A selective small molecule glucagon-like peptide-1 secretagogue acting via depolarization-coupled Ca\(^{2+}\) influx

Jun-ichi Eiki\(^1,2,*\), Kaori Saeki\(^1,*\), Norihiro Nagano\(^1\), Tomoharu Iino\(^1\), Mari Yonemoto\(^1\), Yoko Takayenokilino\(^1\), Satoru Ito\(^1\), Teruyuki Nishimura\(^1\), Yoshiyuki Sato\(^1\), Makoto Bamba\(^3\), Hitomi Watanabe\(^3\), Kaori Sasaki\(^1\), Sumika Ohyama\(^1\), Akio Kanatani\(^1\), Toshio Nagase\(^1\) and Toshihiko Yada\(^2\)

\(^1\)Tsukuba Research Institute, Banyu Pharmaceutical Co., Ltd, 3 Okubo, Tsukuba, Ibaraki 300-2611, Japan
\(^2\)Division of Integrative Physiology, Department of Physiology, Jichi Medical University, 3311-1 Yakushiji, Shimotsuke, Tochigi 329-0498, Japan

Abstract

Glucagon-like peptide-1 (GLP-1) is an incretin hormone that potentiates insulin secretion in a glucose-dependent manner. Selective GLP-1 secretagogue would be one of the potential therapeutic targets for type 2 diabetes. Here, we describe a newly identified small molecule compound (compound A) that stimulates secretion of GLP-1 in murine enteroendocrine cell lines, STC-1 and GLUTag cells, and in primary cultured fetal rat intestinal cells (FRIC). The underlying mechanism by which compound A stimulated GLP-1 secretion was also examined. Compound A stimulated GLP-1 secretion from STC-1 cells in a concentration-dependent manner, and also from GLUTag cells and FRIC. The action of compound A was selective against other tested endocrine functions such as secretion of insulin from rat islets, growth hormone from rat pituitary gland cells, and norepinephrine from rat PC-12 cells. In STC-1 cells, the compound A-stimulated GLP-1 secretion was neither due to cyclic AMP production nor to Ca\(^{2+}\) release from intracellular stores, but to extracellular Ca\(^{2+}\) influx. The response was inhibited by the presence of either L-type Ca\(^{2+}\) channel blockers or K\(^+\) ionophore. Perforated-patch clamp study revealed that compound A induces membrane depolarization. These results suggest that neither G\(_\alpha\)q- nor G\(_\alpha\)s-coupled signaling account for the mechanism of action, but depolarization-coupled Ca\(^{2+}\) influx from extracellular space is the primary cause for the GLP-1 secretion stimulated by compound A. Identifying a specific target molecule for compound A will reveal a selective regulatory pathway that leads to depolarization-mediated GLP-1 secretion.

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Introduction

Glucagon-like peptide-1 (GLP-1) is a hormone secreted from enteroendocrine L-cells, and is well recognized as an incretin that induces insulin secretion in a glucose-dependent manner (Drucker 2006). The functions of GLP-1 have been well studied. Thus, the GLP-1-based mechanism provides one of the promising therapeutic tools for the treatment of type 2 diabetes mellitus. Several approaches are being made to develop therapeutic agents that utilize GLP-1’s action; one is long-acting GLP-1 analogs and the other is inhibitors of dipeptidyl-peptidase 4 (DPP4) that cleaves active GLP-1 into inactive forms (Holst 2006). It is well known that several nutrients induce GLP-1 secretion in vitro and in vivo. Glucose is a well-established stimulus for GLP-1 secretion in animals and humans. Fatty acids also stimulate GLP-1 secretion in vitro (Rocca & Brubaker 1995, Brubaker et al. 1998) and in vivo (Elliott et al. 1993, Rocca & Brubaker 1999). Protein hydrolysates and peptone have also been reported to induce GLP-1 secretion (Cordier-Bussat et al. 1998, Dumoulin et al. 1998). However, the action mechanisms by which these nutrients stimulate GLP-1 secretion are not well understood yet.

