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Activation of muscarinic M-1 cholinoceptors by curcumin to increase glucose uptake into skeletal muscle isolated from Wistar rats

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ABSTRACT

Curcumin, an active principle contained in rhizome of *Curcuma longa*, has been mentioned to show merit for diabetes through its anti-oxidative and anti-inflammatory properties. In the present study, we found that curcumin caused a concentration-dependent increase of glucose uptake into skeletal muscle isolated from Wistar rats. This action was inhibited by pirenzepine at concentration enough to block muscarinic M-1 cholinoceptor (M_1-mAChR). In radioligand binding assay, the binding of [^{3}H]-pirenzepine was also displaced by curcumin in a concentration-dependent manner. In the presence of inhibitors for PLC–PI3K pathway, either U73122 (phospholipase C inhibitor) or LY294002 (phosphoinositide 3-kinase inhibitor), curcumin-stimulated glucose uptake into skeletal muscle was markedly reduced. In Western blotting analysis, the membrane protein level of glucose transporter 4 (GLUT4) increased by curcumin was also reversed by blockade of M_1-mAChR or PLC–PI3K pathway in a same manner. In conclusion, the obtained results suggest that curcumin can activate M_1-mAChR at concentrations lower than to scavenge free radicals for increase of glucose uptake into skeletal muscle through PLC-PI3-kinase pathway.

Diabetes is a disease characterized by chronic hyperglycemia due to a reduction in functional efficacy and/or a deficiency of insulin. In fact, patients with diabetes have a shorter life span and a lesser quality of life, mainly as a result of vascular complications [20]. Hyperglycemia causes the autoxidation of glucose, glycation of proteins, and the activation of ploy metabolism [15]. These changes accelerate the generation of reactive oxygen species (ROS) to increase oxidative modifications of lipids, DNA, and proteins in various tissues. Oxidative stress is believed to play an important role in the development of complications in diabetes such as lens cataracts, nephropathy, and neuropathy [15].

Curcumin [diferuloylmethane, 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], an active principle contained in the rhizome of Curcuma species, is widely used as a yellow coloring and flavoring agent in food [7]. Curcumin is also introduced as a potent scavenger of ROS including superoxide anion, hydroxyl radical, singlet oxygen, nitric oxide and peroxynitrite [9]. Thus, it has the ability to protect lipids, hemoglobin, and DNA against oxidative stress. Also, curcumin has been demonstrated to show anti-inflammatory activity [8] and inhibition of carcinogen-DNA adduct and/or tumor genesis in animal [13]. In recent, curcumin is introduced to be helpful in diabetic control [17]. One of the merits from curcumin to against diabetes is the lowering of blood glucose [19]. However, the action mechanism(s) of this merit remained obscure.

In our previous report, activation of muscarinic M-1 cholinergic receptors (M_1-mAChR) produced an increase of glucose utilization [5]. M1-mAChR is believed as the most common subtype of muscarinic receptor in skeletal muscle [12]. Stimulation of M_1-mAChR could activate phospholipase C (PLC)–phosphoinositide 3-kinase (PI3K) pathway to increase glucose uptake [1,2,6]. Thus, we speculated that curcumin has an ability to activate M_1-mAChR for increase of glucose uptake in muscle. The current study employed rat soleus muscle to investigate this hypothesis.

Male Wistar rats, aged 8–10 weeks weighing 220–250 g, were obtained from the Animal Center of National Cheng Kung University Medical College. They were maintained in a temperature-controlled room ($25 \pm 1\,^{\circ}C$) and kept on a 12:12 light–dark cycle (light on at 06:00 h). Food and water were available *ad libitum*. All animal procedures were performed according to the Guide for the

