Pharmacology, Biochemistry and Behavior 100 (2012) 554–559

Contents lists available at SciVerse ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Exercise-induced galanin release facilitated GLUT4 translocation in adipocytes of type 2 diabetic rats

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ARTICLE INFO

Article history:
Received 14 May 2011
Received in revised form 24 October 2011
Accepted 31 October 2011
Available online 6 November 2011

Keywords:
Adipocytes
GLUT4
M35
Galanin

ABSTRACT

Although galanin has been shown to increase insulin sensitivity in skeletal muscle of rats, there is no literature available about the effect of galanin on Glucose Transporter 4 (GLUT4) translocation from intracellular membrane pools to plasma membranes in adipocytes of type 2 diabetic rats. In the present study M35, a galanin antagonist was used to elucidate whether exercise-induced galanin release increased GLUT4 translocation in adipocytes of streptozotocin-induced diabetic rats. The present findings showed that plasma galanin levels after swimming training in all four trained groups were higher compared with each sedentary control. M35 treatment had an inhibitory effect on glucose infusion rates in the euglycemic–hyperinsulinemic clamp test and GLUT4 mRNA expression levels in adipocytes. Moreover, M35 treatment reduced GLUT4 concentration in both plasma membranes and total cell membranes. The ratios of GLUT4 contents in plasma membranes to total cell membranes in four drug groups were lower compared with each control. These data demonstrate a beneficial role of endogenous galanin to transfer GLUT4 from internal stores to plasma membranes in adipocytes of type 2 diabetic rats. Galanin plays a significant role in regulation of glucose metabolic homeostasis and is an important hormone relative to diabetes.

1. Introduction

Physiological control of blood glucose levels is primarily regulated by increased glucose clearance in response to exercise or insulin release. Skeletal muscle and fat tissue are quantitatively the most important target tissues for exercise and insulin stimulated glucose disposal. Proper exercise is frequently recommended for improving glucose clearance by accelerating Glucose Transporter 4 (GLUT4) translocation and by increasing neuropeptide galanin secretion (Legakis et al., 2000).

Of fourteen members of a glucose transporter family (Augustin, 2010), GLUT4 is the most important one to transport glucose into adipose and muscle tissues to maintain glucose metabolism homeostasis. In the basal state most GLUT4 locates in intracellular vesicular compartments. As stimulated by exercise or insulin it transfers from intracellular sites to plasma membranes which is a key process involving glucose clearance. Only in the plasma membrane can GLUT4 transfer glucose into adipocytes and myocytes.

Seeing that animals with metabolic disorder of galanin easily suffer from type 2 diabetes mellitus (Legalkis, 2005), and galanin gene knockout mice have impaired glucose disposal (Ahren et al., 2004), while the homozygous galanin transgenic mice show a reduction in insulin resistance and an increase in metabolic rates of lipid and carbohydrate (Poritsanos et al., 2009), we postulate that endogenous galanin is able to accelerate GLUT4 translocation. To test this hypothesis, we explored the effect of an increase in exercise-induced galanin release on GLUT4 translocation in skeletal muscles. The result demonstrates that galanin has an important attribute to promote GLUT4 trafficking from intracellular membranes to plasma membranes in myocytes (Jiang et al., 2009; He et al., 2011).

Exercise-induced GLUT4 trafficking takes place at least through two ways in skeletal muscle. One is by contraction-induced elevated AMP/ATP ratio and intracellular Ca^{2+} (Sakamoto and Holman, 2008), the other is by the increase in galanin release. Nevertheless, the contract-induced GLUT4 trafficking takes place only in myocytes, not in adipocytes, as adipose tissue does not contract during exercise. And it is unknown whether galanin promotes GLUT4 translocation to plasma membranes in adipocytes, as it does in myocytes. It is in adipose that the agents and the exact mechanism to elicit the GLUT4 redistribution during exercise are not fully understood yet now.

In the present study, we used M35 [galanin(1–13)-bradykinin(2–9) amide], a galanin receptor antagonist, to evaluate the putative relation between endogenous galanin level and GLUT4 trafficking in adipocytes of type 2 diabetic rats. In this experiment animal exercise was taken as a physiological stimulus to increase endogenous galanin secretion.

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doi:10.1016/j.pbb.2011.10.026

2. Materials and methods

2.1. Materials

M35 was purchased from Sigma-Aldrich Inc, USA. Rat GLUT4 ELISA kit and rat galanin ELISA kit from Santa Cruz Biotechnology Inc, USA. Trizol reagent from Gibco Invitrogen, USA.

