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EFFECT OF HYPOGLYCIN A ON INSULIN RELEASE

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Abstract—Thirty experimental and fifteen control Wistar rats were studied to determine whether hypoglycin A influences insulin levels in the body to contribute to the state of hypoglycemia usually observed in Jamaican vomiting sickness, a condition arising after ingestion of unripe ackees. This fruit also grows in other Caribbean islands, as well as North and Central America. Hypoglycin A is one of the toxic compounds found in unripe ackees and is capable of inducing hypoglycemia. A fall in blood glucose occurred after administration of hypoglycin A. The lowest level of 42.60 ± 4.84 mg/dl was attained 3 hr after administration of the drug. This alteration of blood glucose from the fasting level of 80.31 ± 5.20 mg/dl was significant ($P < 0.01$). The blood glucose level in the control rats showed no significant change from the fasting level. The insulin level in portal and peripheral blood showed no significant change. Results showed that, although hypoglycin A induced severe hypoglycemia after intravenous application, there was no significant change in insulin levels. This observation suggests that hypoglycin A has a mechanism of action other than an alteration in insulin levels to induce hypoglycemia.

Severe hypoglycemia has been reported in patients with Jamaican vomiting sickness [1]. Ingestion of the arillus of unripe ackee has been identified as a cause of this disease [2]. Two toxins, hypoglycin A and hypoglycin B, have been isolated from the seeds and arillus of unripe ackee [3], and it has been demonstrated that hypoglycin A induces severe hypoglycemia in animal experiments [4]. Although ackees grow in Jamaica, West Africa, Central America and southern parts of Florida, it is not commonly eaten outside of Jamaica.

The hypoglycemia observed with ingestion of ackees has been attributed to metabolic changes induced by hypoglycin A. Hypoglycin B inhibits short and medium chain acyl CoA dehydrogenases [5–7]. This action results in inhibition of fluconeogenesis [8, 9]. It remains, however, to be established whether other factors contribute to the hypoglycemia observed with hypoglycin A. This study was, therefore, undertaken to determine if hypoglycin A influences insulin production from the B cell of the endocrinepancreas and, thereby, contributes to the state of hypoglycemia observed in Jamaican vomiting sickness.

MATERIALS AND METHODS

Forty-five Wistar rats of both sexes, weighing between 300 and 350 g, were studied; fifteen of these rats were used as controls. After weighing, the rats were anesthetized with 50 mg/kg body weight of sodium pentobarbital intraperitoneally. The abdominal wall was opened, and the inferior vena cava and portal vein were displayed. A 1-ml syringe with a fine needle attached was used to sample blood from the portal vein and inferior vena cava. After collecting fasting blood samples, hypoglycin A dissolved in physiological saline solution was administered through the inferior vena cava of each of the thirty experimental rats at a dose of 100 mg/kg body weight, and blood from the inferior vena cava and portal vein was sampled at intervals of 15 min, 30 min, and 1, 2, 3, 4 and 5 hr. The control rats received physiological saline solution and were treated otherwise similarly to the experimental animals. The sampled blood was collected into plastic tubes containing EDTA and immersed in a water bath at 4°. The blood was centrifuged, and the plasma was immediately separated and stored at −20° until required.

Insulin was estimated in plasma sampled from both the inferior vena cava and portal vein using a radioimmunoassay kit supplied by the Radiochemical Centre, Amersham. This method is based on the double antibody technique of Hales and Randle [10]. This assay method has been modified in our laboratory to give increased sensitivity in the range 1–20 μU/ml [11]. Rat insulin standard was supplied as a gift from Eli Lilly.

Glucose was determined in plasma sampled from the inferior vena cava using the glucose oxidase method [12].

Statistical analysis of the data was performed using Student's *t*-test. Changes were reported as significant if the P value was less than 0.05. Numerical results are expressed as means ± SEM.