Despite the distribution of L-cells in the distal gut, blood GLP-1 level is rapidly elevated in response to nutrients, suggesting that other endocrine and/or neuronal mechanisms are involved in the process of nutrient-stimulated GLP-1 secretion. Those include glucose-dependent insulinotropic peptide (GIP), a proximal gut hormone, and neurotransmitters such as gastrin-releasing peptide (GRP) and acetylcholine (Roberge & Brubaker 1993, Roberge et al. 1996, Anini et al. 2002, Anini & Brubaker 2003). GIP, GRP, and acetylcholine stimulate GLP-1 secretion via G-protein coupled receptors (GPCRs), indicating a crucial role of GPCR-linked signaling for GLP-1 secretion. However, the GPCR selective for the L-cell has not been identified yet. Therefore, revealing the L-cell-selective mechanism that leads to GLP-1 secretion would provide a novel therapeutic opportunity for treatment of type 2 diabetes mellitus.
We report here the discovery of a novel small molecule that induces GLP-1 secretion from cells that have a phenotype of enteroendocrine L-cells. Selectivity of the novel small-molecule compound for L-cells against other endocrine cell types was examined. The intracellular signaling pathway(s) through which this compound induces GLP-1 secretion was addressed in this report.

**Materials and Methods**

**Reagents**

Compound A, 3-(1-methylethyl) -9b-phenyl-[1,3]oxazolo[2,3-a]isoindole-2,5(3H,9bH)-dione, was synthesized in Tsukuba Research Institute, Banyu Pharmaceutical Co., Ltd, Ibaraki, Japan. Its chemical structure is shown in Fig. 1. Chemicals were purchased from Sigma or Wako Pure Chemicals (Osaka, Japan) unless otherwise stated.

**Cell culture and assay for GLP-1 secretion**

STC-1 cells were provided from Dr D Hanahan at University of California, San Francisco. The cells were grown in DMEM (Gibco) supplemented with 15% v/v horse serum (HS; Gibco), 2-5% v/v fetal bovine serum (FBS; Gibco), and 25 mmol/l glucose. Then, the cells were preincubated with glucose-free DMEM supplemented with 15% v/v HS, 2-5% v/v FBS, and 5-5 mmol/l glucose for 3 h. The medium was replaced with fresh DMEM that contains 0-1% w/v BSA (Fraction V) and 5-5 mmol/l glucose plus test agent. Medium was collected and subjected to determination of GLP-1 concentration.

GLUTag cells were provided from Dr D J Drucker at University of Toronto (Lee et al. 1992). The cells were grown in DMEM supplemented with 10% v/v FBS, and further incubated for 24 h with the same medium except for the glucose concentration at 5-5 mmol/l. Then, the cells were preincubated with serum-free DMEM supplemented with 5-5 mmol/l glucose for 2 h. The medium was replaced with fresh one that contains 0-1% w/v BSA plus test agent. Medium was collected and subjected to determination of GLP-1 concentration.

Primary culture of fetal rat intestinal cells (FRIC) was performed as described by Brubaker (1988). Pregnant Wistar rats (Charles River Laboratories, Yokohama, Japan) at 19 days of gestation were used. Dispersed intestinal cells were suspended in DMEM supplemented with 15% v/v HS, 1% v/v FBS, 50 IU/ml penicillin, and 50 μg/ml streptomycin. The cells were placed into collagen (type I)-coated culture plate, and cultured for 24 h. Then, the cells were washed twice with DMEM supplemented with 20 mmol/l HEPES, pH 7-4, 0-1% w/v BSA, and 2-8 mmol/l glucose, and incubated for 15 min with the same medium plus test agent. Then, the supernatant was subjected to determination of GLP-1 concentration.

RIA for GLP-1 was performed using rabbit anti-GLP-1 (7–36 amide) polyclonal antibody (GA1178; Affinity Research, Nottingham, UK) according to the manufacturer’s instruction. An EC50 value was defined as the concentration that gives half maximal response of hormone secretion.

**Assays for secretion of other hormones**

Preparation of rat islet of Langerhans was described previously (Okeda et al. 1979). Male Wistar rats (Charles River Laboratories; aged 8–9 weeks old) were used. The islet was transferred into culture dish supplemented with DMEM that contained 0-1% w/v BSA, 5-5 mmol/l glucose plus test agent, and incubated for 30 min at 37 °C. Gibenclamide was used as a positive control for the experiment to induce insulin secretion. The medium was subjected to insulin ELISA (Morinaga Seikagaku, Yokohama, Japan).

