Activation of muscarinic M-3 receptor may decrease glucose uptake and lipolysis in adipose tissue of rats

Ting-Ting Yang, Cheng-Kuei Chang, Chiung-Wen Tsao, Ya-Mei Hsu, Chao-Tien Hsu, Juei-Tang Cheng

*Graduate Institute of Basic Medical Science, China Medical University, Taichung City, Taiwan, ROC
b Department of Surgery, Mackay Memorial Hospital, and Graduate Institute of Injury Prevention and Control, Taipei Medical University, Taipei City, Taiwan, ROC
c Department of Nursing, Chung Hua University of Medical Technology, Tainan County, Taiwan, ROC
d Department of Pharmacology, College of Medicine, National Cheng Kung University, Tainan City, Taiwan, ROC
e Department of Pathology, E-Da Hospital/I-Shou University, Yan-Chau Shiang, Kaohsiung Shan, Taiwan, ROC
f Chi Mei Medical Center, Yung Kang City, Tainan Shan, Taiwan, ROC

Keywords: Adipose Muscarinic receptor Acetylcholine Glucose uptake Lipolysis

Abstract

Role of muscarinic receptor in the regulation of glucose uptake or lipolysis in adipose tissue remained unclear. In epididymal white adipose tissue (WAT) isolated from Wistar rats, we observed that acetylcholine (ACh) attenuated the insulin-stimulated glucose uptake and the release of glycerol from WAT in a concentration-dependent manner. Using the blockade of specific antagonists, both actions of ACh were characterized mainly due to an activation of M3 receptors. In the presence of various inhibitors for PLC–PKC pathway, ACh-decreased glucose uptake was also reversed. Taken together, these results suggest that muscarinic M3 receptor is involved in the regulation of glucose uptake and/or lipolysis in adipose tissue.

The energy metabolism is known to be regulated by autonomic nervous system; the sympathetic nervous system is worked via circulating epinephrine or glucagon and the parasympathetic nervous system is probably mediated by insulin [10]. An increase of sympathetic neural activity produces catabolism on glucose and lipid metabolism in liver, muscle, adipose tissue, whereas an increase of parasympathetic neural activity produces anabolism in liver [10]. The autonomic nerves are also involved in the islet adaptation to insulin resistance with possible implication for the development of glucose intolerance and/or type-2 diabetes [1].

Parasympathetic neural activity increases the insulin sensitivity of adipose tissue [10]; however, the main functions for parasympathetic nerves to regulate adipose tissue are still unclear. In the present study, we employ the epididymal white adipose tissue (WAT) isolated from Wistar rats as a material to investigate the effect of acetylcholine (ACh). Ten-week male Wistar rats, weighing 250–300 g, were obtained from the animal center of the National Taiwan University Medical College. Rats were housed in a temperature-controlled room (25 ± 1°C) and kept on a 12:12 light–dark cycle (light on at 06:00 h). Food and water were available ad libitum throughout the experiment. The uptake of 2-[14C]-deoxy-D-glucose (2-DG) (PerkinElmer Life Sciences, Inc., Boston, MA, USA) was determined. Adipocytes were obtained by collagenase digestion (Sigma–Aldrich Co., St. Louis, MO, USA) of the epididymal fat. Adipocytes were incubated with ACh, obtained from Sigma–Aldrich, for 30 min in the presence of insulin as the indicated concentration at 37°C for another 30 min under continuous shaking at 40 cycles/min, subsequently, further incubated with 2-DG (1 μCi/ml) for 5 min at 37°C. Uptake was terminated by addition of ice-cold phosphate-buffer solution (PBS). Radioactivity was determined by lysing the tissue or cells in 1 M NaOH, and the aliquots were neutralized for estimation in a scintillation counter (Beckman LS6000). Non-specific uptake obtained by parallel determinations in the presence of 20 μM cytochalasin B (Sigma–Aldrich) to block transportation was subtracted from total radioactivity. Specific 2-DG uptake was expressed as pmol/mg protein over 5 min. Protein content was determined using the Bio-Rad protein dye binding assay (Richmond, CA, USA). Results showed that ACh decreased insulin-stimulated glucose uptake in a concentration-dependent manner (Fig. 1A). A similar result showing ACh decreased the insulin-stimulated glucose uptake in 3T3-L1 cells was also observed (data not shown).
at 37 °C for 2 h, the adipocytes were incubated for another 1 h by shaking for this study. After treatment with 1 μM ACh on the release of glycerol from WAT was also examined in this group in the presence of insulin.

