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Increased uncoupling protein-2 and -3 gene expressions in skeletal muscle of STZ-induced diabetic rats

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Abstract Streptozotocin (STZ)-induced diabetic animals are vulnerable to cold stress. Uncoupling proteins (UCPs) play an important role in regulating thermogenesis. We investigated the gene expressions of UCPs in brown adipose tissue (BAT), white adipose tissue (WAT), liver and gastrocnemius muscle of STZ-diabetic rats using Northern blot. UCP-1, -2 and -3 mRNA expressions in BAT were all remarkably lower in STZ-diabetic rats than those in control rats. Both UCP-2 and -3 gene expressions in gastrocnemius muscle were substantially elevated in STZ-diabetic rats and insulin treatment restored UCP gene expressions to normal levels. These results suggest that in STZ-diabetic rats, the overexpression of UCP-2 and UCP-3 in skeletal muscle provides a defense against hypothermogenesis caused by decreased UCPs in BAT.

Key words: Streptozotocin; Diabetic rat; Uncoupling protein; Gastrocnemius muscle; Brown adipose tissue; Gene expression

1. Introduction

Brown adipose tissue (BAT) through the action of uncoupling protein (UCP)-1 plays an important role in regulating thermogenesis in rodents [1]. UCP-1 is located at the inner membrane of mitochondria, and translocates protons into the matrix of mitochondria, resulting in heat production without coupling to any other energy-consuming process [2,3].

Diabetes mellitus is known to have reduced cold tolerance in human [4,5]. Alloxan or streptozotocin (STZ)-induced diabetic animals are also vulnerable to cold stress [6,7] because thermogenesis is impaired in these animals. In fact, the weight of BAT was found to be reduced in STZ-diabetic rats [8], and the total amount of uncoupling protein content in BAT mitochondria, the ability for β-oxidation, and mitochondria GDP-binding were also decreased in these animals [9,10]. Insulin treatment reverses the uncoupling protein content and the BAT thermogenesis in STZ-diabetic rats [8].

Recently, two cDNAs encoding the UCP homologs were identified and called UCP-2 [11,12] and UCP-3 [13–16]. Tissue distributions and expression pattern of these two genes were different from UCP-1; e.g. UCP-1 mRNA is expressed specifically in BAT [17,18], while UCP-2 mRNA is expressed in numerous tissues such as white adipose tissue (WAT), BAT, skeletal muscle and liver [11,12]. UCP-3 gene expression appeared to be restricted in human and rodent muscle, and in rodent BAT [13–16]. UCP-3 may act as a mediator of thermogenesis in human, because it is expressed at a high level solely in human skeletal muscle, a major site of thermogenesis. However, neither UCP-2 nor UCP-3 gene expression has been reported in STZ-induced diabetic animals.

To investigate whether UCP gene expressions are altered in STZ-diabetic rats, we determined the mRNA levels of UCP-1, UCP-2 and UCP-3 in BAT, WAT, liver and gastrocnemius muscle in these rats. The effect of insulin treatment on UCP gene expressions was also examined.

2. Materials and methods

2.1. Animals and procedures

Male Sprague-Dawley rats weighing 220–230 g (Japan SCL, Hamamatsu, Japan) were used in all experiments. They were maintained in individual cages on a rotating 12/12 h light/dark cycle with an ambient temperature of 24 ± 1°C, and were provided with free access to both standard rat chow (Oriental Food, Tokyo, Japan) and water.

Animals were divided into three groups: (1) vehicle-treated control, (2) STZ-induced diabetic, and (3) STZ-induced diabetic with insulin treatment. After 5 h fast, each animal received an injection of STZ (Sigma, St. Louis, MO, USA) at 75 mg/kg body weight in sodium citrate buffer (pH 4.0) or sodium citrate buffer (pH 4.0) (vehicle) through the tail vein (day 0 at 2:00 p.m.). Diabetes was diagnosed 24 h later (day 1 at 2:00 p.m.), and defined as a blood glucose level greater than 13.8 mmol/l (250 mg/dl) measured using a drop of blood obtained by tail vein puncture. Insulin-treated STZ-diabetic rats were subcutaneously injected with 4 U and 2 U human insulin (NPH; Novo Nordisk, Copenhagen, Denmark) 29 h (day 1 at 7:00 p.m.) and 42 h (day 2 at 8:00 a.m.) after the STZ injection, respectively.

