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Free fatty acids administered into the colon promote the secretion of glucagon-like peptide-1 and insulin ☆

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Abstract

We examined whether free fatty acids (FFAs) promote glucagon-like peptide-1 (GLP-1) secretion when administered into the intestinal tract. We found that an unsaturated long-chain FFA, α-linolenic acid (α-LA), resulted in increased plasma GLP-1 and insulin levels when administered into the colon. Such stimulatory effects were not apparent with either vehicle or a saturated middle-chain FFA, octanoic acid (OA). Concomitant with GLP-1 secretion, the administration of α-LA, but not vehicle or OA, also resulted in a significant increase in the population of pERK positive cells within the GLP-1 positive cells of the colonic mucosa. Moreover, colonic administration of α-LA into normal C3H/He mice caused a reduction in plasma glucose levels, as well as in type 2 diabetic model NSY mice. Our results indicate that the in vivo colonic administration of α-LA promotes secretion of incretin GLP-1 by activating the ERK pathway in L-cells and thereby enhances the secretion of insulin.

Keywords: GLP-1; G-protein-coupled receptor; Insulin; Diabetes mellitus; Mouse

Glucagon-like peptide-1 (GLP-1) is a naturally occurring hormone (incretin) that is released from L-cells in the gut in response to food [1]. It is well known that GLP-1 acts as a promoter of glucose-dependent insulin secretion in pancreatic β-cells [2]. Post-prandial GLP-1 secretion is important in the secretion of insulin, and thus in the subsequent reduction in plasma glucose concentration. The in vivo biological activity of GLP-1 is critically regulated by dipeptidyl peptidase (DPP-IV)-mediated degradation [3–5], and a DPP-IV inhibitor is now being developed as a new class of oral anti-diabetic agents [6].

GLP-1 is produced as a product of the tissue-specific processing of proglucagon in the L-cells of the intestinal mucosa [1]. Glucagon-like peptide-1 (GLP-1) is released from intestinal L-cells in response to the ingestion of meals. L-cells are shown to be widely localized in the intestine, predominantly in the distal intestine, including the colon segment [7–10]. However, the mechanisms regulating GLP-1 secretion are not clear.

FFAs provide an important energy source and also act as signaling molecules. Administration of a fatty acid mixture directly into the ileum of rats is known to stimulate incretin secretion by the L-cells [11–15]. Recently, we found that unsaturated long-chain FFAs (such as α-linolenic acid) promote the secretion of GLP-1 via G protein-coupled receptor GPR120 in STC-1 enteroendocrine cells [16]. Also, we found that GPR120 is expressed predominantly in the colon [16].

In the present study, we have examined whether FFAs promote GLP-1 secretion when administered into the

☆ *Abbreviations:* DPP-IV, dipeptidyl peptidase IV; ELISA, enzyme-linked immunosorbent assay; GLP-1, glucagon-like peptide-1; α-LA, α-linolenic acid; OA, octanoic acid; PEG, polyethylene glycol.

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intestinal tract and investigated whether this mechanism is specific to only certain parts of the intestinal tract. For this purpose, we first established an experimental system to examine the effect of FFAs administered directly into each segment of the intestine. Using this system, we examined the effects of FFAs upon plasma GLP-1 and insulin levels, ERK activation in the colonic mucosa, and also on plasma glucose levels in normal C3H/He mice and type 2 diabetic model NSY mice. Our results indicate that the in vivo colonic administration of α-LA promotes secretion of GLP-1 and insulin and thereby leads to a reduction in blood glucose levels.

Materials and methods

Chemicals. OA and α-LA (Sigma, St. Louis, MO) were used as test fatty acid regents and polyethylene glycol (PEG) 400 (Sigma) was used as a vehicle solution [16].

Animals. Male C57BL/6J mice (8 weeks old), type 2 diabetic male NSY mice (40 weeks old) [17,18], and normal control C3H/He mice (8 weeks old) were purchased from SLC Japan (Hamamatsu, Japan). The animals were maintained in a temperature-controlled room (23 °C) subjected to a 12-h light/dark cycle. This study was approved by the Kyoto University Animal Care and Use Committee, and conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health. The animals were fed a standard rodent chow diet (MF, Oriental Yeast, Osaka, Japan) and had free access to food and water. The animals fasted for at least 18 h prior to experiments and were anesthetized with sodium pentobarbital (60 mg/kg). Either the duodenum, ileum or colon of each animal was cannulated with a tube 2 mm in diameter to allow for the administration of fatty acid. Then, 100 μl/min of fatty acid was administered via the cannula (300 nmol/100 μl total administration) [11]. Blood samples were collected from the portal vein 5 min after administration and centrifuged to obtain plasma. Plasma samples were then used to measure the levels of GLP-1 and insulin and to determine the activity of dipeptidyl peptidase IV (DPP-IV). Intraperitoneal administration of glucose was performed 5 min before the administration of fatty acid. Blood from the caudal vein was used to measure plasma glucose concentration prior to the administration of glucose and 5 min after the administration of FFAs. Plasma glucose levels were measured by the glucose oxidase method (Glucose C II Test Wako, Wako Pure Chemical, Osaka, Japan). Data were expressed as mean plasma concentration measured 5 min after FFA treatment.

