学位論文要約

論文題目　Evidence for acute activation of 5'-AMP-activated protein kinase by metformin and salicylate in rat skeletal muscles
（ラット骨格筋におけるメトホルミン及びサリチル酸によるAMPキナーゼの急性的活性化に関する検討）

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Skeletal muscle is the principal site of whole-body glucose utilization, and under most physiological conditions, glucose transport across the cell membrane is the rate-limiting step for glucose metabolism by skeletal muscle. 5'-AMP-activated protein kinase (AMPK) has been identified as part of the mechanism leading to the acute and chronic metabolic activation processes in skeletal muscle. Exercise (muscle contraction) is a strong activator of AMPK in skeletal muscle. Skeletal muscle AMPK mediates the antidiabetic effects of exercise including that insulin-independent glucose transport, fatty acid oxidation, glycogen regulation, expression of glucose transporter GLUT4, activation of peroxisome proliferator-activated receptor γ coactivator 1α, mitochondrial biogenesis, and enhanced insulin sensitivity. In addition, recent studies showed that antidiabetic drugs such as metformin (MET) and thiazolidinediones, and functional foods and their natural components such as *Morus alba* leaf extract, caffeine, berberine, *Coptidis rhizoma* extract, and caffeic acid stimulate AMPK activity in skeletal muscle.

MET, a biguanide derived from French lilac (*Galega officinalis*), is the most commonly prescribed medication in the world for type 2 diabetes mellitus (T2DM) patients. MET is known to lower the blood glucose level mainly through hepatic glucose output as a result of increased hepatic insulin sensitivity. The mode of action of MET is complex and not fully understood, but it is demonstrated that MET stimulates AMPK with decreased glucose production in primary cultured rat hepatocytes. A number of studies have documented MET activation of hepatic AMPK. However, although skeletal muscle has been implicated in the antidiabetic effect of MET, only a few studies have examined skeletal muscle AMPK.

Salicylate (SAL), a willow bark product, has been used as an anti-inflammatory agent since ancient times. SAL induces systemic anti-inflammatory effects by suppressing cyclooxygenase, resulting in decreased production of proinflammatory mediators such as prostaglandins. Interestingly, a number of clinical studies have

suggested that SAL stimulates metabolic processes and improves glucose homeostasis in humans. The molecular mechanism of SAL action is not understood. We hypothesized that AMPK in skeletal muscle plays a pivotal role in the antidiabetic effect of SAL.

Thus, the aims of the present study was to reevaluate whether MET directly acts on skeletal muscle AMPK (Study1), and to elucidate whether AMPK is involved in the mechanism leading to the SAL-induced activation of glucose metabolism (Study2). For this purpose, we used rat fast- and slow-twitch muscles incubated in vitro to eliminate systemic confounders including circulatory, humoral, and neural factors and intestinal absorption of drugs.

Study1

Background and aims: MET is the most commonly prescribed medication in the world for T2DM patients. The molecular mechanism of MET action is not fully understood. AMPK is a key molecule of metabolic enhancement in skeletal muscle. We investigated whether MET acts directly on different muscle types, and activates both AMPK and glucose transport under reduced energy status. We also examined the possibility that MET is acutely transported into skeletal muscle via organic cation transporters (OCTs), and activates AMPK.

Materials and Methods: Isolated rat fast-glycolytic epitrochlearis and slow-oxidative soleus muscles were incubated in Krebs buffer containing MET, and activation of AMPK, the intracellular energy status, and glucose and MET transport activity were then evaluated. The effect of cimetidine, which is an OCT inhibitor, on AMPK activation and glucose transport were also examined.

