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学位論文名	膵β細胞 Kv2.1 チャンネルによるインスリン分泌制御機構の解明と糖尿病治療展開
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論文内容の要旨

1　研究目的

Voltage-dependent potassium channels are involved in repolarization of excitable cells. In pancreatic β-cells, activation of voltage-gated K^+ (Kv) channels possibly repolarizes cells and attenuates glucose-induced action potentials to suppress insulin secretion. Among Kv channel families, Kv2.1 is reportedly expressed in islet β-cells as the major component in rodents. It is expected that inhibition of the β-cell Kv current prolongs action potentials and enhances glucose-induced insulin secretion.

Glucagon-like peptide-1 (GLP-1)-based medicines have recently been widely used to treat type 2 diabetic patients, while adverse effects of nausea and vomiting have been documented. Inhibition of Kv channel subtype Kv2.1 in pancreatic β-cells has been suggested to contribute to mild depolarization and promotion of insulin release. This study aimed to determine the effects of pharmacological or genetic blockade of Kv2.1 channels on the glucose-induced increases in insulin release in islets and cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in β-cells of mice. Furthermore, I aimed to determine whether blockade of Kv2.1 channels potentiates insulinotropic effect of GLP-1 agonists.

2　研究方法

1. Animals

   Wild-type C57BL/6J mice, $Kv2.1^{+/-}$ mice, db/db mice, and NSY mice were housed in accordance with our institutional guidelines.
2. Preparation of pancreatic islets and single β-cells

   Islets of Langerhans were isolated by collagenase digestion. For β-cell experiments, islets were dispersed into single cells and maintained for 1 day at 37°C in Eagles minimal essential medium.
3. Measurements of insulin release in mouse islets

Groups of 10 islets were incubated at 37°C in HEPES-added Krebs-Ringer bicarbonate buffer (HKRB) solution with 2.8 mM glucose for stabilization, followed by test incubation for 1 hr in HKRB with 2.8 or 8.3 mM glucose. Guangxitoxin-1E (GxTx), exendin-4 (Ex-4), and GxTx with Ex-4 were present throughout the incubation. Insulin release in islets was determined by ELISA.

4. Measurements of $[Ca^{2+}]_i$ in single β-cells

   Dissociated single β-cells on coverslip were mounted in an open chamber and superfused in HKRB. $[Ca^{2+}]_i$ in single β-cells was measured by dual-wavelength fura-2 microfluorometry.

5. Patch-clamp experiments in mouse single β-cells

   Conventional whole-cell currents were recorded using an amplifier (Axopatch 200B; Molecular Devices, Foster, CA), in a computer using pCLAMP10.2 software. Single β-cells were voltage-clamped at a holding potential of -70 mV and then shifted to the test potential to 0 mV with the pulses of 100 milliseconds duration for Kv channel currents at room temperature (25°C).

6. Intraperitoneal glucose tolerance tests (IPGTT) and oral glucose tolerance tests (OGTT)

   An IPGTT were performed with male $Kv2.1^{+/-}$ mice and wild-type littermates (10 weeks-old), db/db mice (7 weeks-old) or NSY mice (20 weeks-old) fasted overnight. One g/kg glucose into db/db mice or 2 g/kg glucose into $Kv2.1^{+/-}$ mice, wild-type mice and NSY mice was injected intraperitoneally (i.p.), followed by blood sampling from the tail vein. Saline (0.1 ml/10 g body weight), GxTx (100 nmol/kg, i.p.), liraglutide (3 nmol/kg, i.p.) or liraglutide with GxTx was administrated 30 minutes before the glucose challenge. In OGTT, 2 g/kg glucose was orally injected into wild-type and $Kv2.1^{+/-}$ mice. Blood glucose concentrations were measured using a GlucoCard DIA meter (Arkray, Kyoto, Japan), and insulin concentrations using an ELISA kit (Morinaga Institute of Biological Sciences).

3 研究成果

In islets isolated by collagenase digestion, GxTx, a Kv2.1 channel blocker, significantly increased glucose (8.3 mM)-induced insulin release without altering basal insulin release at 2.8 mM glucose. Glucose-induced insulin release from isolated islets of $Kv2.1^{+/-}$ mice was significantly greater than that of wild-type mice. Likewise, blockade of Kv2.1 channels by GxTx potentiated glucose (8.3 mM)-induced $[Ca^{2+}]_i$ increases in β-cells without altering basal $[Ca^{2+}]_i$ levels at 2.8 mM glucose. These results suggest that Kv2.1 channels physiologically restrict glucose-induced Ca^{2+} influx and thereby attenuate insulin secretion in β-cells.

When Kv2.1 channel blocker GxTx and GLP-1 agonist Ex-4 at the concentrations ineffective on insulin release (sub-threshold doses) were added together, this combination markedly increased insulin release and $[Ca^{2+}]_i$ in a glucose-dependent manner in mouse islets and β-cells. In $Kv2.1^{+/-}$ mice, Ex-4 at sub-threshold concentration alone increased insulin

release and $[Ca^{2+}]_i$ in islets and β-cells. The $[Ca^{2+}]_i$ response to sub-threshold Ex-4 and GxTx in combination was attenuated by pretreatment with protein kinase-A (PKA) inhibitor H-89, indicating PKA-dependency of the cooperative effect.

