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...
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/Ks-J-Leprdb/db mice, a genetic model of obesity [6] 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 Hercules 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.
treatment. They were anesthetized with sodium pentobarbital (60 mg/mL in sterile saline, 0.2 mL i.p.), and blood collected by cardiac puncture and adipose tissue harvested from the various sites and were also to be assayed for glucose, insulin, leptin and adiponectin. The assay data for blood sera and adipose tissue for Groups 1, 2, 3 and 4 will be presented in a separate paper.

2.4. Statistical analysis

Mean values of BG and change in BG for Groups 1, 2 and 3 on Days 1, 3, 5, 8, 10 and 12, and also over Weeks 1 and 2, were analyzed by analysis of variance (ANOVA) and when a significant p value was found, a Tukey post hoc test was performed for pairwise comparisons between the means. Mean values of BG and change in BG for Weeks 1 and 2 were compared for each group using Student unpaired t test. Significance was taken to be at the level of p < 0.05.

3. Results

3.1. Choice of anesthetic

In a pilot study, halothane anesthesia (4% induction, 2% maintenance) of fasted male obese diabetic ZDF rats caused an increase in BG over 30 minutes of 2.4 (0.4) mmol/L, while isoflurane (4% induction, 2% maintenance) raised BG over 30 minutes by 3.3 (0.6) mmol/L. The use of 1% halothane in 3:1 nitrous oxide:oxygen mixture was found to be satisfactory for anesthetizing the animals sufficiently to enable blood samples to be collected from the lateral saphenous vein of the hind limbs and to perform EA. It also minimized the amount of halothane that each animal was exposed to over the course of the study. It was less satisfactory using 1% isoflurane in 3:1 nitrous oxide:oxygen mixture, as the animals were not sufficiently anesthetized for blood sampling or EA.

3.2. Monitoring of animals

None of the animals showed any physical signs of discomfort or of being stressed when examined prior to withdrawing food or treatment the following day. They were all physically active in their cages, their eyes were bright and without any discharge, and their coat was clean and well groomed. No change in body weight occurred in Week 2 compared to Week 1. The means (SE) of body weight in Weeks 1 and 2 were, for Group 1, 423 (7) and 420 (8) g; Group 2, 359 (9) and 361 (10) g; and Group 3, 418 (7) and 411 (6) g, respectively. The mean ages at the start of the study were, for Group 1, 15.8 (0.3) weeks; Group 2, 12.3 (1.1) weeks; and Group 3, 15.0 (0.0) weeks. All the animals completed the study.

3.3. Effect of EA treatment on BG

In this study, the baseline glucose level was taken to be at 20 minutes after insertion of the nose of animal into the nose cone of the anesthetic apparatus, and at this time the animals were well relaxed. Only a small increase in BG occurred from 10 minutes to 20 minutes (mean value of all measurements at 10 minutes was 8.17 mmol/L and at 20 minutes was 8.66 mmol/L), and the change in BG over a 30 minute period was measured from 20 minutes to 50 minutes.

The mean BG levels on the various days of treatment for Groups 1, 2 and 3 are shown in Table 1. For Group 2, the baseline BG level at 20 minutes on Days 5, 8 and 12 was significantly lower than for Group 1 (p < 0.05), as was also the BG level at 50 minutes on Days 3, 5 and 12 (p < 0.05). A significant modulation of the change in BG measured over a 30 minute period was found for Group 2 compared to Group 1 on Days 3 and 5 (p < 0.05). No other significant changes were found on comparing Group 2 or Group 3 with Group 1 (p > 0.05).

The baseline BG level for Group 2 at 20 and 50 minutes on Day 12 was significantly lower than for Group 3 (p < 0.05). In addition, the change in BG over a 30 minute period for Group 2 was significantly less than for Group 3 on Days 3 and 5 (p < 0.05).