Results: MET (10 mmol/L, ≥ 60 min) increased the phosphorylation of Thr^{172} at the catalytic α subunit of AMPK, an essential step for full kinase activation, in both epitrochlearis and soleus muscles. Phosphorylation of Ser^{79} of acetyl CoA carboxylase, an endogenous substrate of AMPK, increased similarly. Two distinct α isoforms (α1 and α2) of catalytic subunit exist in skeletal muscle. MET activated both the α1 and α2 isoforms. AMPK is implicated in the mechanism of insulin-independent glucose transport, which is elicited by energy-depriving stimuli. To determine the energy status of skeletal muscle, we measured the ATP, phosphocreatine (PCr), and glycogen content. MET significantly decrease ATP, PCr and glycogen content in both muscles. Correspondingly, MET increased rates of 3-*O*-methyl-D-glucose (3MG) transport. We examined whether MET affects the activation status of insulin receptor signaling molecules in skeletal muscle, but MET did not change the basal phosphorylation status

of insulin receptor substrate (IRS)-1 Tyr^{612}, Akt Ser^{473}, glycogen synthase kinase-3β Ser^{9}, mammalian target of rapamycin Ser^{2448}, p70 ribosomal protein S6 kinase (p70S6K) Thr^{389} , or eukaryotic initiation factor 4E-binding protein 1 Thr$^{37/46}$. MET did not affect GLUT4 content in either muscle. MET has been identified as a substrate of OCTs. OCT1 and OCT3 protein were clearly detected in epitrochlearis and soleus muscles. Furthermore, MET was transported into the cytoplasm in a time-dependent manner, and cimetidine suppressed MET-induced AMPK phosphorylation and 3MG transport.

Conclusion: Our data provide fundamental evidence that confirms the stimulatory actions of MET on AMPK signaling. These results suggest that MET is acutely transported into skeletal muscle by OCTs, and stimulates AMPKα1 and α2 activity in both fast- and slow-twitch muscle types, at least in part by reducing the energy state.

Study2

Background and aims: SAL has been used as an anti-inflammatory agent since ancient times. SAL has been recently implicated in the antidiabetic effect in humans. The molecular mechanism of SAL action is not understood, therefore we assessed whether AMPK in skeletal muscle is involved in the effect of SAL on glucose homeostasis.

Materials and Methods: Isolated rat fast-glycolytic epitrochlearis and slow-oxidative soleus muscles were incubated in Krebs buffer containing SAL. The activation status of AMPK, the intracellular energy status, and glucose and SAL transport activity were then evaluated.

Results: To our knowledge, no study has shown that SAL is taken up into skeletal muscle tissue. We found that the intracellular concentration of SAL increased rapidly (<5 min) in both epitrochlearis and soleus muscles in a time-dependent manner. SAL increased the Thr^{172} phosphorylation of the α subunit of AMPK increased in a dose- and time-dependent manner. SAL increased both AMPKα1 and AMPKα2 activities. To determine the energy status of skeletal muscle, we measured the ATP, PCr, and glycogen content. SAL significantly decreases ATP, PCr and glycogen content in both muscles. Correspondingly, SAL increased rates of 3MG transport. We examined whether SAL affects the activation status of insulin receptor signaling molecules in skeletal muscle, SAL did not change the phosphorylation of insulin receptor signaling including IRS-1 Tyr^{612}, Akt Ser^{473} and p70S6K Thr^{389}. SAL did not affect GLUT4 or actin content in either muscle.

Conclusion: Our data provide fundamental evidence that confirms the stimulatory actions of SAL on AMPK signaling. These results suggest that SAL may be transported

into skeletal muscle and may stimulate AMPK and glucose transport via energy deprivation in multiple muscle types. We proposed that skeletal muscle AMPK might be part of the mechanism responsible for the metabolic improvement induced by SAL.

In the present study, we investigated the mechanism of the antidiabetic effect of plant-derived drugs. Two studies showed that both MET and SAL directly act on skeletal muscle and increase muscle AMPK activity accompanied with energy deprivation in fast-glycolytic and in both fast- and slow-twitch muscle types, and that both MET and SAL increase glucose transport in both muscle types. We proposed that skeletal muscle AMPK plays an important role in the MET- and SAL-induced hypoglycemic effects. Further research, especially *in vivo* study, is required to clarify whether MET and SAL are beneficial compounds in reducing the risk of T2DM.