PC-12 cells were grown as described previously (Matsuzawa et al. 1996). The differentiated neuron-like cells were incubated with RPMI1640 (Gibco) supplemented with 10 mmol/l HEPES, pH 7-4, 1 mmol/l pyruvate, 10% v/v HS, 5% v/v FBS, and [3H]-norepinephrine (37 kBq/ml; NEN, Boston, MA, USA) for 4 h at 37 °C. The cells were washed thrice with RPMI 1640 medium that contained 0-1% w/v BSA, and incubated with the same medium plus test agent for 30 min at 37 °C. Carbachol was used as a positive control for norepinephrine secretion. Then, culture
supernatant was collected. Cells were treated with 1% w/v SDS solution to obtain cell extract. Both culture supernatant and cell extract were mixed with Clear-sol II (Nacalai Tesque, Kyoto, Japan), and the radioactivity was counted. The percentage of norepinephrine release was calculated by the following equation: secretion % = (radioactivity of supernatant (c.p.m.)/radioactivity of supernatant (c.p.m.) + radioactivity of cell extract (c.p.m.)) × 100.

Methods for primary culture of rat pituitary gland cells and growth hormone (GH) secretion assay were described previously (Cheng et al. 1989). A small molecule agonist for ghrelin receptor MK-677 (Merck Research Laboratories) was used as a positive control for the GH secretion.

Cytotoxicity assay
STC-1 cells were cultured as described earlier. Culture media after incubation with test agent was subjected to the measurement of lactate dehydrogenase (LDH), a stable cytosolic enzyme that is released upon cell damage. LDH activity in the culture media was determined by CytoTox 96 Non-Radioactive Cytotoxicity assay (Promega) with a standard LDH as a positive control (Promega).

Measurements of cytosolic-free Ca²⁺ concentration
STC-1 cells were seeded on the coverglass and cultured as described earlier. On the day of experiment, the cells were incubated with assay buffer (Hanks balanced salt solution (HBSS), pH 7·4, supplemented with 0·5 mmol/l CaCl₂, 5·6 mmol/l glucose, and 0·1% w/v BSA) containing 2 µmol/l of fura-2AM for 1 h at 37 °C. Then, the cells on the coverglass were washed with either assay buffer or calcium-free assay buffer (HBSS, pH 7·4, supplemented with 0·5 mmol/l EGTA, 5·6 mmol/l glucose, and 0·1% w/v BSA), placed on a cuvet filled with either assay buffer or calcium-free assay buffer, put into a fluorescence spectro-photometer CAF110 (JASCO, Tokyo, Japan), and incubated at 37 °C for a few minutes to stabilize the fluorescence. Test agent was added to the cuvet, and the fluorescence at 500 nm due to excitation at 340 nm and that due to excitation at 380 nm were recorded followed by calculation of the ratio of these two fluorescence. For the measurements of cytosolic-free Ca²⁺ concentration by the use of Fluorometric Imaging Plate Reader (FLIPR), STC-1 cells were seeded in poly-d-lysine-coated 96-well plates as described earlier. Two days after, medium was replaced with assay buffer (HBSS supplemented with 20 mmol/l HEPES, pH 7·4, and 0·5% w/v BSA) that contains 4 µmol/l Calcium Green-1-AM (Molecular Probes, Eugene, OR, USA), 0·04% v/v pluronic acid (Molecular Probes), and 1% v/v FBS, and incubated for 1 h at 37 °C. Then, the plate was washed thrice with assay buffer and put into FLIPR tetra apparatus (Molecular Devices, Sunnyvale, CA, USA). Test agent was added, and the fluorescence intensity detected at 515–575 nm with excitation at 470–495 nm was recorded.

Measurements of intracellular cAMP
To measure intracellular cAMP, cells were exposed to ice-cold 65% v/v ethanol/distilled-water solution for 1 h at 4 °C to extract cAMP from the cells. The ethanol solution was collected and dried up at 42 °C. The samples were subjected to determination of cAMP concentration using BIORAD cAMP EIA assay system (Amersham Pharmacia Biotech).

[^H]-glibenclamide binding assay
Preparation of plasma membrane fraction and binding assay was described previously (Nelson et al. 1992), except that 1 nmol/l of [^H]-glibenclamide (NET1024; NEN) was used and incubation was performed for 2 h at room temperature. Aliquots were filtered through Unifilter GF/C, and then washed thrice. Radioactivity was counted by TopCount (Packard Instruments, Meriden, CT, USA).