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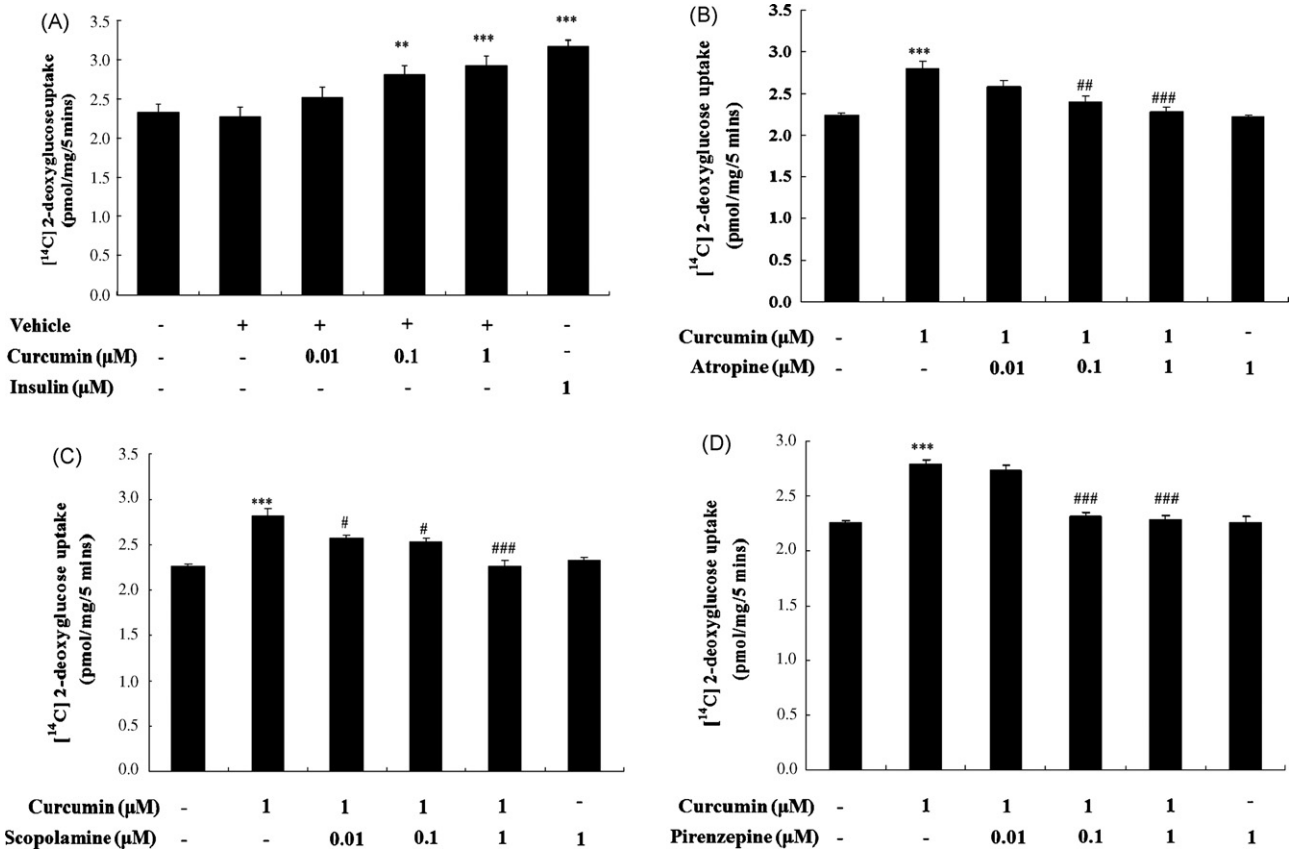

Fig. 1. Concentration-dependent stimulatory effect of curcumin on radioactive 2-deoxyglucose (2-DG) uptake in skeletal muscle isolated from Wistar rats. Insulin treated group as positive control (A). Inhibitory effect of atropine (B), scopolamine (C) or pirenzepine (D) on curcumin-stimulated radioactive 2-DG uptake in skeletal muscle isolated from Wistar rats. Results are the mean ± SEM of eight experiments. $^{**}p < 0.01$ and $^{***}p < 0.001$ vs. basal glucose uptake.

