Communication

Cinnamtannin A2, a Tetrameric Procyanidin, Increases GLP-1 and Insulin Secretion in Mice

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Procyanidins are oligomers and polymers of flavan-3-ols consisting of (−)-epicatechin subunits. In this study, we isolated and purified dimeric, trimeric and tetrameric procyanidins from cacao liquor and investigated their influence on the “incretin effect” as compared to the monomer, (−)-epicatechin in mice. Cinnamtannin A2 specifically increased the glucagon-like peptide-1 (GLP-1) and insulin secretion levels in the plasma after 60 min administration. As evidence of the action of insulin, activation of insulin receptor and insulin receptor substrate-1 was observed in the soleus muscle. These results indicate that the intake of cinnamtannin A2 may improve hyperglycemia through an incretin-like effect, accompanied by activation of the insulin-signaling pathway.

Key words: procyanidin; cinnamtannin A2; glucagon-like peptide-1 (GLP-1); insulin; hyperglycemia

Procyanidins are oligomers and polymers of flavan-3-ols consisting of epicatechin subunits and are usually found in fruits and other plants. There is accumulating evidence that procyanidins possess various beneficial health effects, including prevention of diabetes mellitus. For example, a grape-seed procyanidin extract suppressed hyperglycemia in animals of type 2 diabetic model, and cacao extracts containing abundant procyanidins also suppressed hyperglycemia and obesity in high-fat diet-fed mice. Most of these studies describing the beneficial effects of procyanidins have shown peripheral activity. However, there are few data addressing whether procyanidins have central effects on the endocrine system, a key component of metabolic control. Moreover, the underlying molecular mechanisms by which procyanidins suppress hyperglycemia are not yet fully understood.

The importance of endocrine hormones in whole-body nutrient equilibrium is highlighted by their suppression in several pathologies of nutrient metabolism, such as hyperglycemia and diabetes. Glucagon-like peptide 1 (GLP-1), which is released from distal intestinal endocrine L cells after food intake, is a potent glucose-dependent stimulant of insulin secretion. GLP-1 exerts important effects on regulating glucose metabolism, stimulating glucose-dependent insulin secretion, promoting β-cell proliferation, enhancing glucose transport to the peripheral tissue, as well as inhibiting glucagon release, gastric emptying, and food intake. The action of GLP-1 is short-lived because of rapid catabolism by the dipeptidyl peptidase-4 (DPP-4) enzyme. Recently, DPP-4 inhibitors have been used as novel drugs for the treatment of diabetes. However, only a few studies have reported that food components have inhibitory effects against DPP-4; for example, a grape-seed procyanidin extract and chalcone inhibited DPP-4. Moreover, there is little information on the effect of food components or extracts increasing GLP-1 secretion. A recent paper has shown that resveratrol treatment for five weeks enhanced GLP-1 secretion with oral glucose loading in high-fat diet-fed mice. Thus polyphenols may have potential for modulation of GLP-1 activation, although these effects are highly dependent on the polyphenol structures, the experimental conditions, and models used. It is unclear yet whether or not procyanidins stimulate the secretion of GLP-1 and insulin. In the present study, we isolated and purified dimeric, trimeric, and tetrameric procyanidins from cacao liquor, and investigated whether a single oral administration of these procyanidins increased GLP-1 and insulin levels in the plasma, and influenced phosphorylation of the insulin target molecules insulin receptor (IR) and its substrate-1 (IRS-1) in muscle.

(−)-Epicatechin was purchased from Sigma-Aldrich (St. Louis, MO). Procyanidin oligomers procyanidin B2 and C1, and cinnamtannin A2 were prepared as previously reported. K597, a DPP-4 inhibitor, was purchased from Wako Pure Chemical Industries (Osaka, Japan). Primary antibodies against IR-β, IRS-1, and β-actin were purchased from Cell Signaling Technology (Danvers, MA). Anti-GLP-1R was purchased from Abcam (Cambridge, UK). Anti-phosphotyrosine was purchased from Becton, Dickinson (Franklin Lakes, NJ). Horseradish peroxidase-conjugated anti-goat, anti-rabbit, and anti-mouse IgG antibodies and protein A/G plus-agarose were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). All other reagents used were of the highest grade available from commercial sources.