2.2. Animals

2.2.1. Animal model of type 2 diabetes

140–160 g male Wistar rats were housed in individual cages at 22 ± 2 °C with a 12 h light/dark cycle and fed with high-fat diet (59% fat, 21% protein and 20% carbohydrate). Eight weeks later, some of animals were treated with an i.p. of streptozotocin (30 mg/kg) dissolved in citric buffer. The tail blood of the rats was weekly taken to determine the blood glucose level with a Glucometer (HMD Biomedical, Taiwan) during the study. After another four weeks, animals with glucose levels over 16.7 mmol/l on two consecutive measurements were taken as models of type 2 diabetes. Sixty-four diabetic rats and sixty-four nondiabetic rats were used in this study. This research was approved by the Animal Study Committee of Yangzhou University.

2.2.2. Animal grouping

The experimental protocol is a combination of 2 states (diabetic, non-diabetic) × 2 activity (sedentary, swimming) × 2 drugs (saline, M35). All animals tested were assigned to either four exercise groups or four sedentary groups. The nondiabetic rats were randomly distributed into four experimental groups: sedentary control (S, n = 16), trained control groups (T, n = 16), sedentary control with M35 (S-M35, n = 16) and trained control with M35 (T-M35, n = 16). While the diabetic animals into diabetic sedentary control (Diab-S, n = 16), diabetic trained control (Diab-T, n = 16), diabetic sedentary group with M35 (Diab-S-M35, n = 16) and diabetic trained group with M35 (Diab-T-M35, n = 16). Rats from four non-drug groups were injected i.p. with 0.3 ml citrate buffer, while rats from four drug groups with M35 (25 mg/kg) in the citrate buffer. The injection was given before swimming training or at corresponding time.

2.2.3. Exercise training

To familiarize with the swimming area, the rats in four exercise groups adaptively exercised for 3 days first, 15, 30 and 45 min/day respectively. Then animals were trained for 1 h/day, 5 days/week for 4 weeks as previously described (Jiang et al., 2009). Swimming training was performed in square tanks filled with warm water at 32 ± 2 °C (45 cm in depth). During the period the rats were continuously supervised. After swimming animals were carefully dried up with towels and air blowers.

2.2.4. Taking animal samples

At the end of the swimming protocol, all animals were fasted overnight (12 h) and then were anesthetized by 3% amobarbital sodium (50 mg/kg i.p.). Half of rats in every group (n = 8) were subjected to Euglycaemic hyperinsulinaemic clamp tests, and the remains (n = 8) were collected 4 ml artery blood for the measurement of galanin concentrations and 4–6 g epididymal fat pad for the determination of GLUT4 protein level and GLUT4 gene expression level. Once above experiments were completed, all rats were humanely killed under anesthesia, by infusion of amobarbital sodium and saturated potassium chloride.

2.3. Measurement of galanin concentrations

Plasma samples were obtained through centrifuge. The galanin levels were measured by using an ultrasensitive rat galanin ELISA kit.

2.4. Hyperinsulinemic–euglycemic clamp

A hyperinsulinemic–euglycemic clamp technique was performed according to previous work (Legakis et al., 2007). After an overnight fast of 12 h, rats were anesthetized as above. One cannula was inserted into the right jugular vein for infusion of glucose and insulin, and the other cannula into the left carotid artery for blood sampling. The first blood sample was taken to determine baseline blood glucose concentrations. The glucose clamp was started by insulin infusion at a constant rate of 2 mU/kg·min until the end of the clamp. Thereafter, a 10% glucose solution was infused simultaneously at a variable rate to maintain plasma glucose values at a clamp level (5.0 ± 0.5 mmol/l). Blood samples were drawn at 5-minute intervals to measure plasma glucose concentrations. Insulin sensitivity was calculated as the glucose infusion rates corresponding to the last 6 samplings.

2.5. GLUT4 mRNA expression

As previously described (Komatsu et al., 2003), the GLUT4 mRNA expression level was evaluated by reverse transcription followed by polymerase chain reaction (RT-PCR). The total RNA was isolated from 100 mg of the frozen adipose tissue with the Trizol reagent. The concentration and integrity of the RNA were assessed by spectrophotometric assays of 260 nm. cDNA was synthesized from 1 μg RNA using MMLV reverse transcriptase. The cDNA obtained after reverse transcription was amplified with a Exicycler™ 96 PCR machine (LG company, Korea). The primers used for gene amplification were: sense, 5′-ACAGGGCAAGGATGGTAGA-3′, reverse 5′-TGGAGGG GAACAAGAAAGT-3′.