Care and Use of Laboratory Animals of the National Institutes of Health, as well as the guidelines of the Animal Welfare Act.

The uptake of 2-[^{14}C]-deoxy-D-glucose (2-DG) (PerkinElmer Life Sciences, Inc., Boston, MA, USA) was determined. The soleus skeletal muscle isolated from rat was incubated with curcumin or porcine insulin monocomponent (Novo Industrias; Bagsvaerd, Denmark) at indicated concentrations for 30 min at 37 °C under continuous shaking at 40 cycles/min. Then, they were further incubated with 2-DG (1 μCi/mL) for 5 min. Uptake was terminated by addition of ice-cold phosphate-buffer solution (PBS). Radioactivity was determined by lysing the samples in 1 M NaOH, and the aliquots were neutralized for estimation in a scintillation counter (Beckman LS6000). Non-specific uptake was obtained by parallel determinations in the presence of 20 μmol/mL cytochalasin B (Sigma–Aldrich, St. Louis, MO, USA). Specific 2-DG uptake was then expressed as pmol/mg protein over 5 min. Protein content was determined using the Bio-Rad protein dye binding assay (Richmond, CA, USA).

Data are expressed as the mean ± SEM for the number (n) of samples in the group as indicated in tables and figures. Repeated measures analysis of variance (ANOVA) was used to analyze the changes in testing parameters. The Dunnett range post hoc comparisons were used to determine the source of significant differences where appropriate. A P-value <0.05 was considered statistically significant.

As shown in Fig. 1A, curcumin enhanced the glucose uptake into rat soleus muscle in a concentration-dependent manner. However, the maximal effect of curcumin was still lower than insulin (1 μM) using as positive control.

The parasympathetic regulation of glucose homeostasis through muscarinic receptor has been indicated [22]. Chinese herbs, such as Die-Huang-Wan [10] and syringin [11], can induce the release of ACh from nerve terminal to activate muscarinic receptors, leading to plasma glucose lowering action. Thus, we investigated the subtype of muscarinic receptors involved in this curcumin-stimulated glucose uptake into skeletal muscle. The muscarinic antagonists including the non-selective antagonist, atropine or scopolamine, and M1-mAChR specific antagonist, pirenzepine, were all purchased from Sigma–Aldrich. In the presence of atropine, as shown in Fig. 1B, action of curcumin was reduced at concentrations enough to block muscarinic receptors. Also, scopolamine produced similar inhibition (Fig. 1C). Activation of muscarinic receptor by curcumin can thus be considered. Moreover, as shown in Fig. 1D, pirenzepine inhibited the action of curcumin for increase of glucose uptake at concentrations sufficient to block M1-mAChR [4].

Then, we employed the radioligand binding study to confirm the ability of curcumin binding on M_1-mAChR. Following the previous method [21], effect of curcumin on radioactive pirenzepine (specific ligand for M_1-mAChR) binding was performed in myoblast (C_2C_{12}) cell line. The C_2C_{12} cell line (ATCC CRL-1772) was purchased from Bioresource Collection and Research Center (Food Industry Research and Development Institute, Hsinchu, Taiwan) and subcultured in our lab. As described previously [21]. The radioligand binding displacement study was carried out using [N-methyl-^{3}H]-pirenzepine (PerkinElmer Life Sciences, Boston, MA, USA). The C_2C_{12} cells were homogenized in 20 mL Tris–HCl buffer (pH 7.4). The homogenate was centrifuged at 1660 rpm for 5 min. The supernatant was then collected and centrifuged at 28,000 rpm for 20 min. The pellet containing cell membranes was further homogenized in Tris–HCl buffer. The membrane fraction (0.5 mL) was incubated with various concentrations of curcumin, purchased from Sigma Chemical Co. (St. Louis, MO, USA), for 30 min. After addition of [N-methyl-^{3}H]-pirenzepine, the reaction was allowed to proceed for