The baseline BG values and the change over 30 minutes for Weeks 1 and 2 are presented in Table 2. For Group 1, the baseline BG value for Week 2 was significantly lower than for Week 1 (p < 0.05) and may have been due, in part, to being deprived of food, but there was no significant change in BG over 30 minutes for Week 2 compared to Week 1 (p > 0.05). No significant differences were found for Groups 2 and 3 in Week 2 compared to Week 1 (p > 0.05).

For Group 2, the baseline BG value was significantly decreased compared to Group 1 for both Week 1 and Week 2 (p < 0.05), and the change over 30 minutes in Week 1 was also significantly lower than for Group 1 (p < 0.05). For Group 3, a significant decrease in baseline BG value compared to Group 1 was found in Week 1 (p < 0.05). No other significant changes were found on comparing values for Groups 2 and 3 with Group 1 (p > 0.05).

4. Discussion

This is the first reported study of repeated EA treatment in an adult obese animal model of type 2 diabetes, the ZDF rat. It was made possible by developing an anesthetic procedure that used 1% halothane in a 3:1 mixture of nitrous oxide and oxygen. The male obese diabetic ZDF rats were sufficiently anesthetized using this gaseous anesthetic, to enable repeated blood sampling from the lateral saphenous vein and with successive EA treatment given three times per week on alternate weekdays. The only other reported animal studies that used repeated EA treatment were performed in conscious male Wistar rats 4 weeks of age [9] or conscious male Sprague Dawley rats 16 weeks of age, restrained in a plastic holder [10]. All the other animal studies involved a single EA treatment in anesthetized animals (usually with sodium pentobarbital), which were mainly type 1 diabetic or normal and were terminal studies. Only one other study has used an adult rat model of type 2 diabetes, which was the non-obese Goto-Kakizaki rat [5].

EA was performed in fasted male diabetic ZDF rats at two different sets of acupoints, i.e., Zhongwan and Guanyuan (Group 2) and bilateral Zusanli (Group 3), and BG data compared with animals not receiving any EA treatment (Group 1). EA treatment at the abdominal acupoints had a
much greater effect on BG levels than EA at the hind limb acupoints. Baseline BG levels for Group 2 were significantly lowered at Days 5, 8 and 12 compared to Group 1 and on Day 12 compared to Group 3, and the change in BG over a 30 minute period for Group 2 was decreased on Days 3 and 5 compared to Group 1 and Group 3. Thus, it would appear that EA treatment at the abdominal acupoints is able to decrease baseline BG levels and modulate the increase in BG that occurred over a 30 minute period. There was no change in baseline BG levels or modulation in the rise of BG over a 30 minute period for Group 3 compared to Group 1.

Analysis of the data for Weeks 1 and 2 showed that the baseline BG for Group 2 was significantly less than for Group 1 in both Weeks 1 and 2, and that the change in BG over 30 minutes in Week 1 was significantly decreased compared to Group 1. For Group 3, a significant change occurred in baseline BG level in Week 1 compared to Group 1, but there was no significant alteration in the change in BG over 30 minutes in Week 1. Thus, EA at the abdominal acupoints, brought about glycemic control in both Weeks 1 and 2 whereas EA at the hind limb acupoints was less effective, bringing about significant change only over Week 1. While it is recognized that Group 2 comprised animals which were slightly younger than those in Groups 1 and 3, there was no significant difference in baseline BG values on Day 1 and Day 3 for this group compared to the other two groups. Group 2 was the only group in which the rise in BG that occurred over 30 minutes in the presence of halothane, was completely inhibited and replaced by a negative change on Days 3 and 5. Furthermore, it was only for Group 2 that the baseline BG level on Day 12 was significantly lower than that for Group 1 and also significantly decreased from its level on Day 1 (unpaired t test, p < 0.05). The finding that EA at the abdominal acupoints had a more pronounced hypoglycemic action than EA at the hind limb acupoints is significant, as there were no previous studies of EA treatment in this animal model of type 2 diabetes.