On the day of experiments (day 2, 48 h later), food was withdrawn at 8:00 a.m. and experiments were carried out after a 6-h fast. All animals were killed by decapitation. Tissues (interscapular BAT, epididymal WAT, liver and gastrocnemius muscle) were dissected and blood was taken. Tissues were immediately frozen in liquid nitrogen and stored at −80°C until use. The blood was centrifuged at 4°C, and plasma was stored at −80°C until assayed.

2.2. Measurements

Plasma glucose levels were determined by glucose oxidase method (Glucose B-test, Wako Pure Pharmaceutical, Osaka, Japan). Immunoreactive insulin (IRI) concentrations were determined by a radioimmunoassay kit (Cat#RI-13K, Linco Research, St. Charles, MO, USA) standardized against rat insulin. Ketone bodies (acetoacetate and β-hydroxybutyrate) were determined by the enzyme method using

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Table 1
Characteristic features of control (vehicle), STZ diabetic (STZ) and STZ diabetic rats with insulin treatment (STZ+insulin)

	Vehicle ($n=5$)	STZ ($n=5$)	STZ+insulin ($n=5$)
Initial BW (g) (0 h)	225 ± 3	222 ± 3	221 ± 2
% BW change (24 h)	1.96 ± 0.66	−9.2 ± 0.50[a]	−7.42 ± 0.45[a]
% BW change (48 h)	5.05 ± 0.90	−19.0 ± 2.07[a]	−2.34 ± 0.91[a,b]
Final BW (g) (48 h)	236 ± 3	180 ± 4[a]	216 ± 4[a,b]
Epididymal fat pads (g)	1.6 ± 0.1	1.2 ± 0.1[a]	1.4 ± 0.1
Food intake (0–24 h) (g)	20.0 ± 1.2	11.4 ± 0.4[a]	11.9 ± 1.3[a]
Food intake (24–48 h) (g)	18.5 ± 0.9	5.0 ± 2.5[a]	23.5 ± 1.6[b]
Plasma glucose (mmol/l)	4.27 ± 0.64	35.04 ± 6.78[a]	4.14 ± 1.59[b]
Total cholesterol (mmol/l)	0.86 ± 0.02	1.15 ± 0.22	0.80 ± 0.10
Triglyceride (mmol/l)	0.92 ± 0.08	4.32 ± 1.90	1.66 ± 0.23
NEFA (μmol/l)	1139 ± 67	1900 ± 340[a]	1489 ± 101
Total ketone bodies (μmol/l)	91.7 ± 7.1	5295.8 ± 148.7[a]	145.6 ± 12.3[b]
Plasma insulin (ng/ml)	2.90 ± 0.43	0.06 ± 0.03[a]	3.86 ± 0.73[b]

Data represent the means ± standard error (S.E.M.). Superscripts indicate statistical significance by one-way ANOVA at $P<0.05$: [a] vs. vehicle-control, [b] vs. STZ-diabetes. NEFA, non-esterified fatty acid.

a commercially available kit (Ketone Teat Sanwa, Sanwa Kagaku Laboratory, Nagoya, Japan). Plasma non-esterified fatty acid (NEFA) concentration, plasma triglyceride concentration and plasma total cholesterol concentration were determined by the enzyme method using a kit (NEFA-C test, triglyceride-G test and total cholesterol-C test, respectively, all from Wako Pure Pharmaceutical).

