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Chronic exercise enhances insulin secretion ability of pancreatic islets without change in insulin content in non-diabetic rats

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ABSTRACT

We evaluated the effect of chronic exercise on insulin secretion in response to high-glucose by using a perifusion method with isolated pancreatic islets from normal rats. Male Wistar rats were assigned to one of two groups: a sedentary group and a trained group. Running exercise was carried out on a treadmill for one hour per day, five days per week, for six, nine, or 12 weeks. The chronic exercise significantly enhanced the insulin secretion ability of pancreatic islets in response to the high-glucose stimulation upon nine and 12 weeks of exercise. The insulin content in the pancreas and the weight of the pancreas did not change upon nine weeks of exercise. Potassium-stimulated insulin secretion was also increased in the islets isolated from rats that trained for nine weeks compared with that in sedentary rats, suggesting that insulin secretion events downstream of membrane depolarization are involved in targets of the exercise effect. These findings suggest that chronic exercise could be a useful strategy not only for the maintenance of peripheral insulin sensitivity but also for the promotion of islet function to secrete insulin in non-diabetics.

1. Introduction

Physical exercise is well recognized as an effective strategy to improve blood glucose control in both normal and diabetic individuals [1–3]. For example, chronic exercise improves overall glucose homeostasis by increasing the rate of whole-body glucose disposal. Many lines of evidence show that an increase in the sensitivity of skeletal muscle glucose transport to insulin is one of the mechanisms by which exercise produces this effect. On the other hand, whether chronic exercise affects the ability of the pancreas to secrete insulin is still poorly understood. Generally, chronic exercise is associated with a lowered basal insulin concentration in the circulation. This lower blood insulin level does not directly indicate an attenuated ability of the pancreas to secrete insulin, but is instead interpreted as a result of an increase in insulin sensitivity in peripheral tissues, such as skeletal muscle, due to exercise repetitions, which enables a reduction in the amount of insulin required to maintain glucose homeostasis [1–3].

Although the effect of exercise on insulin secretion has been of considerable interest to investigators, a number of conflicting results on this issue have been reported to date. When considering only studies that used rats as an animal model, physical exercise has been found to have no [4], negative [5–10], and positive [10–16] effects on the potential for insulin secretion from the pancreas, pancreatic islets, or beta-cells. One possible reason for this is variation in the method used to evaluate insulin secretion ability: hyperglycemic clamp *in vivo* [7,11,13,14], static incubation of isolated islets [9,10,17], single beta-cell assay [4,8], and perifusion of isolated islets [5,6,12,16].

Since its introduction, perifusion assay has been used as a reliable method to evaluate the insulin secretion potential of isolated pancreatic islets [18]. This is an effective and straightforward method to evaluate the ability of islets to secrete insulin against various secretagogues without an influence of the extracellular environment *in vitro*. To our knowledge, only three studies have used this method to determine the effects of chronic exercise on insulin secretion ability. In the early 1980s, it was reported that chronic exercise training with swimming [5] and voluntary wheel running [6] attenuates insulin secretion from isolated islets in normal rats in response to exposure to a high concentration of glucose. On the other hand, Delghingaro-Augusto et al. recently observed with the method that exercise prevents beta-cell failure in susceptible islets of Zucker diabetic rats [16]. As the perifusion method has been progressively improved for more than 30 years compared with the approach used in the 1980s, it might be worth re-evaluating the effect of chronic exercise on the insulin secretion ability of

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islets. In this context, this study was designed to evaluate the effect of chronic exercise training on insulin secretion in response to high-glucose by using a perifusion method with isolated pancreatic islets of normal non-diabetic rats.

2. Materials and methods

2.1. Animal care and exercise training program

Male Wistar rats (Japan CLEA) with an initial body weight of approximately 120 g were housed in a cage in a temperature-controlled room at 23 °C with a 12-h (5:00 a.m.–5:00 p.m.) light–dark cycle. The animals were randomly assigned to one of two experimental groups: sedentary control and chronic exercise-trained groups. Prior to the chronic exercise protocol, the rats in the trained group were habituated to treadmill running for 15 min from 0 to 15 m/min for five days. After adaptation, the trained group was subjected to chronic exercise on a treadmill for 60 min at 25 m/min, five days per week, for six, nine, or 12 weeks. When the trained rats exercised, the sedentary rats were placed beside the treadmill and exposed to the same environment, but did not run on a treadmill. Trained rats were sacrificed 36 h or later after the last exercise session. All experiments conducted in this study were approved by the Animal Care Committee of Tokyo Metropolitan University.