For each assay, a hotstart at 95 °C for 30 min was performed before the following PCR cycling parameters: denaturation at 95 °C for 30 s, annealing for 30 s at 60 °C and extension at 72 °C for 60 s. Each sample underwent 40 cycles. PCR products were analyzed by ethidium bromide staining and visualization using ethidium bromide staining (100 mg EB in 1 l PBS). The $2^{-\Delta\Delta CT}$ method was use to analyze the real-time PCR data (Livak and Schmittgen, 2001). All values were normalized to β-actin levels, which was used as the internal control.

2.6. Subcellular fractionation of rat adipocytes

Membranes of adipocytes were fractionated as described previously (Beard et al., 2006). Briefly, fat pads were washed, minced and homogenized in ice-cold homogenization buffer (250 mmol/l sucrose, 2 mmol/l EDTA, 2.5 mmol/l Tris–HCl, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 100 μmol/l phenylmethylsulfonyl fluoride, pH 7.4), and then centrifuged at 13,000 *g* for 20 min at 4 °C to remove the fat cake. The infranatant was centrifuged at 31,000 *g* for 1 h to yield the low-density intracellular membranes. The pellet from the first spin was layered over a sucrose cushion and centrifuged at 75,000 *g* for 1 h. The interphase was removed and spun at 39,000 *g* for 20 min to yield the plasma membranes.

2.7. Western blotting

Fifty micrograms of plasma membranes or intracellular membranes was subjected to 12% sodium dodecylsulfate polyacrylamide gel electrophoresis according to the method described before (Geiger et al., 2006). Separated proteins in gel were electrophoretically transferred to a polyvinylidene difluoride filter membranes. Immunodetection was performed with a polyclonal antibody contained in GLUT4 ELISA

kit against GLUT4 C-terminal peptide, washed with Tris-buffered saline with 0.1% Tween and incubated with horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. Bands were revealed by using enhanced chemiluminescence. Visualization and quantification were performed with a HPIAS-2000 Image Analysis System (ChampionImages, China). The sum of the GLUT4 concentration in plasma membranes and in intracellular membranes was taken as the GLUT4 concentration of total cell membranes.

2.8. Statistical analysis

All values are expressed as means ± SEM. Statistical comparisons between the means of multiple groups were made using a 2×2 ANOVA and 2×2×2 mixed factor ANOVA analysis with the SPSS software for Windows (version 10.0). When a statistically significant effect was found, the galanin levels in pre and post swimming training were analyzed with paired samples Student's t-tests. Statistical significance was defined as $P<0.05$.

3. Results

3.1. Swimming elevated plasma galanin concentration

The plasma galanin contents before and after the experiments in the eight groups were obviously different respectively ($F[7,64]=7.08$, $P=4.72e^{-6}$ and $F[7,64]=37.25$, $P=7.69e^{-19}$). The basal concentrations of galanin before the experiments did not significantly differ among four nondiabetic groups ($F=0.30$, $P=0.83$) and among four diabetic groups ($F=1.89$, $P=0.15$) respectively. As shown in Table 1, the four week training significantly increased the galanin release. The plasma galanin concentration after training in T, T-M35, Diab-T and Diab-T-M35 groups was increased compared with each preswimming level and each sedentary control. Besides, the galanin concentration before and after the swimming was lower in Diab-S and Diab-T groups than each nondiabetic control respectively.

