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Effect of bosentan (ET_A/ET_B receptor antagonist) on metabolic changes during stress and diabetes

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Abstract

Elevated plasma ET-1 levels have been reported in several conditions such as stress and diabetes. ET-1 is found to cause insulin resistance and to stimulate liver glycogenolysis. The question arises whether ET-1 has a role in the metabolic changes occurring in such conditions. To test this, we studied the possible effect of the endothelin receptor antagonist, bosentan (50 and 100 mg kg^{-1}) on serum glucose and insulin levels as well as on liver glycogen contents in normoglycemic stressed animals. In addition, the effect of bosentan on serum glucose and insulin levels in both mild and severely diabetic rats and its effect on insulin-induced hypoglycemia were also determined. Restraining water immersion stress was used as a model for severe stress reported to elevate plasma ET-1 level. Mild diabetes was induced in rats by intraperitoneal injection of a low dose of streptozotocin (38 mg kg^{-1}) while severe diabetes was induced by intraperitoneal injection of a higher dose of streptozotocin (45 mg kg^{-1}). Bosentan partially prevented stress-induced both hyperglycemia and decrease in glycogen content while it completely blocked the stress-induced decrease in insulin level in normoglycemic stressed rats. Bosentan also decreased serum glucose level without any effect on insulin secretion in mild diabetic rats and potentiated the hypoglycemic action of insulin.

Keywords: Bosentan; Endothelin; Insulin; Stress; Streptozotocin; Glycogenolysis; Hyperglycemia

1. Introduction

It is well-established that hyperglycemia and glucose intolerance may occur under conditions of severe stress, such as trauma, burn, operation, or myocardial infarction in patients without prior history of diabetes [1]. Such circumstances are accompanied by marked increases in plasma glucagon, catecholamines (epinephrine and norepinephrine), and glucocorticoids as well as decrease in plasma insulin, all of which may contribute to hyperglycemia. Recent findings incorporate endothelin-1, the most potent constrictor discovered to date, as a new mediator in stress [2,3]. Increase in endogenous endothelin-1 due to stress suggested the possibility that endothelin may participate in the metabolic changes caused by stress. Elevated ET-1 levels in the plasma have been reported in patients with insulin resistance, such as that resulting from type II diabetes [4]. In addition, ET-1 is reported to induce insulin resistance in rat adipocytes [5] and rat arterial smooth muscle cells in vitro [6] and in conscious rats in vivo [7]. In healthy humans, exogenous administration of ET-1 has also been found to induce insulin resistance by reducing insulin-dependent glucose uptake in skeletal muscle, without decreasing skeletal muscle blood flow [8]. ET-1 also caused a rapid and sustained stimulation of glycogenolysis and resulted in a dose-dependent hepatic glucose production [9,10]. Interestingly, a negative correlation between total glucose uptake and circulating ET-1 levels was demonstrated by Ferri et al. [11]. All these in vitro studies demonstrated that ET-1 could antagonize insulin actions of facilitating glucose transport or inhibiting glycogenolysis. These considerations prompted us to investigate the effect of bosentan, an orally active non-peptide mixed ET_A/ET_B receptor antagonist, on serum glucose and insulin levels in both stressed and diabetic rats.

2. Materials and methods

2.1. Experimental animals

Adult male Sprague–Dawley rats weighing 150–200 g (purchased from National Organization of Vaccine and Biological Products, El Dokky, Egypt) were housed six per cage in a light-controlled room with an alternating 12 h light/12 h dark cycle.

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2.2. Drugs and chemicals

Bosentan (RO 47-0203) was obtained as a generous gift from Dr. Martine Clozel (F. Hoffman La Roche Ltd., Basel, Switzerland).

Crystalline (Regular) insulin (Actrapid, Novo Nordisk, Bagsvaerd, Denmark) was used in this study.

Streptozotocin (STZ) was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and dissolved in citrate buffer, pH 4.5 [12]. Anthrone was purchased from Sigma Chemical Co. All other chemicals used in this study are of analytical grade.