GLP-1 and insulin level. Plasma levels of GLP-1 and insulin were measured from blood samples using the GLP-1 ELISA Kit (Wako Pure Chemical, Osaka, Japan) and Revis Insulin Kit (Shibayagi, Maebashi, Japan), respectively.

DPP-IV activity. Plasma DPP-IV activity was determined according to Pederson et al. [19], using Gly-Pro *p*-nitroanilide (Nacalai Tesque, Kyoto, Japan) as a substrate. Enzyme activity is given herein as the ratio of *p*-nitroanilide production in the fatty acid treatment group relative to that in the vehicle treatment group.

Immunohistochemistry. A 2 cm portion (approximate) of the colon was removed from the fatty acid administration site 5 min after injection of FFA. Tissue samples were then fixed in 4% paraformaldehyde solution (Wako Pure Chemical) and then embedded in OCT compound (Sakura Finetechnical, Tokyo, Japan). Serial cryosections (10 μm) of each segment were then immunostained using an anti-GLP-1 antibody (Peninsula Laboratories, San Carlos, CA) and anti-phosphorylated p42/p44 (anti-pERK) (Cell Signaling Technology, Beverly, MA). The total number of anti-GLP-1 positive cells and anti-GLP-1 positive + anti-pERK positive cells was determined in a series of random fields from 10 separate regions. Data were expressed as the ratio of anti-GLP-1 positive + anti-pERK positive cells/total anti-GLP-1 positive cells.

Statistical analysis. Data were expressed as means ± SEM. Differences in mean values were compared by use of Dunnett's test using statistical software (StatView-J, SAS Institute, Cary, NC). Statistical significance was defined as $p < 0.05$.

Results

Colonic administration of α-LA induces the secretion of GLP-1

Either the duodenum, ileum or colon of C57BL/6J mice was cannulated, and α-LA or vehicle was administered into each segment. Five minutes after administration, there was no significant difference between α-LA and vehicle-injected animals in terms of plasma GLP-1 level when administered via the duodenum or ileum. However, when administered into the colon, α-LA resulted in significantly ($p < 0.01$) increased plasma GLP-1 levels when compared to levels seen with vehicle-injected animals (Fig. 1).

α-LA, but not OA, induces secretion of GLP-1 and insulin

Next, we examined GLP-1 secretion after administration of either OA or α-LA. FFAs were administered into the colon of C57BL/6J mice and blood samples were collected from the portal vein. There was no significant difference in plasma GLP-1 level between animals administered with either OA or vehicle. However, the α-LA treatment led to significantly ($p < 0.01$) higher plasma GLP-1 levels than either the OA or vehicle treatments (Fig. 2A). Moreover, significantly higher plasma insulin levels were observed following treatment with α-LA than with either OA or vehicle (Fig. 2B). We also examined the plasma DPP-IV activity to determine whether the increased GLP-1 levels associated with α-LA treatment were due to the suppression of DPP-IV or not. We found no significant differences in plasma DPP-IV activity between α-LA, OA, and vehicle treatments (Fig. 2C).

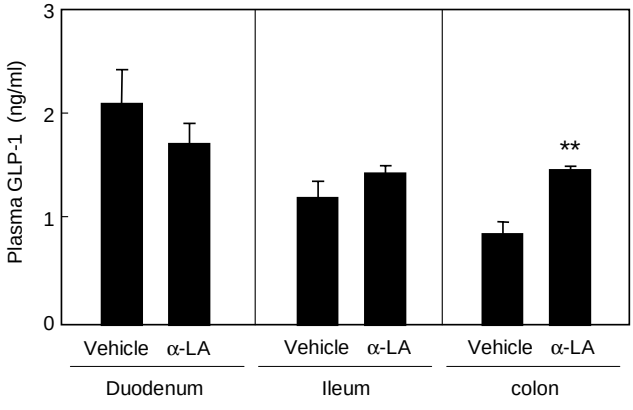

Fig. 1. Plasma GLP-1 levels 5 min after administration of α-LA or vehicle into the duodenum, ileum or colon using an intestinal cannulation method. Data are shown as means ± SEM ($n = 5$). $^{**}p < 0.01$ compared with vehicle administration.