3.2. M35 treatment reduced glucose infusion rates in hyperinsulinemic–euglycemic clamping test

The assessment of the glucose infusion rate in steady state during the hyperinsulinemic–euglycemic clamping involves the determination of insulin sensitivity which is closely relative to GLUT4 translocation. The glucose infusion speed during the clamping test was obviously reduced by M35 treatment ($F[7,64]=37.35$, $P=7.24e^{-19}$). Fig.1 showed that the glucose infusion rate in S-M35, T-M35, Diab-S-M35 and Diab-T-M35 groups was slower compared with each no-drug control respectively during the clamping test (6.99 ± 0.69 mg/kg·min vs. 11.09 ± 0.92 mg/kg·min, $P<0.01$; 3.37 ± 0.56 mg/kg·min vs.8.05 ± 0.73 mg/kg·min, $P<0.05$; 11.92 ± 0.76 mg/kg·min vs. 18.09 ± 1.38 mg/kg·min, $P<0.01$ and 8.64 ± 0.79 mg/kg·min vs. 12.14 ± 0.61 mg/kg·min, $P<0.05$). However, exercise enhanced the infusion rates. The swimming training resulted in an increase in the infusion rates during the clamping test in T, T-M35, Diab-T and Diab-T-M35 groups when compared with each sedentary control (18.09 ± 1.38 mg/kg·min vs. 11.09 ± 0.92 mg/kg·min, $P<0.01$; 11.92 ± 0.76 mg/kg·min vs.6.99 ± 0.69 mg/kg·min, $P<0.01$; 12.14 ± 0.61 mg/kg·min vs. 8.05 ± 0.73 mg/kg·min, $P<0.01$ and 8.64 ± 0.79 mg/kg·min vs. 3.37 ± 0.56 mg/kg·min, $P<0.01$). In addition, the rates in Diab-S, Diab-T and Diab-T-M35 groups were lower compared with each nondiabetic control respectively ($P<0.05$ and $P<0.01$).

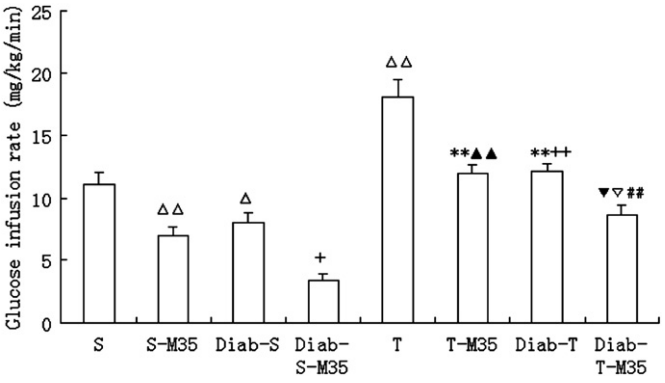

Fig. 1. The exercise elevated the glucose infusing rate, but M35 treatment reduced it in hyperinsulinemic–euglycemic clamp tests. The glucose infusing rates in four trained groups were higher compared with each sedentary control respectively. While the rates in all of four M35 treatment groups were lower compared with each control (n = 8, Freedom degree = 7). All data shown are the means ± SEM. △$P<0.05$, △△$P<0.01$ vs. sedentary control (S); +<0.05, ++$P<0.01$ vs. diabetic sedentary control (Diab-S); **$P<0.01$ vs. trained control (T); ▲▲<0.01 vs. sedentary control with M35 (S-M35); ▼<0.05 vs. trained control with M35 (T-M35); ▽<0.05 vs. diabetic trained control (Diab-T); ##$P<0.01$ vs. diabetic sedentary group with M35 (Diab-S-M35).

Table 1
The change of plasma galanin concentrations before and after swimming in rats (pM, n = 8, Freedom degree = 7, $\bar{x}$ ± SEM).

Groups	Preswimming	After swimming	t	P
S	6.02 ± 0.43	5.91 ± 0.45	0.64	0.54
S-M35	5.74 ± 0.33	4.47 ± 0.42	1.54	0.17
Diab-S	3.67 ± 0.34$^{\triangle\triangle}$	3.88 ± 0.38$^{\triangledown}$	1.02	0.34
Diab-S-M35	4.49 ± 0.43	4.65 ± 0.34	0.36	0.73
T	6.32 ± 0.45	10.62 ± 0.48$^{**\triangledown\triangledown}$	8.99	$4.29e^{-5}$
T-M35	5.89 ± 0.49	9.50 ± 0.52$^{**\bigcirc\bigcirc}$	7.36	$1.55e^{-4}$
Diab-T	4.56 ± 0.39$^{\blacktriangle}$	8.48 ± 0.59$^{**\blacktriangledown++}$	6.27	$4.18e^{-4}$
Diab-T-M35	4.49 ± 0.29	7.79 ± 0.39$^{**\#\#}$	5.58	$8.34e^{-4}$

△△$P<0.01$ vs. sedentary control (S) before the swimming; ▽$P<0.05$, ▽▽$P<0.01$ vs. S after the swimming; **$P<0.01$ vs. each preswimming level; ○○<0.01 vs. sedentary control with M35 (S-M35) after the swimming; ▲$P<0.05$ vs. trained control (T) before the swimming; ▼<0.05 vs. T after the swimming; ++$P<0.01$ vs. diabetic sedentary control (Diab-S) after the swimming; ##$P<0.01$ vs. diabetic sedentary group with M35 (Diab-S-M35) after the swimming.