In wild-type mice, administration of GLP-1 agonist liraglutide (3 nmol/kg i.p.) failed to significantly alter blood glucose and plasma insulin levels during IPGTT. In contrast, administration of this low dose of liraglutide in $Kv2.1^{+/-}$ mice significantly suppressed blood glucose increases and enhanced insulin responses during IPGTT. In OGTT, $Kv2.1^{+/-}$ mice exhibited improved glucose tolerance and increased plasma insulin levels after glucose challenge, compared to wild-type mice. Furthermore, sub-threshold doses of GxTx and liraglutide in combination markedly increased plasma insulin and improved glucose tolerance in diabetic db/db mice and NSY mice. These results suggest a modest suppression of Kv2.1 channels dramatically raises insulinotropic potency of GLP-1 agonists, which opens a new avenue to reduce the doses of GLP-1 agonist and associated adverse effects while achieving the same glycemic control in type 2 diabetes.

4　考察

It has been reported that Kv2.1 channels are involved in repolarization of pancreatic β-cells and thereby limit insulin release. In this study, I examined the physiological role of Kv2.1 channel by using pharmacological and genetic blockade of Kv2.1 channel. The results showed that either pharmacological blockade of Kv2.1 channel by using GxTx or genetical blockade by $Kv2.1^{+/-}$ mice, enhances the glucose-induced $[Ca^{2+}]_i$ increases and insulin release in pancreatic islet β-cells. These results confirmed previous studies that this channel physiologically limits glucose-induced insulin release in pancreatic β-cells.

GLP-1 agonists have been shown to improve glucose intolerance in type 2 diabetic patients with a lower risk of hypoglycemia than ATP-sensitive K^+ channel blocker sulfonylureas. It has been shown, however, that GLP-1 agonists often causes gastrointestinal adverse events such as nausea and vomiting, and that their incidence is dose-dependent. The present study showed that the use of Kv2.1 channel blocker, even at sub-threshold concentrations, markedly reduces the dose of GLP-1 agonists required for their insulinotropic and blood glucose-lowering effects. This finding opens a new clinical avenue to reduce the dose and associated adverse effects of GLP-1 agonists, while achieving the same glycemic control in type 2 diabetes.

The effects of GLP-1 are mediated by stimulation of adenylate cyclase and subsequent increase in intracellular cyclic AMP, which lead to activation of PKA and/or exchange proteins directly activated by cAMP (Epac) pathways. Present study indicates that synergistic effect of sub-stimulatory concentrations of GxTx and Ex-4 to increase $[Ca^{2+}]_i$ in β-cells was attenuated by pretreatment with a PKA inhibitor H-89. These results suggest that the Kv2.1 channel blockade interacts with the PKA, but not Epac, branch of cAMP signaling in β-cells.

5　結論

In conclusion, Kv2.1 channels physiologically limit glucose-induced Ca^{2+} influx and thereby attenuate insulin secretion in pancreatic β-cells. Combination of low doses of GLP-1 agonist and Kv2.1 channel blocker had strong *in vivo* insulinotropic and blood glucose-lowering effects in type 2 diabetic mice. Blockade of Kv2.1 channels enhances potency of GLP-1-based drugs, which can lower their dose required to improve glucose tolerance in type 2 diabetes and thereby reduce the frequency of their adverse effects. This study provides a novel avenue to make the GLP-1-based diabetes therapy more effective and reliable.

論文審査の結果の要旨

電位依存性 K^+チャネルを阻害すると、β細胞の糖刺激に対する細胞内 Ca^{2+}濃度の上昇反応が促進され膵島からのインスリン放出が促進されることを、単離したβ細胞と膵ランゲルハンス島を用いて示唆した報告である。電位依存性 K^+チャネルを阻害するために、guangxitoxin 或いは Kv2.1 ヘテロ遺伝子欠損マウスを用いている。即ち、単独では効果をもたらさない低濃度の GLP-1 アゴニストの exendin-4 と、低濃度の Kv2.1 阻害剤 guangxitoxin とを両方同時に与えると、糖刺激に対するβ細胞の細胞内 Ca^{2+}濃度の上昇とインスリン放出が促進されることを示した。さらに、Kv2.1 ヘテロ欠損マウスのβ細胞において、野生型動物のβ細胞と比較し、比較的低濃度の exendin-4 の投与により、細胞内 Ca^{2+}濃度の上昇とインスリン放出がより促進されることを示した。さらに、in vivo においても、グルコースを腹腔内投与した場合は、GLP-1 アゴニスト liraglutide 投与を組み合わせたると Kv2.1 ヘテロ欠損マウスでは野生型に比較しインスリン分泌が亢進し血糖値の上昇が抑えられることを示した。これに対し、糖を経口で投与した場合には Kv2.1 ヘテロ欠損マウスでは野生型に比較し、インスリン分泌が促進され、血糖値の上昇が少ないことが示された。さらに、糖尿病モデルマウス db/db マウス、NSY マウスにおいて、低用量の liraglutidoe と guangxitoxin の両方の投与により、単独投与群あるいは偽薬投与群に比較し、インスリン分泌が亢進し、血糖値の上昇が抑えられることが示された。以上から、電位依存性 K^+チャネル阻害を組み合わせると、低用量の GLP-1 アゴニストで糖依存性のインスリン分泌を促進させることが明らかとなった。

本研究は、電位依存性 K^+チャネル阻害を組み合わせて投与することで GLP-1 の必要量を減少させることが可能であることを示したもので、今後の臨床応用につながる発見と思われる。本論文の内容は、既に Endocrinology に受理されている。

タイプミスを修正したうえで、博士論文に値すると審査員全員一致で判断した。

最終試験の結果の要旨

最初に膵β細胞のインスリン放出の細胞内機序について概説し、博士論文の内容について発表を行った。発表は明快で、様々な質問に対し真摯な態度で適切に答えた。申請者は、本研究領域に関して十分な知識と見識を備えており、審査員全員一致で、申請者は学位を授与するに値すると判断した。