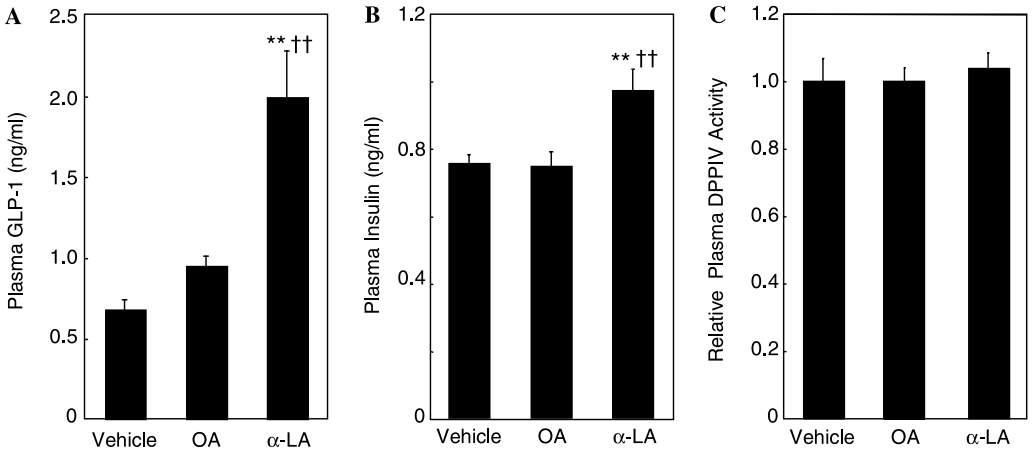

Fig. 2. Plasma GLP-1 (A) and insulin (B) levels, along with DPP-IV activity (C), 5 min after administration of α-LA, OA or vehicle via the colon using an intestinal cannulation method. Data are shown as means ± SEM ($n = 5$). $^{**}p < 0.01$ compared with vehicle administration. $^{\dagger\dagger}p < 0.01$ compared with OA administration.

Administration of α-LA enhances the ERK response in L-cells

We performed immunohistochemical analysis to demonstrate the effects of α-LA administration on cellular signaling within intestinal GLP-1-containing cells (L-cells). Fig. 3A shows immunostaining of the intestine 5 mins after administration of vehicle, OA, and α-LA, using anti-GLP-1 and anti-pERK antibodies. The ratio of anti-GLP-1 positive + anti-pERK positive cells/total anti-GLP-1 positive cells indicated that the population of anti-pERK positive cells had significantly ($p < 0.01$) increased following α-LA administration but not after either vehicle or OA administration (Fig. 3B).

Administration of α-LA enhances GLP-1 and insulin secretion, and lowers glucose level in normal C3H/He and diabetic NSY mice

As shown above, the administration of α-LA into the colon segment increased plasma GLP-1 levels in C57BL/6J mice. Furthermore, in order to determine the effect of colonic administration of α-LA on the glucose metabolism of diabetic mice, we administered α-LA into normal C3H/He and type 2 diabetic NSY mice after intraperitoneal glucose treatment, and then measured plasma GLP-1, insulin, and glucose levels. Plasma GLP-1 levels increased significantly ($p < 0.01$) higher following α-LA administration than after either vehicle or OA administration, in both normal and diabetic mice (Figs. 4A and D). In addition to the