2.3. RNA analysis

Total RNA was prepared from the tissue of each rat using TRIzol reagent (Gibco-BRL, Frederick, MD, USA). The concentration of RNA was determined spectrophotometrically. Total RNA was loaded at 15 μg per lane on a 1% agarose/formaldehyde gel and transferred onto a nylon membrane (Hybond N^+, Amersham, Arlington Heights, IL, USA). Membranes were hybridized with [α-^{32}P]dCTP-labeled cDNA probe for rat UCP-1, mouse UCP-2 and rat UCP-3. The blot was exposed to X-ray film (Fuji Photo Film, Tokyo, Japan) at −80°C using an intensifying screen. Signals were quantified with FUJIX Bio-Imaging Analyzer BAS2000 (Fuji Photo Film). Equivalent loading was verified by the density of the methylene blue-stained rRNAs bands after transfer.

2.4. Statistical analysis

Data are expressed as means ± standard error (SEM). Statistical significance was assessed by one-way ANOVA.

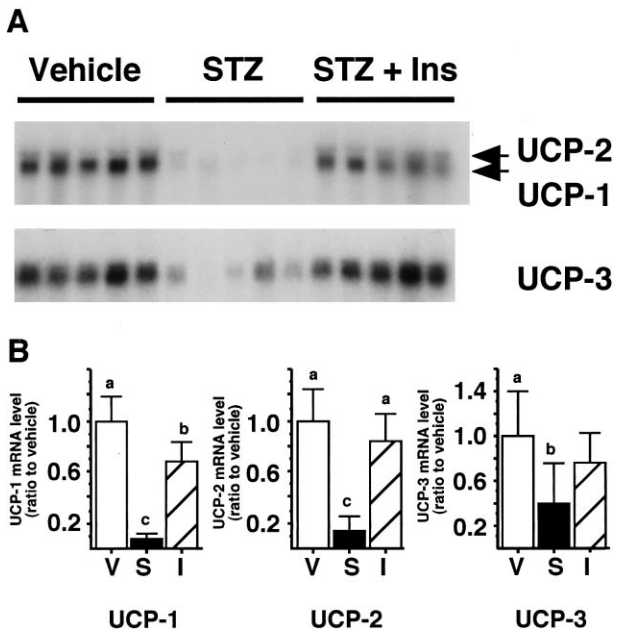

Fig. 1. Northern blot analysis and quantification of the relative levels of UCP mRNA in BAT. A: Autoradiograph of Northern blot analysis. Vehicle: vehicle-treated control; STZ: STZ-induced diabetic rats; STZ+Ins: STZ-induced diabetic rats with insulin treatment. B: UCP mRNA level. UCP mRNA levels normalized to total RNA loaded. The *y*-axis indicates the relative level of UCP mRNA (level of UCP mRNA of vehicle-treated control was designated as 1.0). V: vehicle-treated control rats; S: STZ-induced diabetic rats; I: STZ-induced diabetic rats. Data are mean ± S.E. Different superscripts show that the means are significantly different from each other: [ab] $P<0.005$; [bc] $P<0.0001$; [ac] $P<0.0001$. Columns not sharing the same superscript are significantly different.