In several recent studies, it was found that regular administration of insulin-sensitizing drugs like the thiazolidinediones rosiglitazone or pioglitazone, to male obese ZDF rats on Purina 5008, fully prevented hyperglycemia or restored normoglycemia, if the treatment was initiated at a young age of <9 weeks. More severe hyperglycemia prevailing at ages 10–12 weeks could only be ameliorated by thiazolidinediones, and intervention at 21 weeks of age was without a glucose-lowering effect [2]. Our finding of a hypoglycemic effect in male obese ZDF rats on Purina 5008 at a mean age of 12 weeks (Group 2) using EA at Zhongwan and Guanyuan acupoints, is therefore very significant, and provides a possible explanation for the less pronounced effect of EA at Zusanli acupoints in animals at a mean age of 15 weeks (Group 3), which would have been at a later stage of metabolic derangement.

In conclusion, repeated EA treatment at the Zhongwan and Guanyuan acupoints lowered the fasted baseline BG level from 10.25 mmol/L on Day 1 to 7.53 mmol/L and 6.75 mmol/L on Day 3 and Day 12, respectively, and did not have any apparent adverse effect on the wellbeing of the animals. The BG of fasted non-diabetic lean ZDF rats of a comparable age was reported to be 5 mmol/L [2]. Further

### Table 1
Blood glucose measurements on days of treatment for Groups 1, 2 and 3.

<table>
<thead>
<tr>
<th>Treatment, Day</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 min</td>
<td>12.18 (1.16)</td>
<td>10.25 (0.55)</td>
<td>9.85 (1.27)</td>
</tr>
<tr>
<td>50 min</td>
<td>13.35 (1.00)</td>
<td>10.98 (0.50)</td>
<td>12.03 (1.87)</td>
</tr>
<tr>
<td>Change</td>
<td>1.18 (0.72)</td>
<td>0.73 (0.54)</td>
<td>2.18 (0.74)</td>
</tr>
<tr>
<td>20 min</td>
<td>9.95 (0.88)</td>
<td>7.53 (0.76)</td>
<td>8.00 (0.43)</td>
</tr>
<tr>
<td>50 min</td>
<td>11.65 (1.26)</td>
<td>7.18 (0.75)</td>
<td>9.80 (0.58)</td>
</tr>
<tr>
<td>Change</td>
<td>1.70 (0.50)</td>
<td>-0.35 (0.31)</td>
<td>1.75 (0.30)</td>
</tr>
</tbody>
</table>

Table 2
Blood glucose measurements over Weeks 1 and 2 for Groups 1, 2 and 3.

<table>
<thead>
<tr>
<th>Week 1 Baseline</th>
<th>Change in 30 min</th>
<th>Week 2 Baseline</th>
<th>Change in 30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>10.63 (0.60)</td>
<td>1.49 (0.30)</td>
<td>9.13 (0.30)</td>
</tr>
<tr>
<td>Group 2</td>
<td>8.16* (0.57)</td>
<td>0.08* (0.24)</td>
<td>7.08* (0.27)</td>
</tr>
<tr>
<td>Group 3</td>
<td>8.62* (0.51)</td>
<td>1.70 (0.29)</td>
<td>8.34 (0.28)</td>
</tr>
</tbody>
</table>

Values are means (SE). Means tested for significant differences between Groups 1, 2 and 3 by ANOVA and Tukey test.

* Denotes significant difference for Group 2 compared to Group 1 (p < 0.05).

Additional notes:

- Denotes significant difference between Group 2 and Group 3.
- Denotes significant difference for Group 2 or Group 3 compared to Group 1 (p < 0.05).
- Denotes significant difference for Week 2 compared to Week 1 by Student unpaired t test (p < 0.05).
studies are warranted to test the effect of repeated EA at the abdominal and hind limb acupoints in male obese diabetic ZDF rats over a longer period, and also of different ages. EA has been given to human patients with type 2 diabetes and the reduction in BG was more pronounced with an increasing number of treatments, with fasted BG lowered from 8.1 to 6.2 mmol/L after 3 months of treatment [11].

**Disclosure statement**

The author affirms there are no conflicts of interest and the author has no financial interest related to the material of this manuscript.

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**References**