All animal experiments were approved by the Institutional Animal Care and Use Committee (Permission...
In the present study, the plasma insulin levels were measured 60 min after oral administration of procyanidins. It was found that only cinnamtannin A2 significantly increased the plasma insulin level without a glucose load (Fig. 1A). Other procyanidins, including the monomer (−)-epicatechin, did not alter the insulin level. These results suggest that cinnamtannin A2 induced insulin secretion from pancreatic beta-cells. In the same animals, procyanidins did not affect plasma glucose levels (Fig. 1B).

Because cinnamtannin A2 increased plasma insulin levels, the secretion levels of glucagon-like peptide-1 (7−36 amid) (GLP-1) were measured in plasma in the presence of K597, a DPP-4 inhibitor. As shown in Fig. 2A, cinnamtannin A2 significantly increased the GLP-1 secretion levels in the plasma 60 min after oral administration, although we did not measure GLP-1 secretion levels in the plasma of the mice treated with the other procyanidins. These results suggest that cinnamtannin A2-induced insulin secretion is, at least in part, contributing to the observed increase in GLP-1 secretion. Under our experimental conditions, expression of GLP-1 receptor in muscle did not change (Fig. 2B).

To confirm the action of insulin, which was probably secreted in response to increased GLP-1 level, we investigated phosphorylation of IRβ and IRS-1. As shown in Fig. 3, cinnamtannin A2 significantly promoted phosphorylation of both IRβ and IRS-1. Other procyanidins also slightly increased phosphorylation of these proteins, although this was not significant. These results strongly suggest that cinnamtannin A2 promotes insulin secretion, resulting in the activation of insulin signaling pathways in muscle.

We also investigated the involvement of the Jak-Stat pathway, which is known to activate IRS6,17 without IRβ activation. Oral intake of procyanidins did not promote phosphorylation of either Jak2 or Stat3 (data not shown).

In generally, GLP-1 is secreted from enteroendocrine L cells localized in the distal ileum and colon.18 GLP-1 acts through a specific G protein-coupled receptor to potently stimulate glucose-dependent insulin secretion.
In vivo bioavailability of propanidin B2 has been reported using a radioactively labeled molecule in male rats. Radioactivity was observed to increase, and its T_max in blood was approximately 6 h after oral administration, indicating that the parent compound and its microbial metabolites are incorporated into the body. Using an apple polyphenol extract containing abundant as well as to inhibit glucagon secretion, gastric emptying, and food intake. Because of its pleiotropic actions in nutrient homeostasis, GLP-1 is now under investigation as a potential treatment for patients with type 2 diabetes mellitus. Here we have demonstrated that a single oral ingestion of cinnamtannin A2, one of the tetrameric propanidins, increased the GLP-1 secretion levels and insulin secretion in plasma without an oral glucose load (Figs. 1 and 2). In this study, we measured GLP-1 (7–36) amide which is considered the most important incretin hormone. Its action is mediated by receptors expressed by the endocrine pancreatic β-cells. We also confirmed that cinnamtannin A2 promoted the phosphorylation of IRβ and IRS-1 in skeletal muscle as a result of the action of insulin (Fig. 3). To our knowledge, this is the first report that a non-nutrient can increase GLP-1 secretion with a single oral ingestion of cinnamtannin A2. Further study is needed to clarify the roles of these hormones in propanidin-caused incretin effect.