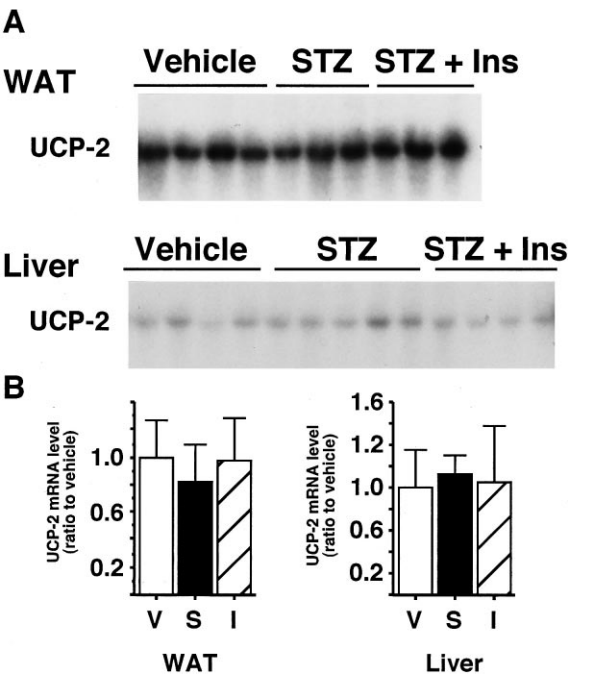

Fig. 2. Northern blot analysis and quantification of the relative levels of UCP-2 mRNA in WAT and liver. A: Autoradiograph of Northern blot analysis. Upper panel shows UCP-2 gene expression in WAT. Lower panel shows UCP-2 gene expression in liver. Vehicle: vehicle-treated control; STZ: STZ-induced diabetic rats; STZ+Ins: STZ-induced diabetic rats with insulin treatment. B: UCP mRNA level. UCP-2 mRNA level normalized to total RNA loaded. The *y*-axis indicates the relative level of UCP-2 mRNA (level of UCP mRNA of vehicle-treated control was designated as 1.0). V: vehicle-treated control rats; S: STZ-induced diabetic rats; I: STZ-induced diabetic rats. Data are mean ± S.E.

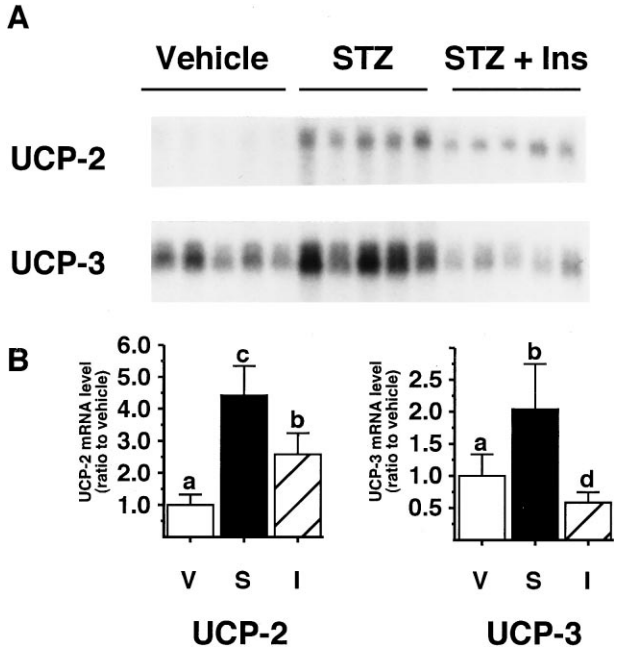

Fig. 3. Northern blot analysis and quantification of the relative levels of UCP mRNA in gastrocnemius muscle. A: Autoradiograph of Northern blot analysis. Vehicle: vehicle-treated control; STZ: STZ-induced diabetic rats; STZ+Ins: STZ-induced diabetic rats with insulin treatment. B: UCP mRNA level. UCP mRNA levels normalized to total RNA loaded. The *y*-axis indicates the relative level of UCP mRNA (level of UCP mRNA of vehicle-treated control was designated as 1.0). V: vehicle-treated control rats; S: STZ-induced diabetic rats; I: STZ-induced diabetic rats. Data are mean ± S.E. Different superscripts show that the means are significantly different from each other: $^{ab}P < 0.001$; $^{cd}P < 0.001$; $^{bc}P < 0.0001$; $^{ac}P < 0.0001$.

3. Results

3.1. Group characteristics

Table 1 shows characteristic features in three groups of rats. Body weight decreased about 45 g in STZ-induced diabetic rats, while plasma concentrations of glucose, NEFA and ketone bodies significantly increased ($P < 0.05$). Plasma insulin levels in STZ-induced diabetic rats were significantly lower than in control rats ($P < 0.05$). Body weight and plasma glucose, NEFA and ketone body levels were restored to normal by insulin treatment.