3.3. M35 treatment decreased GLUT4 mRNA expression levels

In the present study, the GLUT4 mRNA expression levels in M35 treated rats were significant decreased as compared with their no-drug controls as shown by the densitometric analysis ($F[7,64]=39.97$, $P=5.00e^{-19}$, Fig. 2). The expression levels in S-M35, T-M35, Diab-S-M35 and Diab-T-M35 groups were lower than each control (0.225 ± 0.015 vs. 0.295 ± 0.013, $P<0.05$; 0.153 ± 0.012 vs. 0.237 ± 0.015, $P<0.01$; 0.367 ± 0.026 vs. 0.400 ± 0.028, $P<0.05$ and 0.257 ± 0.023 vs. 0.363 ± 0.020, $P<0.01$). As expected, exercise enhanced GLUT4 mRNA expression levels in adipose tissues. The expression levels increased in T, T-M35, Diab-T and Diab-T-M35 groups in comparison to each sedentary control (0.400 ± 0.028 vs. 0.295 ± 0.013, $P<0.01$; 0.367 ± 0.026 vs. 0.225 ± 0.015, $P<0.01$; 0.363 ± 0.020 vs. 0.237 ± 0.015, $P<0.01$ and 0.257 ± 0.023 vs. 0.153 ± 0.012, $P<0.01$). In addition, the GLUT4 gene expression in Diab-S, Diab-S-M35, Diab-T and Diab-T-M35 groups was significantly lower compared with each nondiabetic control ($P<0.05$ and $P<0.01$).

3.4. M35 treatment attenuated GLUT4 contents in membranes of adipocytes

The GLUT4 levels in membranes of adipocytes were significantly lower in all drug groups than each control. As shown in Fig.3, the M35 treatment significantly attenuated GLUT4 protein contents in plasma membranes ($F[7,64]=80.93$, $P=5.97e^{-27}$) and in total cell membranes ($F[7,64]=44.26$, $P=1.47e^{-20}$) of adipocytes. Comparison of S-M35, T-M35, Diab-S-M35 and Diab-T-M35 groups with each control showed that the GLUT4 immunoreactivities in plasma

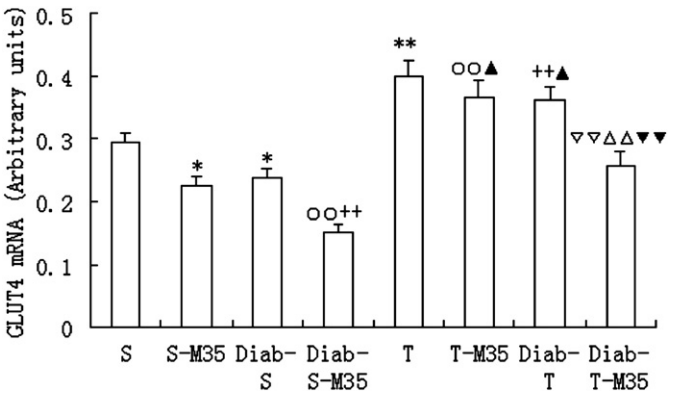

Fig. 2. The exercise enhanced the Glucose Transporter 4 (GLUT4) mRNA expression level, but M35 treatment reduced it in adipocytes of type 2 diabetic rats. M35 treatment reduced GLUT4 mRNA expression levels in four drug groups compared with each control, whereas exercise elevated the expression levels in all of four trained groups compared with each sedentary control (n = 8, Freedom degree = 7). The data shown are the means ± SEM. *P<0.05, **P<0.01 vs. sedentary control (S); ○○<0.01 vs. sedentary control with M35 (S-M35); ++P<0.01 vs. diabetic sedentary control (Diab-S); ▲P<0.05 vs. trained control (T); ▽▽P<0.01 vs. diabetic group with M35 (Diab-S-M35); △△P<0.01 vs. trained control with M35 (T-M35); ▼▼P<0.01 vs. diabetic trained control (Diab-T).