RESULTS

Figure 1 shows that a fall in blood glucose occurred after administration of hypoglycin A. The lowest level of 42.60 ± 4.84 mg/dl was attained 3 hr after administration of the drug. This alteration of blood

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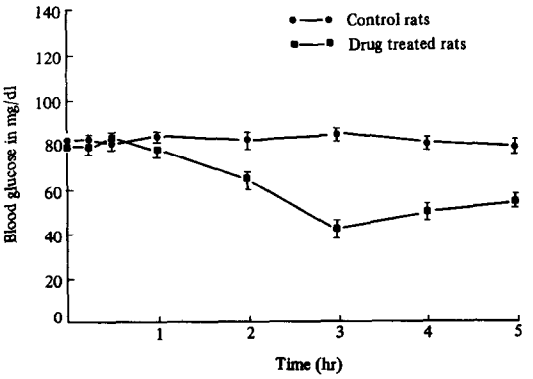

Fig. 1. Blood glucose levels in control and hypoglycin-treated rats. Results are expressed as mean ± SEM. The 3-hr value was significantly different from control ($P < 0.01$).

glucose concentration from the fasting level of 80.31 ± 5.20 mg/dl was significant ($P < 0.01$). The blood glucose level in the control rats showed no significant change from the fasting level.

The insulin level in portal and peripheral blood showed no significant change despite the decrease in blood glucose levels in the treated rats (Fig. 2).

DISCUSSION

Determination of insulin levels at early stages after administration of hypoglycin A was of importance in this study in order to detect any changes in insulin levels that might occur before the appearance of hypoglycemia. Latency for appearance of hypoglycemia in rats given hypoglycin A is 2–3 hr, depending on the dose administered [13, 14]. Tanaka *et al.* [4] used 100 mg/kg body weight intramuscular injection of hypoglycin A in rats and observed peak hypoglycemia in 4.5 hr. The 100 mg/kg body weight intravenous injection of hypoglycin A in this study was capable of inducing hypoglycemia in 3 hr. Although the dose of hypoglycin A used in this study was capable of inducing hypoglycemia in animals, in human cases of ackee poisoning, hypoglycemia may either be absent or occur only at a late stage of the acute illness [15]. This observation suggests that hypoglycin A may influence factors controlling the blood glucose level in such a way that either homeostasis or a derangement prevails. The results of the present study showed that, although hypoglycin A induced a significant change in the blood glucose level, the insulin production from the pancreas and the insulin level in peripheral blood were not altered significantly.

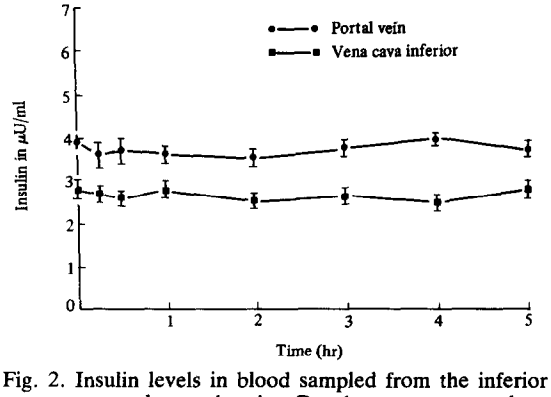

Fig. 2. Insulin levels in blood sampled from the inferior vena cava and portal vein. Results are expressed as mean ± SEM.

The hypoglycemia induced by hypoglycin A has been attributed by earlier investigators [5–7] to inhibition of gluconeogenesis. The inhibition of several short chain acyl CoA dehydrogenases was reported by these investigators to be the major mechanism of action of hypoglycin A. The acyl CoA dehydrogenases which are inhibited by hypoglycin A include butyryl CoA dehydrogenase and glutaryl CoA dehydrogenase. As a result of the inhibition of butyryl CoA dehydrogenase, oxidation of long chain fatty acid stops at the level of hexanoyl CoA and butyryl CoA [7, 16]. This leads to decreased production of NADH and acetyl CoA. Since NADH and acetyl CoA are respectively required as cofactor of 3-phosphoglyceraldehyde phosphate dehydrogenase [17] and an activator of pyruvate carboxylase [18, 19], their diminished concentration would contribute to the inhibition of gluconeogenesis [20–23]. Furthermore, the inhibition of glutaryl CoA dehydrogenase results in accumulation of glutaryl CoA. This could inhibit transmitochondrial malate transport, a rate-limiting step in the early phase of gluconeogenesis, and, consequently, suppress gluconeogenesis [8, 24].

The present findings from experimental animals suggest that hypoglycemia associated with hypoglycin action is not caused by altered levels of circulating insulin.

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