3.2. Gene expression levels of UCP-1, UCP-2 and UCP-3

In STZ-induced diabetic rats, UCP-1, UCP-2 and UCP-3 mRNA significantly decreased in BAT (Fig. 1), while expression of UCP-2 mRNA did not change in WAT or liver (Fig. 2). Levels of UCP-2 and UCP-3 mRNA in gastrocnemius muscle were 4.4 and 2.0 times higher in STZ-diabetic rats than in control rats, respectively (Fig. 3). The alteration of UCP mRNA expressions in BAT and gastrocnemius muscle in STZ diabetic rats were almost restored to normal by insulin treatment (Figs. 1 and 3).

4. Discussion

Two new members of the UCP gene family have recently been cloned, UCP-2 and UCP-3. Both diminish mitochondrial membrane potential when they are transfected into yeast [11,12,14], suggesting that, similar to UCP-1, these proteins also contribute to both uncoupled-oxidative phosphorylation and thermogenesis.

Géloën et al. [8] reported that total UCP content in BAT was decreased in STZ-diabetic rats, and suggested that this contributes to their vulnerablility to cold stress. In this study, we found that UCP-1, UCP-2 and UCP-3 gene expressions in BAT were all remarkably diminished in STZ-diabetic rats, which may explain hypothermogenesis in these animals [6,7].

Since there is little BAT in adult human [19], UCP-1 is not likely to play a major role in thermogenesis in adult human. UCP-3 may be involved in thermogenesis in human as well as in rodent, because UCP-3 mRNA is expressed at a high level in skeletal muscle [13,16]. Our novel findings in the present study are that UCP-3 mRNA in gastrocnemius muscle was significantly elevated in STZ-diabetic rats, and that insulin treatment normalized this overexpression. Tsuboyama-Kasaoka et al. [20] reported that UCP-3 gene expression is positively associated with glucose transporter-4 (GLUT4) gene expression in gastrocnemius muscle. According to their observation, UCP-3 gene expression must be decreased because it is well known that GLUT4 mRNA is reduced in STZ-diabetic animals [21]. We found, however, that UCP-3 gene expression was markedly increased in these animals. Therefore, it is likely that hyperglycemia itself does not increase UCP-3 gene expression. An increase in plasma NEFA level by high-fat diet feeding [15] or intralipid infusion [22] leads to overexpression of UCP-3 mRNA in gastrocnemius muscle of rodents. Therefore, it is possible that an increase in NEFA enhances the expression of UCP-3 mRNA, because STZ-diabetic rats had substantially elevated NEFA levels. Ketone bodies are another candidate for UCP-3 mRNA overexpression in these insulin-deficient diabetic animals. Indeed, Guerin et al. [23] reported that diabetic ketoacidosis is associated with hypothermia. The issue of expression of UCP-3 mRNA by ketone bodies needs further investigation.

Very recently Krook et al. [24] reported that UCP-3 mRNA in femoral muscle was decreased in type 2 diabetic patients. Their results do not contradict with our results: reduction of UCP-3 with hyperinsulinemia in their patients vs. elevation of UCP-3 with hypoinsulinemia in our diabetic rats.

Enerbäck et al. [25] reported that UCP-2 mRNA was overexpressed in BAT in UCP-1 null mice. Thus, compensatory increase in UCP-2 may also contribute to prevention of hypothermogenesis in these mice. In fact, UCP-2 gene expression in the gastrocnemius muscle of STZ-diabetic rats significantly increased, although the mechanism of overexpression of UCP-2 mRNA is unknown.

In summary, we demonstrated for the first time that UCP-2 and UCP-3 gene expressions were increased in skeletal muscle of STZ-induced diabetic rats, while UCP-1, UCP-2 and UCP-3 gene expression were reduced in BAT of these rats, and insulin treatment reversed all these abnormalities. We hypothesize that overexpression of UCP-2 and UCP-3 gene may change reciprocally between gastrocnemius muscle and BAT to maintain thermogenesis.

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