Perforated whole-cell patch clamp
STC-1 cells were seeded on the surface of coverglass. On the day of the experiment, culture medium was changed to DMEM containing 15% v/v HS, 2·5% v/v FBS, and 5·5 mmol/l glucose, and incubated for 3–5 h. Then the cells grown on the surface of coverglass were placed in to a bath filled with external solution containing (in mmol/l) 10 HEPES, pH 7·4, 137 NaCl, 5·9 KCl, 1·2 MgCl₂, 2·2 CaCl₂, and 14 glucose for 10–60 min. The bath was continuously perfused with the external solution. To make perforated-patch configuration, a glass electrode (pipette resistance: 2–5 MΩ) filled with internal solution containing (in mmol/l) 10 HEPES, pH 7·2, 140 KCl, 0·25 MgCl₂, and 200 µg/ml nystatin was attached to the cell. After establishing perforated-patch configuration, access resistance was dropped to a level sufficient to record membrane potential. Membrane potential of the cells was recorded under current clamp condition.

Statistical analysis
Data were expressed by mean ± S.E.M. Statistical significance was examined by ANOVA and by Student's t-test. A P value of < 0·05 was considered statistically significant.

Result
GLP-1 secretion
A mouse cell line, STC-1, is known to have a phenotype of intestinal endocrine L-cells that secrete GLP-1 (Rindi et al. 1990). Screening of in-house compound library for stimulation of GLP-1 secretion from STC–1 cells yielded compound A. This compound stimulated GLP-1 secretion
Figure 2 Effects of compound A and ionomycin on GLP-1 secretion (A) and lactate dehydrogenase (LDH) release (B) into culture media in STC-1 cells. STC-1 cells were incubated with vehicle (0.05% v/v DMSO), compound A at 3 μmol/l, or ionomycin at 20 μmol/l for 60 min at 37 °C. Then, GLP-1 concentration and LDH activity in culture media were determined. Values are mean ± S.E.M., n = 3–6. **Significantly (P < 0.01) different from vehicle control group.

in a concentration-dependent manner with an EC_{50} value of 180 nmol/l (Fig. 1A). Analysis of time profiles showed that the compound A-induced GLP-1 secretion reached nearly maximal levels within 10 min of administration (Fig. 1B). Next, the effect of compound A was examined in another mouse enteroendocrine cell line, GLUTag cells. Under the condition that GRP stimulates GLP-1 secretion in the cells, compound A at 100 nmol/l also induced GLP-1 secretion (Fig. 1C). Furthermore, we assessed the ability of compound A to induce GLP-1 secretion in primary culture of FRIC prepared from rat embryo (E-18). As shown in Fig. 1D, compound A at 100 nmol/l stimulated GLP-1 secretion from the FRIC. Thus, compound A induced GLP-1 secretion from both established mouse cell lines and primary cultured rat intestinal cells. The results also suggest that a similar mechanism is conserved in both mouse and rat species.

### Cytotoxicity

In order to address potential for cytotoxicity of compound A, we employed LDH assay in culture media of the STC-1 cells. LDH is a cytosolic enzyme and known to be released from the damaged cells. Both compound A at 3 μmol/l (the highest concentration in Fig. 1A) and ionomycin, a calcium ionophore, at 20 μmol/l demonstrated significant GLP-1 secretion at 60 min after incubation in the STC-1 cells (Fig. 2A). Under the same conditions, ionomycin but not compound A treatment significantly induced LDH release from the STC-1 cells (Fig. 2B). The result indicated that the compound A-induced GLP-1 secretion does not result from cytotoxicity, but is mediated by a certain physiological mechanism.

### Selectivity of compound’s action against other endocrine cell types

To study whether the ability of compound A to stimulate hormone secretion is selective to GLP-1-secreting cells, we examined the effects of compound A on insulin secretion from isolated rat islets of Langerhans, on norepinephrine secretion from rat pheochromocytoma cell line, PC-12, and on GH secretion from primary cultured rat pituitary gland cells. Glibenclamide, carbachol, or a small molecule agonist for ghrelin receptor MK-0677 (Howard et al. 1996) induced respective hormone secretion in the corresponding cell type by more than threefold. Under these conditions, compound A had no effect on hormone secretions in those preparations even at 1 μmol/l, the concentration that demonstrated maximal GLP-1 secretion in STC-1 cells (Fig. 1A and Table 1). From the data, the action of compound A was at least selective to GLP-1 secretion over other tested endocrine cell types.