There are three possibilities for the increase in the GLP-1 levels: 1, Cinnamtannin A2 directly or indirectly stimulates GLP-1 secretion from L cells; 2, Cinnamtannin A2 inhibits DPP-4, and thus prevents the rapid degradation of GLP-1 by this prolyl peptidase; 3, Cinnamtannin A2 modulates the expression and affinity of the GLP-1 receptor in target tissues. Regarding the first possibility, GLP-1 secretion from L cells is thought to be essentially a glucose-dependent action. Recently, it was reported that GLP-1 secretion occurs not only in response to glucose intake, but also that of fatty acids and amino acids. All of these inducers of GLP-1 secretion are nutrients. Although we are not aware of any reports of non-nutrient food components such as polyphenols increasing the GLP-1 secretion levels in the absence of a glucose load, our data suggest that a single oral administration of cinnamtannin A2 may stimulate L cells and increase GLP-1 secretion. Recently, it was shown that administration of resveratrol for 5 weeks increased glucose-induced GLP-1 secretion in mice. This report suggested that resveratrol exerts its actions in part through modulation of the entero-endocrine system in vivo. Several studies have demonstrated that administration of nutrients into the duodenum stimulates GLP-1 secretion indirectly through the pathway which includes glucose-dependent insulinotropic polypeptide (GIP) secretion from endocrine K cells. Moreover, a recent report has also demonstrated that muscarinic receptors, particularly the M1 and M2 subtypes expressed in L cells, indirectly control GLP-1 secretion from human L cells, although the mechanism by which this might occur is unclear.

With respect to the second possibility, it has been reported that grape seed-derived propanidins decrease DPP-4 activity and expression. In the current study, although we did not measure any inhibitory effects on DPP-4, there remain a possibility that cinnamtannin A2 inhibits DPP4 activity. For the last possibility, we measured the expression levels of GLP-1R, even though the muscle samples were collected only 60 min after the administration of propanidins. Our data clearly demonstrate that there was no change in GLP-1R levels (Fig. 2). We assume that any change in the receptor affinity would also be negligible, because of the short treatment time. However, cinnamtannin A2 may stimulate GLP-1R in pancreatic pancreatic β-cells. Further study is also needed to clarify these remaining issues.

Regarding the bioavailability of propanidins, the extent of absorption and metabolism are controversial. Pharmacokinetic studies of the monomeric flavan-3-ols, (-)-epicatechin and (+)-catechin, are well documented. These compounds are, at least in part, absorbed into the body and are thereby able to function in vivo. In vivo bioavailability of propanidin B2 has been reported using a radioactively labeled molecule in male rats. Radioactivity was observed to increase, and its T_max in blood was approximately 6 h after oral administration, indicating that the parent compound and its microbial metabolites are incorporated into the body.
procyanidin oligomers, dimeric and trimeric procyanidins were detected by LC-MS/MS in rat plasma 2 h after administration. The same report also indicated that procyanidin tetramers and pentamers might have been in the plasma. However, there is no available evidence for the absorption of tetramers, or procyanidins with a higher degree of polymerization, in the body. At the minimum, procyanidins are stable during gastric transit in humans. Our results indicate that cinnamtannin A2 specifically increased the GLP-1 and insulin levels in plasma. These results suggest that microbial metabolites of procyanidins in the intestine are not effective in the induction of GLP-1 secretion, but dimeric and trimeric procyanidins did not increase plasma insulin levels, and microbial metabolites of cinnamtannin A2 are most likely also dimeric and trimeric procyanidins. Thus, insulin secretion due to cinnamtannin A2 is probably as a result of GLP-1 secretion, but not of incorporation and/or metabolism of cinnamtannin A2.

Intestinal function is linked to metabolism of the whole body. Our previous report demonstrated that feeding a procyanidin-rich cacao liquor extract for 13 weeks prevented hyperglycemia through promotion of GLUT4 translocation in the plasma membrane of mouse skeletal muscle. We also demonstrated that this cacao liquor extract promoted phosphorylation of AMPKα, without phosphorylation of phosphatidylinositol 3-kinase, and this was the molecular mechanism of GLUT4 translocation. Our current findings suggest a new possibility, namely that promotion of GLUT4 translocation by a single oral administration of procyanidins, particularly cinnamtannin A2, is due to activation of the insulin-signaling pathway (Fig. 3) through increased the GLP-1 secretion levels (Fig. 2). These results strongly suggest that procyanidins possess anti-hyperglycemic activities with at least two different mechanisms. Dimeric and trimeric procyanidins also showed evidence of activating the insulin-signaling pathway, although these effects were not significant. They may possibly increase GLP-1 secretion at an earlier or later time-point. Further study is needed to understand the effects of procyanidins on GLP-1 secretion in more detail.

In conclusion, our findings suggest that procyanidins are attractive food compounds for prevention of hyperglycemia and diabetes mellitus by the “incretin effect.”

References

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