Human adipose tissue using the in situ microdialysis[2]. The effect of ACh can thus be considered.

Wistar rats were incubated with different concentrations of ACh for 30 min in the presence of 1 μM insulin. The effect of various muscarinic receptor blockers on glucose uptake induced by ACh in rat adipocytes. After pre-incubation with various blockers at 1 μM or vehicle for 30 min, the adipocytes were exposed to 0.1 μM ACh in the presence of 1 μM insulin for 30 min as described in (A). Data are expressed as the means (±S.E.) of six experiments. **P < 0.01 and ***P < 0.001 as compared to the medium control. *P < 0.05 and **P < 0.01 as compared to cells incubated with the insulin-treated group.

These results were similar to that when adipose tissue was pre-treated with phentolamine plus propranolol to block alpha and beta-adrenoceptors, the subsequent addition of carbachol, a cholinergic agonist, induced a decrease in dialysate glycerol levels as antilipolytic action[2]. Also, we observed that 4-DAMP pretreatment reversed the ACh-induced antilipolysis at low dose from 0.01 to 0.1 μM and it totally abolished the ACh-induced action at high dose of 1 μM (Fig. 2B and C). However, pirenzepine or methotramine at same concentration did not influence this effect of ACh (Fig. 2B). Thus, an activation of adipose M3 muscarinic receptor is related to involve in lipolysis. However, previous study showed that 4-DAMP had low affinity for M1 and M5 cholinoreceptors[14]. Therefore, it is hard to exclude the possible involvement of M1 and M5 cholinoreceptors for the complete antagonism of ACh response by 4-DAMP at high concentrations. This view needs a further investigation in advance.

PKC subfamily was related to insulin signaling[11], as well as translocation and expression of glucose transporter[9,11]. The M3 muscarinic receptor was coupling to Gq protein[9] and through PLC–PKC pathway[9]. Thus, we examined the PLC and PKC signal pathway relating to glucose uptake induced by ACh. The adipocytes...
The hypothalamus has autonomic connection with brain stem nuclei that innervated adipose tissue via both sympathetic and parasympathetic fibers [4]. The physiological role of this parasympathetic input has been assessed using combination of selective vagotomy of a unilateral retroperitoneal fat pad with a hyperinsulinemic euglycemic clamp through reverse transcriptase-polymerase chain reaction analysis [4]. It showed that parasympathetic denervation of adipose tissue shifts the metabolism to a catabolic state: uptake of substrate is reduced with an increase of lipolysis [6]. Recent studies with the M3 receptor-deficient mice showed greatly impairment in ameliorations of glucose homeostasis and insulin sensitivity, reduced food intake, and a significant elevation in basal and total energy expenditure, due to increased central sympathetic outflow and increased rate of fatty acid oxidation [5]. However, the direct effect of ACh on adipose tissue is still unknown. In the present study, we found that ACh acted M3 muscarinic receptor to cause a direct reduction of glucose uptake and lipolysis in adipose tissue. Although the PLC–PKC pathway was identified to be involved in the decrease of glucose uptake by ACh, the detailed mechanisms are required more investigations in the future.

In conclusion, the obtained results suggest that muscarinic M3 receptor is involved in the regulation of glucose uptake and/or lipolysis in adipose tissue. This finding might be helpful to apply in handling of obesity and associated metabolic disorders.

Acknowledgements

We appreciate Miss M.J. Wang for research assistance. The present study was supported in part by a grant from China Medical University (CMU96–235).

References