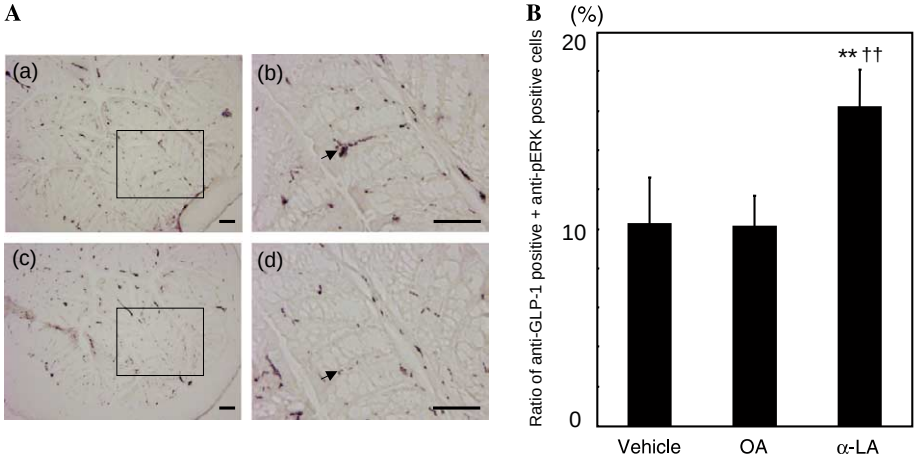

Fig. 3. Immunohistochemical analysis of colon specimens. (A) Immunostaining of serial sections taken from the colon 5 min after administration of α-LA, using anti-GLP-1 (a,b) and anti-pERK (c,d). (b,d) Magnifications of the square areas of (a,c), respectively. Arrows show anti-GLP-1 positive and anti-pERK positive cells. Bar: 100 μm. (B) The ratio of anti-GLP-1 positive + anti-pERK positive cells/total anti-GLP-1 positive cells. In a series of random fields from 10 regions, the number of total anti-GLP-1 positive cells and anti-GLP-1 positive + anti-pERK positive cells was counted. Data are shown as means ± SEM ($n = 5$). $^{**}p < 0.01$ compared with vehicle administration. $^{\dagger\dagger}p < 0.01$ compared with OA administration.

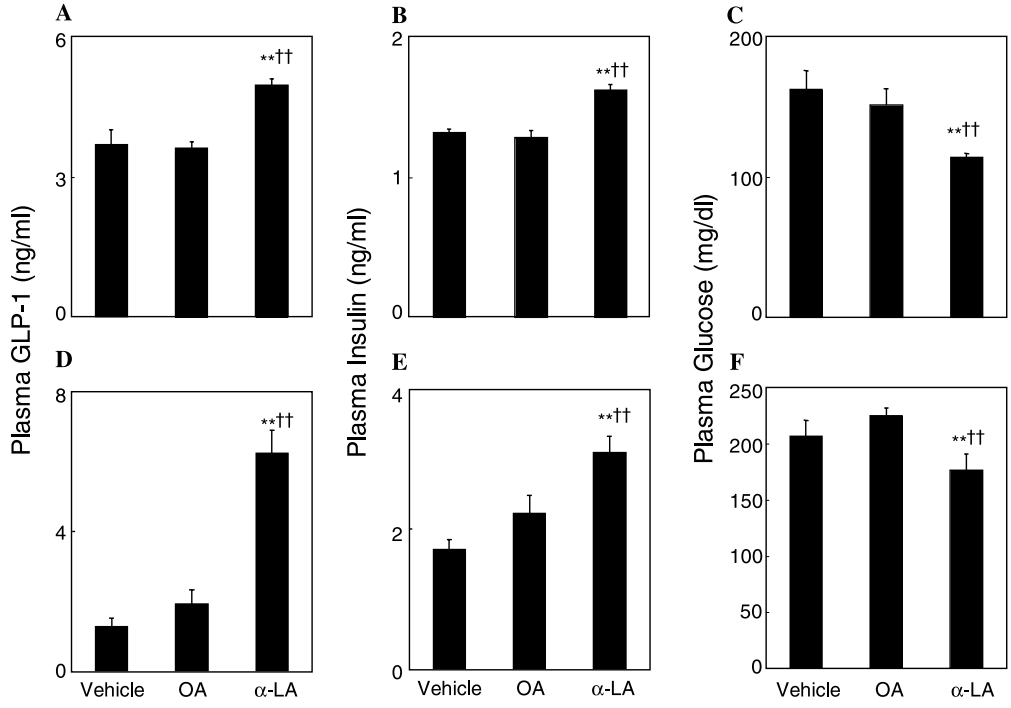

Fig. 4. Plasma GLP-1 (A,D), insulin (B,E), and glucose (C,F) levels of normal C3H/He (A–C) and diabetic NSY mice (D–F) 5 min after administration of α-LA, OA or vehicle into the colon, using an intestinal cannulation method. Data are shown as means ± SEM ($n = 5$), and data were expressed as mean levels 5 min subsequent to FFA treatment. $^{**}p < 0.01$ compared with vehicle administration. $^{††}p < 0.01$ compared with OA administration.

enhanced GLP-1 levels, greater increases in plasma insulin level were observed in these mice after α-LA administration than after either vehicle or OA administration (Figs. 4B and E). Moreover, plasma glucose level was reduced much more following α-LA administration than after the administration of either vehicle or OA (Figs. 4C and F).

