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RESEARCH ARTICLE

Repeated Application of Electroacupuncture Ameliorates Hyperglycemia in Obese Zucker Diabetic Fatty Rats

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Abstract
Electroacupuncture (EA) was investigated for lowering the blood glucose (BG) in fasting male obese Zucker fatty diabetic (ZDF) rats aged 10—17 weeks. Anesthesia provided satisfactory chemical restraint to enable repeated EA. Animals in Groups 1, 2 and 3 were anesthetized on Days 1, 3, 5, 8, 10 and 12. Group 1 ($n = 4$) received no EA (controls), Group 2 ($n = 4$) EA at Zhongwan and Guanyuan acupoints, and Group 3 ($n = 4$) EA at both Zusanli acupoints. BG was measured at 10 and 20 minutes, and EA was applied for 30 minutes, after which BG was measured again. Group 2 had a significantly lower baseline BG at 20 minutes on Days 5, 8 and 12 and significantly less change in BG over 30 minutes on Days 3 and 5 than Group 1 ($p < 0.05$). Group 3 showed a significant decrease in the mean baseline BG compared to Group 1 in Week 1 ($p < 0.05$). Thus, repeated EA using Zhongwan and Guanyuan acupoints was effective in lowering the baseline BG and modulating the change in the BG in anesthetized animals.

1. Introduction

Male obese Zucker diabetic fatty (ZDF) rats are much more prone to developing hyperglycemia than females, and in combination with the defined diet Purina 5008, have emerged as a standard model of human type 2 diabetes. The diabetes-like syndrome of ZDF rats is caused by a mutation in the gene encoding the leptin receptor, resulting in severe dysregulation of appetite and body weight [1]. The predominant factor driving metabolic derangement is severe adiposity-associated insulin resistance. Insulin-sensitizing drugs such as the thiazolidinediones (e.g., pioglitazone) are effective in preventing hyperglycemia or restoring normoglycemia in ZDF rats, if the treatment is

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started at a young age of <9 weeks [2]. Daily administration of pioglitazone (12 mg/kg/day) in 2 mL/kg 5% gum arabic initiated at 7 weeks of age for male obese ZDF rats fed with Purina 5008, completely suppressed the development of hyperglycemia in vehicle-treated ZDF rats over the entire treatment period, with the fasted blood glucose (BG) being 6.4 mmol/L. The same intervention at 10–11 weeks caused only a transient decrease of hyperglycemia [2].

A single application of electroacupuncture (EA) has been reported to have an insulin-sensitizing effect at Zusanli acupoints in male Wistar rats, in which type 1-like diabetes had been induced by injection with streptozotocin [3,4]. In addition, a single treatment of EA at the Zhongwan acupoint in male Goto-Kakizaki rats, a genetic model of non-obese type 2 diabetes, improved glucose tolerance by enhancing insulin sensitivity [5]. A similar effect was found in C57BL/KsJ-Lep$^{db/db}$ mice, a genetic model of insulin resistance and type 2 diabetes, with EA performed with needles inserted into Zusanli and Guanyuan acupoints and five treatments given weekly for 8 weeks [6].

This paper reports on a preliminary study of the effect of repeated EA application in fasted male obese ZDF rats. Treatment was given on alternate weekdays over 12 days, with two different sets of acupoints, Zhongwan and Guanyuan and bilateral Zusanli, being trialed. Application of EA at the Zhongwan and Guanyuan acupoints had been shown to cause a significant hypoglycemic response in fasted male Wistar rats [7]. It seemed pertinent to test whether successive EA treatment in fasted male obese ZDF rats could suppress hyperglycemia in this standard model of human type 2 diabetes.

2. Materials and methods

2.1. Animals

Genetically obese male ZDF rats, 10–17 weeks of age, were obtained from a breeding colony maintained at the Taieri Animal Station, University of Otago. The animals were fed with Purina LabDiet Formulab 5008 (Purina LabDiet, Richmond, Indiana, USA) soon after weaning and also following delivery to the Hercus Research Unit, University of Otago. They were housed in individual cages with free access to food and water, in a room with an artificial 12-hour light/12-hour dark cycle, at constant room temperature. The obese diabetic animals were usually physically identifiable from their lean nondiabetic littermates by 4–5 weeks of age and 'Clinistix' testing of their urine was done at 8–10 weeks of age. Animals were deprived of food at 4:00 PM on the day before the experiment, to ensure an overnight fast of at least 17 hours. This study was approved by the University of Otago Animal Ethics Committee.