membranes were attenuated (12.54 ± 2.13 vs. 22.39 ± 2.95, P<0.01; 8.08 ± 2.08 vs. 16.13 ± 3.83, P<0.05; 27.36 ± 2.23 vs. 47.35 ± 3.81, P<0.01 and 15.44 ± 2.77 vs. 40.68 ± 2.95, P<0.01), and in total cell membranes (68.05 ± 3.27 vs. 80.07 ± 3.75, P<0.05; 52.39 ± 4.01 vs. 66.56 ± 4.35, P<0.05, 94.71 ± 5.69 vs. 108.94 ± 6.11, P<0.05 and 80.73 ± 5.07 vs. 94.90 ± 4.59, P<0.05).

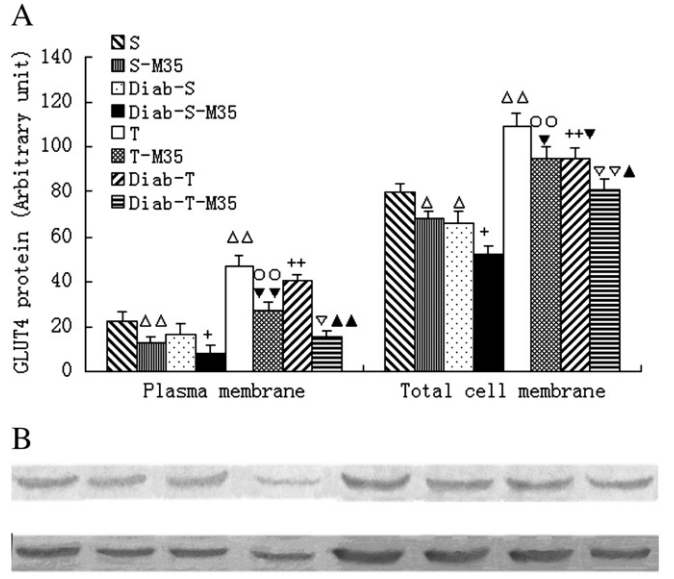

Fig. 3. The swimming training elevated GLUT4 concentration in plasma membranes and in total cell membranes, but M35 treatment decreased it in type 2 diabetic rats. A M35 treatment reduced GLUT4 immunoreactivity in plasma membranes and in total cell membranes of adipocytes in four drug groups, whereas exercise increased the immunoreactivity in all trained groups (n = 8, Freedom degree = 7). The data shown are the means ± SEM. △P<0.05, △△<0.01 vs. sedentary control (S) of each division; +P<0.05, ++P<0.01 vs. diabetic sedentary control (Diab-S) of each division; ○○<0.01 vs. sedentary control with M35 (S-M35); ▼<0.05, ▼▼P<0.01 vs. trained control (T) of each division; ▽<0.05, ▽▽<0.01 vs. diabetic sedentary group with M35 (Diab-S-M35) of each division; ▲<0.05, ▲▲<0.01 vs. diabetic trained control (Diab-T) of each division. B Representative Western blots. The bands of GLUT4 protein in plasma membranes are shown in the upper panel and that in intracellular membranes in the lower panel. The sum of the GLUT4 content in plasma membranes and in intracellular membranes is taken as the GLUT4 concentration of total cell membranes. The sequence of a series of lines in each panel is S, S-M35, Diab-S, Diab-S-M35, T, T-M35, Diab-T and Diab-T-M35.

Besides, the swimming procedure led to a significant increase in GLUT4 densities in plasma membranes and in total cell membranes of adipocytes. Comparison of T, T-M35, Diab-T and Diab-T-M35 groups with corresponding sedentary groups showed that the GLUT4 concentrations after the swimming training were elevated in plasma membranes (47.35 ± 3.81 vs. 22.39 ± 2.95, P<0.01; 27.36 ± 2.23 vs. 12.54 ± 2.13, P<0.01; 40.68 ± 2.95 vs. 16.13 ± 3.83, P<0.01 and 15.44 ± 2.77 vs. 8.08 ± 2.08, P<0.05), and in total cell membranes respectively (108.94 ± 6.11 vs. 80.07 ± 3.75, P<0.01; 94.71 ± 5.69 vs. 68.05 ± 3.27, P<0.01; 94.90 ± 4.59 vs. 66.56 ± 4.35, P<0.01 and 80.73 ± 5.07 vs. 52.39 ± 4.01, P<0.01).