2.3. Effect of bosentan on serum glucose and insulin levels as well as liver glycogen contents of normoglycemic rats subjected to restraining-water immersion stress

Rats were divided into four groups each of six rats. They were fasted for 16 h and treated with bosentan or the vehicle orally as follows. The first group received bosentan (50 mg kg^{-1}) as 2% suspension in 5% gum arabic, the second group received bosentan (100 mg kg^{-1}) as 4% suspension in 5% gum arabic, the third group received equivalent volume (2.5 ml kg^{-1}) of 5% gum arabic (control stressed group). The three groups of rats were subjected to restraining-water immersion stress (RWIS) as described by Said and El-Mowafy [3] after 2 h of bosentan or vehicle treatment. Rats were lightly anesthetized with ether, restrained on a wooden plate and immersed vertically in water to the level of xiphoid process in a water bath thermostatically controlled at 23 ± 1 °C, for 60 min. Immersion of the animals started after the ether anesthesia was stopped, to prevent any effect of the anesthetic. Blood samples were collected, from retro-orbital sinus, before bosentan or vehicle treatment, after 2 h treatment and at the end of stress period. The determination of serum glucose was performed by enzymatic method described by Trinder [13] using glucose oxidase kit (Bio Merieux, Marcy L' Etoile, France) and serum insulin by using EIA kit (DRG Instruments GmbH, Germany). Liver was taken for glycogen determination by anthrone [14]. The fourth group of rats received equivalent volume (2.5 ml kg^{-1}) of 5% gum arabic (control non-stressed group). Rats in this group were also killed at the end of the experiment and liver was excised for glycogen determination.

2.4. Effect of bosentan on serum glucose and insulin levels in mild and severe diabetic rats

Mild diabetes was produced in rats aged 9–10 weeks by i.p. injection of a low dose (38 mg kg^{-1}) of STZ [15], where partial destruction of pancreatic beta cell mass occurred mimicking the clinical picture of type II non-insulin-dependent diabetes mellitus (NIDDM). Severe diabetes was produced in rats aged 9–10 weeks by i.p. injection of a higher dose (45 mg kg^{-1}) of STZ [16], where nearly complete destruction of pancreatic beta cell mass occurred mimicking the clinical picture of type I insulin-dependent diabetes mellitus (IDDM). Three groups each comprised of six mild diabetic rats were treated with 50 mg kg^{-1} bosentan, 100 mg kg^{-1} bosentan, or the vehicle and another three groups each of six severe diabetic rats received the same previous treatment. Blood samples were collected before treatment, after 2, 4, and 6 h from treatment for determination of serum glucose and insulin levels.

2.5. Effect of bosentan on oral glucose tolerance test in mild diabetic rats

Three groups each of six mild diabetic rats treated with 50 mg kg^{-1} bosentan, 100 mg kg^{-1} bosentan, or the vehicle and one group of six normoglycemic rats received the vehicle before the oral glucose tolerance test. Rats underwent an oral-glucose-tolerance test (OGTT) after an overnight fast [7]. Two hours after vehicle or bosentan administration, a zero-min blood sample was taken from each rat. Then without delaying, rats were given a glucose solution, 2 g kg^{-1} of 20% glucose solution administered by gavage. Five more blood samples were collected at 30, 60, 90, 120, and 180 min. Serum glucose level was determined.

2.6. Effect of bosentan on insulin-induced hypoglycemic effect in severe diabetic rats

Two groups of severe diabetic rats each comprised 11 animals were used in this study. The first group received bosentan 50 mg kg^{-1} orally. The second group received equivalent volume of the vehicle. Two hours after treatment, each rat was injected subcutaneously with 4 units kg^{-1} of crystalline insulin [17]. Blood samples were collected 2 h after bosentan or vehicle treatment, 0.5, 1, 2, 4, 6, and 8 h after insulin treatment for determination of serum glucose levels.

2.7. Statistical analysis

Results are expressed as mean ± S.E.M. Statistical analysis was performed by the aid of the computer program (Instat GraphPad) by using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparisons test in case of more than two groups. Student's unpaired *t*-test was used in case of comparing two groups. Paired *t*-test was used as a test of significance for comparison between two arithmetic means of the same subject before and after treatment. Significance was assumed when *P*-value was less than 0.05.

3. Results

3.1. Effect of bosentan on glucose, insulin levels and glycogen in stressed rats

In both 50 and 100 mg kg^{-1} bosentan-treated groups, the percentage increase in serum glucose levels was significantly