2.2. Anesthesia

A pilot study of different anesthetics in male obese ZDF rats was performed and the alteration in BG over 30 minutes was measured. The change in BG for isoflurane (4% induction, 2% maintenance) in oxygen 1 L/min was 3.3 mmol/L; for halothane (4% induction, 2% maintenance) in oxygen 1 L/min was 2.4 mmol/L; for halothane (1%) in 3:1 mixture of nitrous oxide:oxygen 1.2 L/min was 0.9 mmol/L; and for isoflurane (1%) in 3:1 mixture of nitrous oxide:oxygen 1.2 L/min was 1.5 mmol/L. The animals were not sufficiently anesthetized with the latter for EA and this was also found using ketamine (120 mg/kg, s.c.) together with 3:1 mixture of nitrous oxide:oxygen 1.2 L/min. For this study halothane (1%) in 3:1 mixture of nitrous oxide:oxygen 1.2 L/min was used as the anesthetic agent.

2.3. Treatment of animals

The rats were divided into four groups and those in Groups 1–3 were anesthetized with halothane (1%) in nitrous oxide:oxygen on alternate weekdays, giving a total of six applications over 2 weeks. This involved placing the nose of each animal in the nose cone of the anesthetic apparatus and measuring BG with a hand-held glucometer (Accu-Chek Advantage, Roche, Roche Diagnostics NZ Ltd., Mt Wellington, Auckland, New Zealand) after needle pricking the lateral saphenous vein of one of the hind limbs at 10 and 20 minutes. The animals in Group 1 ($n = 4$) were not treated using EA, whereas for Group 2 ($n = 4$) EA was applied using the Zhongwan (CV12) and Guanyuan (CV4) acupoints and for Group 3 ($n = 4$) using both Zusanli acupoints. The acupoints were located using the acupoint detector of the EA unit. The Zhongwan acupoint is 9/14 above the pubic crest of the distance measured between the top of the xiphoid process and the pubic crest, while the Guanyuan acupoint is 2/14 of this distance above the pubic crest [8]. The Zusanli (ST36) acupoints are located 5 mm below and lateral to the anterior tubercle of the tibia. The hair at the acupoint sites was removed with electric clippers. Sterile acupuncture needles (Seirin (Seirin Corporation, Shizuoka City, Shizuoka, Japan), 0.25 × 15 mm) were inserted into the muscle layer at the chosen acupoints to a depth of 2 mm. Electrical stimulation was carried out for 30 minutes at frequency 10 Hz, pulse width 200 mS, and intensity 15 mA, using Hans E600 EA unit (Han's Healthtronics, Likon, Taipei, Taiwan). The positive and negative charges were connected to the Zhongwan and Guanyuan acupoints, respectively. For Zusanli acupoints, the positive and negative charges were introduced to the left and right hind limbs, respectively. The frequency and intensity of the electrical stimulation were monitored with an oscilloscope located at both ends of a resistor (20 Ω) inserted into the circuit. On each day at the completion of EA treatment, BG was measured by sampling blood from the lateral saphenous vein (at 50 minutes following the insertion of the nose of the animal in the nose cone). The weights of the animals were recorded after each treatment.

At the completion of the last application of EA and measuring BG, the rats were given sodium pentobarbitone (60 mg/mL in sterile saline, 0.2 mL i.p.) and blood was collected by cardiac puncture and adipose tissue harvested from the anterior abdominal wall, posterior abdominal wall, mesentery and pelvic region and frozen immediately in liquid nitrogen. The blood samples were centrifuged and the sera stored at −20°C, and the frozen adipose tissue samples stored at −80°C, until assays for glucose, insulin, leptin and adiponectin were performed. Animals in Group 4 ($n = 6$) with mean age 17 (0.2) weeks, were not exposed to halothane (1%) in nitrous oxide:oxygen or given EA