The ratios of GLUT4 concentrations in plasma membranes to total cell membranes were 27.96%, 18.42%, 24.23%, 15.42%, 43.46%, 28.89%, 42.87% and 19.13% in S, S-M35, Diab-S, Diab-S-M35, T, T-M35, Diab-T and Diab-T-M35 groups respectively.

4. Discussion

Recent researches have demonstrated that adipose tissue is not only a fat depot and endocrine organ, but also plays an important role in energy regulation and energy balance (Trayhurn, 2005). Adipose tissue can buffer excess of energy and control metabolic homeostasis (Eijk et al., 2009). Much evidence supports that dysregulation of lipid metabolism is a cause of insulin resistance and is closely relative to impaired GLUT4 translocation and downregulated GLUT4 mRNA level (Griesel et al., 2010). These are various hormones and metabolic states that influence GLUT4 function, including leptin (Geiger et al., 2006), neuropeptide Y (Hohmann et al., 2004) and mitochondrial capacity for fatty acid oxidation (Koves et al., 2008). Here we tried to establish an association between galanin concentrations with GLUT4 translocation in adipocytes of type 2 diabetic rats. These findings that galanin receptors exist in adipocytes (Geiger et al., 2006) and M35 has an inhibitive effect on GLUT4 trafficking in myocytes suggest such a possibility (Jiang et al., 2009; He et al., 2011).

The neuropeptide galanin is widely distributed in nervous and digestive system. Galanin is colocalized with corticotropin (ACTH) in pituitary and with epinephrine (E) and norepinephrine (NE) in chromaffin cells of the adrenal medulla (Ceresini et al., 1997). These colocalizations suggest that galanin participates in stress responses, including physical exercise.

Experiments prove that physical exercise is effective to increase galanin secretion. Murray et al. (2010) found wheel running significantly elected galanin concentration and GAL mRNA expression level in the locus coeruleus region. And the overall running distance of the rats correlated with the GAL mRNA expression level. Besides, Legakis et al. (2000) observed that exercise induced an increase in galanin release in middle-aged individuals. In line with these, we found that the four week swimming training significantly elevated galanin concentration in type 2 diabetic rats. Besides, the four trained groups also displayed higher GLUT4 mRNA expression levels, GLUT4 concentration in membranes of adipocytes and glucose infusion rates in the hyperinsulinemic–euglycemic clamp test. These results prove that proper exercise is not only an effective stimulus to increase endogenous galanin secretion in normal and diabetic states, but also a good way to treat diabetes mellitus. The latter is relative to the mechanism of exercise. First, contraction of skeletal muscle elevates both AMP/ATP ratio and intracellular Ca^{2+} to accelerate GLUT4 translocation and to reduce the blood glucose level (Sakamoto and Holman, 2008). Next, exercise may improve the capacity for fatty acid oxidation and increase release of fatty acids from adipocytes to elevate insulin sensitivity and to reduce body weight (Krämer et al., 2007). On the contrary, impaired oxidation rates of fatty acid are associated with insulin resistance as fatty acids can interfere with insulin signaling (Bonen et al., 2006). Last, the increase in exercise-induced galanin release may upregulate GLUT4 transcription and accelerate GLUT4 trafficking to transfer more glucose into adipocytes and myocytes

(Jiang et al., 2009; He et al., 2011). So is proper exercise beneficial to diabetes mellitus.

The hyperinsulinemic–euglycemic clamp test is a method to assess the capacity of glucose uptake which mainly depends on the effect of GLUT4 in the plasma membrane (Thien et al., 2003). During the clamp test, a constant insulin infusion increases the uptake of circulating glucose into insulin-sensitive tissues and inhibits endogenous glucose production by liver. The decline in plasma glucose is prevented by a concomitant variable rate of glucose infusion. The amount of exogenous glucose required to maintain plasma glucose at its clamp level is quantified by the glucose infusion rate. Thus, the infusion rate is a measure of the ability of insulin to increase glucose uptake and to suppress glucose production in a given subject, i.e., a measure of the insulin sensitivity of this subject. An elevation of the glucose infusion speed suggests an increase in GLUT4 quantity in the plasma membrane. Fig.1 demonstrated that the glucose infusion rate was lower in all of four M35 treatment groups in the clamp tests. It suggested that GLUT4 translocation was attenuated by M35 treatment, i.e. endogenous galanin may facilitate GLUT4 trafficking to accelerate glucose clearance in both rest and exercise conditions.