2.2. Perifusion assays of isolated islets

Islets were isolated from Wistar rats by pancreatic duct injection of 1.33 mg/ml collagenase solution, followed by digestion at 37 °C for 9–12 min with gentle shaking, as reported previously [19]. Islets were picked up by hand selection under a dissecting microscope. A 2.5-ml syringe was cut to make its volume 700 μl, and the bottom was plugged with Sephadex G-25 gel (GE Healthcare). Fifty islets were placed on the gel in the handmade syringe column. This column was eluted with standard low-glucose Krebs–Ringer (KR) buffer (15 mM HEPES pH 7.4, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 24 mM $NaHCO_3$, 0.1% bovine serum albumin, and 2.8 mM glucose) at a constant flow rate of 1.0 ml/min for 30 min, which stabilized the basal insulin secretion from the islets. For glucose stimulation, islets were perifused with high-glucose KR buffer (16.7 mM) for 15 min, followed by low-glucose buffer for 10 min. For stimulation by high-potassium, islets were perifused with buffer containing 30 mM KCl plus 95 mM NaCl for 15 min, followed by low-glucose buffer for 20 min. During the entire perifusion period, samples were collected every minute or two for insulin assay. The insulin concentration of the sample buffer was measured by mouse insulin ELISA. Area under the curve of the perifusion assay was calculated using the obtained insulin concentration results.

2.3. Quantification of insulin content in pancreas

For the quantification of insulin content, the pancreases were exercised and weighed, and each was then cut into small pieces and frozen using liquid nitrogen. Insulin was extracted by homogenization using a glass-Teflon homogenizer (1300 rpm, 30 strokes) in an acid–ethanol solution (70% ethanol and 0.18 M HCl) and then by sonication for 20 s. After centrifugation at 3000*g* for 10 min, the immunoreactive insulin in the supernatant was measured.

2.4. Immunoblotting

Islet proteins (40 μg) were separated by SDS–PAGE and transferred to nitrocellulose membranes. The nitrocellulose membranes were blocked in Tris-buffered saline with either 5% milk or 5% bovine serum albumin and immunoblotted using antibodies against glucose transporter 2 (GLUT2; 07-1402, MILLIPORE), potassium inwardly rectifying channel, subfamily J, member 11 (Kir6.2; AB5495, MILLIPORE), calcium channel, voltage-dependent, P/Q type, alpha 1A subunit (Cav2.1; AB5152, MILLIPORE), synaptosomal-associated protein 25 (SNAP25; ab5666, abcom), vesicle-associated membrane protein 2 (VAMP2; 104211, Synaptic Systems), and syntaxin 1 (S1172, Sigma–Aldrich). The blots were then incubated with secondary antibody (donkey anti-rabbit IgG horseradish peroxidase, Amersham Biosciences) for one hour at room temperature, followed by enhanced chemiluminescence (PerkinElmer). The intensity of the bands was quantified by densitometry.

2.5. Phenotypic characterization

For insulin tolerance test (ITT), human insulin (Humulin R; Eli Lilly) was injected intraperitoneally into the sedentary and trained rats in the eighth week of the training session (0.75 U/kg body weight). Blood samples were collected at 0, 15, 30, 60, 90, 120, 150, and 180 min after the injection of insulin. A drop of tail blood was applied to a One Touch Ultra glucometer (LifeScan Johnson & Johnson, CA) to measure blood glucose levels. Body weight, blood glucose level during the feeding period, and food intake were measured every two weeks until the eighth week of the nine-week exercise period. Since the ITT test was expected to influence the measurements, these variables were not measured in the ninth week.

2.6. Statistics

Data are expressed as means ± SEM. Statistical analysis of the data was performed using Student's *t*-test. A *p*-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Effect of chronic exercise on high-glucose-stimulated insulin secretion in perifused islets

To evaluate the effect of chronic exercise on the ability of islets to secrete insulin, rats were subjected to treadmill running for one hour per day, five days per week, for six, nine, or 12 weeks. Pancreatic islets were isolated from sedentary and trained rats 36 h or later after the last exercise session, and were then used for the perifusion assay. As shown in Fig. 1 (left column), insulin secretion in response to high-glucose (16.7 mM) was enhanced in trained rats compared with that in sedentary rats for all exercise durations. Areas under the curve of insulin secretion obtained from the perifusion assay for the different exercise periods were significantly larger in the groups that trained for nine ($p < 0.01$) and twelve weeks ($p < 0.05$) than in the sedentary group (Fig. 1, right column). Even with six weeks of exercise, the area under the curve of perifusion analysis tended to be larger than in the sedentary group ($p = 0.07$). These results show that chronic exercise enhances the ability of islets to secrete insulin in response to high-glucose in normal rats. Since statistically significant differences were obtained for the area under the curve from nine weeks, we chose nine weeks of exercise for the subsequent analyses.

3.2. Quantification of pancreatic insulin content

To test whether the enhancement of insulin secretion ability by exercise is due to increased insulin storage, insulin content of the pancreas was measured in rats trained for nine weeks and their sedentary counterparts. There was no significant difference in pancreatic insulin content between the sedentary and trained