotoxicity (Aizenman, *et. al.*, 1961).

To elucidate the effects of hydroxybrazilin on glycogen synthesis, responsiveness and sensitivity of insulin were investigated at 10^{-5}M hematoxylin. As shown in Fig. 2, hematoxylin did not affect the insulin responsiveness.

Data-presentation as the percent of maximal insulin stimulation, showed that insulin sensitivity was increased by hydroxybrazilin treatment (Fig. 3). In hydroxybrazilin treated group, half maximal stimulation was achieved at the insulin concentration of 0.1 mU/m*l*, but it was reached to half maximal stimulation at somewhat higher concentration in control group than in hematoxylin treated group. And maximal stimulation was also achieved at lower insulin concentration in hydroxybrazilin treated group than in control group. As the increased sensitivity reflects the increase in receptor number and/or affinity (Kasuga *et. al.*, 1978), it is suggested that hydroxybrazilin might have effects on receptor level at this concentration.

In conclusion, hydroxybrazilin improved insulin sensitivity and stimulated glycogen synthesis without any effects on insulin dependent steps.

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