GLUT4 expressed mainly in adipocytes and myocytes plays an important role in regulation of whole body glucose homeostasis. A reduction in GLUT4 gene expression and GLUT4 protein level in pathophysiological states represents a decreased glucose clearance. As insulin deficiency such as streptozotocin-induced diabetes, GLUT4 mRNA expression level and GLUT4 protein level are reduced (Olson, 2005). Contrarily, mice that express exogenous GLUT4 gene in skeletal muscle or adipose tissue alone, displayed enhanced insulin sensitivity and peripheral glucose utilization (Olson et al., 1993). In humans and rodents, diabetes leads to downregulation of GLUT4 gene transcription in adipose tissue (Carlson et al., 2003). Interestingly, streptozotocin-dependent changes in GLUT4 mRNA expression occur much more rapidly in adipose tissue than skeletal muscle (Richardson et al., 1991). The GLUT4 mRNA level most likely reflects the change in the rate of GLUT4 mRNA synthesis rather than its half-life (Geiger et al., 2006). An evidence by nuclear run-on assays showed that transcription was decreased in both adipose tissue and skeletal muscle in streptozotocin-induced diabetic animals. In the current study, we demonstrated that M35 treatment down-regulated GLUT4 mRNA expression levels in diabetic animals, i.e. endogenous galanin may elevate the level to increase GLUT4 synthesis in adipocytes. We reason that galanin may generate metabolic signals to upregulate GLUT4 transcription. This responsible signaling pathway remains elusive now.

The intracellular distribution of GLUT4 is concentrated in the trans-Golgi region or in the cytosol, often close to the cell surface but not in the cell surface under resting conditions (Sakamoto and Holman, 2008). Only in the cell surface can GLUT4 transport glucose into cells. The maximal glucose clearance activity by adipose and skeletal muscle tissues is in directly proportional to the GLUT4 concentration in plasma membranes of rats (Geiger et al., 2006). Studies in both 3T3–L1 adipocytes and adipose cells suggest that GLUT4 is continuously cycled between intracellular membrane compartments and plasma membranes (Leney and Tavaré, 2009). A combination of rapid endocytosis and slow exocytosis maintains its intracellular localization under the basal state. As stimulus of exercise or insulin the continuous recycle reaches a new equilibrium of releasing and capturing GLUT4 vesicles. In this state of new equilibrium more GLUT4 moves to plasma membranes. Glucose homeostasis is exquisitely sensitive to the level of GLUT4 protein in plasma membranes. The greater the GLUT4 protein in the cell surface, the higher the insulin sensitivity is observed (Konrad et al., 2002).

Quantitative densitometry of adipocytes revealed that M35 treatment caused an obvious decrease in GLUT4 levels both in plasma membranes and in total cell membrane fractions. And the change ratios of GLUT4 contents in plasma membranes to total cell membranes were lower in S-M35, T-M35, Diab-M35 and Diab-T-M35 groups compared with each control respectively (18.4% vs. 28.0%, 15.4% vs. 24.2%, 28.9% vs.43.5% and 19.1% vs. 42.9%). The former reductive ratios were greater than the latter. This result suggests that M35 treatment inhibits GLUT4 vesicular trafficking to cellular surface of adipocytes of type 2 diabetic rats, i.e. endogenous galanin may accelerate GLUT4 translocation from intracellular membrane compartments to plasma membranes to reach a new equilibrium between exocytosis and endocytosis. Therefore galanin is an important hormone to promote glucose clearance. These data contribute to our understanding of type 2 diabetes.

To date, three galanin subtype receptors have been cloned and characterized, GALR1, GALR2 and GALR3, which have different function in different tissues. As yet it is unknown which one(s) of the three subtype receptors is most effective to increase GLUT4 translocation. This is a significant subject for further exploration.

5. Conclusion

Our experiment suggests that exercise-induced the galanin release may enhance the GLUT4 mRNA expression level and accelerate GLUT4 translocation from intracellular membrane compartments to cell surfaces in adipocytes of type 2 diabetic rats. Consequently galanin plays a significant role in regulation of glucose metabolic homeostasis and in elevation of insulin sensitivity to promote glucose clearance.

Acknowledgments

This work was supported in part by Protein Research Grant of Yangzhou University to Mingyi Shi.

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