Discussion

In the present study, we administered FFAs directly into the large intestine. We chose this approach because of the fact that oral administration of foods or chemicals, such as FFAs, stimulates the secretion of incretins and insulin [20,21]. Administration of α-LA directly into the colon, but not to the duodenum and ileum, induced a significant increase in circulating levels of GLP-1 (Fig. 1). We have previously reported that FFA receptor GPR120 is expressed predominantly in the colon segment of the intestine [16]. Our present results suggest that GLP-1 secretion in response to α-LA administration into the colon may be induced via GPR120.

In a recent in vitro study involving mouse enteroendocrine STC-1 cells, we showed that long-chain unsaturated FFAs, such as α-LA, promoted GLP-1 secretion [16]. In the present study, we extended these findings by administering OA, α-LA, and vehicle directly into the colon segment, and we monitored plasma GLP-1 levels and the activity of DPP-IV, which is known to be an enzyme involved in the degradation of GLP-1 [3–5] and whose inhibition promotes improvement in cases of diabetes mellitus [6,22]. Administration of α-LA resulted in increased plasma GLP-1 levels, whilst treatment with OA and vehicle did not (Fig. 2A). Moreover, α-LA administration resulted in increased plasma insulin levels (Fig. 2B). On the other hand, DPP-IV activity remained unchanged following administration of α-LA, OA or vehicle treatment (Fig. 2C). Since it has been reported that exogenously administered GLP-1 exerts a glucose-dependent insulinotropic effect on pancreatic β-cells [23], our results suggest that increased secretion of insulin may be due to the enhanced plasma GLP-1 levels after the administration of α-LA into the colon.

We previously reported that α-LA administration activates GPR120 and stimulates ERK transduction in STC-1 cells [16]. To determine whether administration of α-LA into the colon promotes ERK phosphorylation in GLP-1 positive cells, we immunostained the colonic segment of the intestine using an anti-pERK and anti-GLP-1 antibody. We found that the administration of α-LA via the colon markedly increases the population of pERK-positive cells within GLP-1-positive cells, while treatment with vehicle and OA treatment did not have such an effect (Figs. 3A and B). Similar to the observation in STC-1 cells, these results may indicate that the in vivo colonic α-LA administration activates ERK transduction probably via GPR120.

Post-prandial hyperglycemia in cases of impaired glucose tolerance and diabetes mellitus is closely associated with metabolic disorders, resulting in the induction of diabetic complications. The control of post-prandial plasma glucose levels, hence, is important in the improvement of

metabolic function. Using type 2 diabetic model NSY mice, we performed an intraperitoneal glucose tolerance test either with or without α-LA administration into the colon. Colonic administration of α-LA increased plasma GLP-1 and insulin levels, and decreased plasma glucose concentration in both diabetic NSY mice and normal C3H/He (Figs. 4A–F). Our results may suggest that colonic administration of α-LA improves glucose metabolism even in type 2 diabetic model NSY mice, which were previously reported to have an impaired insulin secretion response to high concentrations of glucose [18].

GLP-1 is rapidly released into the circulation after ingestion of a mixed meal [23–25]; thus, oral administration of nutrients produces a biphasic increase in plasma GLP-1, with an early peak within 15–20 min after nutrient ingestion, followed by a second peak in GLP-1 secretion approximately 1–2 h later [7,11,26]. As GLP-1 producing L-cells reside predominantly in the distal small intestine and colon [7,11,26], it is unlikely that the rapid increase in plasma GLP-1 is mediated by direct action of nutrients on intestinal L-cells, rather the existence of a proximal-distal loop that transmits nutrient-induced stimulatory signals via neural or endocrine effectors to the distal L cell has been proposed [11]. Our present study indicated that among the nutrients FFAs can be one of stimuli, as especially FFAs in the colon potently promote GLP-1 secretion. Also, we had previously observed that the oral administration of α-LA induces GLP-1 secretion 30 min after administration [16]. Taken together, we speculate that FFAs contained in food may contribute to the later phase, if not at all to the early phase, of GLP-1 secretion.

In summary, the present study for the first time shows that the in vivo colonic administration of α-LA promotes ERK transduction in L-cells, enhances secretion of GLP-1 and insulin, and thereby leads to a reduction in blood glucose levels even in diabetic animals.

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