### Intracellular signaling

Several GPCR ligands are reportedly involved in the GLP-1 release from L-cells, which include GIP, GRP, and acetylcholine (Roberge & Brubaker 1993, Roberge et al. 1996, Anini et al. 2002, Anini & Brubaker 2003). Most recently, several new GPCRs exemplified as GPR119, GPR120, and TGR5 have been shown to regulate GLP-1 secretion (Hirasawa et al. 2005, Katsuma et al. 2005, Chu et al. 2008). These findings clearly indicate that the mechanism mediated by GPCRs plays an important role in the regulation of GLP-1 secretion. We therefore tried to identify the mechanism that accounts for the compound A-induced GLP-1 secretion in STC-1 cells, a representative enteroendocrine cell line. Compound A at 300 nmol/l, which gives near a maximal response in the GLP-1 secretion experiments in the STC-1 cell line, had no effect on hormone secretions in those preparations even at 1 μmol/l, the concentration that demonstrated maximal GLP-1 secretion from the FRIC (Howard et al. 1996, Chu et al. 2008). These findings clearly indicate that the mechanism mediated by GPCRs plays an important role in the regulation of GLP-1 secretion. We therefore tried to identify the mechanism that accounts for the compound A-induced GLP-1 secretion in STC-1 cells, a representative enteroendocrine cell line. Compound A at 300 nmol/l, which gives near a maximal response in the GLP-1 secretion experiments in the STC-1 cell line, had no effect on hormone secretions in those preparations even at 1 μmol/l, the concentration that demonstrated maximal GLP-1 secretion from the FRIC (Howard et al. 1996, Chu et al. 2008).

### Table 1 Effect of compound A on insulin secretion from rat islets, norepinephrine secretion from PC-12 cells, and GH secretion from rat pituitary gland cells.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Insulin secretion (ng/islet-60/min)</th>
<th>[3H]-norepinephrine secretion (% secretion-30/min)</th>
<th>GH secretion (ng/ml-15/min)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>0.31 ± 0.069</td>
<td>3.8 ± 0.18</td>
<td>31 ± 4.6</td>
</tr>
<tr>
<td>Compound A at 1 μmol/l</td>
<td>0.21 ± 0.044</td>
<td>4.1 ± 0.17</td>
<td>39 ± 5.8</td>
</tr>
<tr>
<td>Glibenclamide at 100 nmol/l</td>
<td>0.99 ± 0.067*</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Carbachol at 5 mmol/l</td>
<td>−</td>
<td>22 ± 0.12*</td>
<td>−</td>
</tr>
<tr>
<td>MK-0677 at 10 mmol/l</td>
<td>−</td>
<td>≥ 200*</td>
<td>−</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., n = 4–5 (rat islets), n = 3 (PC-12 cells) and n = 5 (rat pituitary gland cells).

*Significantly (P < 0.01) different from respective vehicle control group; *Some of the samples exceeded upper limit of quantification (= 200 ng/ml-15/min).
Compound A increased cytosolic-free Ca\(^{2+}\) agonistic action on G\(_{\text{STC-1}}\) cells, suggesting that compound A does not have an effect on cAMP concentrations, while forskolin elevated it in the absence of extracellular Ca\(^{2+}\). Compound A-mediated GLP-1 secretion was examined to determine the potential involvement of GPCR mechanisms in GLP-1 secretion. GRP at 100 nmol/l or compound A at 300 nmol/l in STC-1 cells. Values are mean ± S.E.M., n=3. (B–E) Effects of compound A and gastrin-releasing peptide (GRP) on [Ca\(^{2+}\)]\(_i\) in STC-1 cells in the presence (B and C) or absence (D and E) of extracellular Ca\(^{2+}\) at 0.5 mmol/l. Each arrow indicates application of either GRP at 100 nmol/l or compound A at 300 nmol/l. **Significantly (P<0.01) different from vehicle control group.

Since compound A induced Ca\(^{2+}\) influx, upstream mechanisms including Ca\(^{2+}\) channels are potentially involved. We therefore examined the effects of several agents that affect ion fluxes. Treatment with high KCl induced GLP-1 secretion from STC-1 cells, and the response was inhibited by L-type Ca\(^{2+}\) channel blockers, verapamil, and diltiazem (Fig. 4A). The data indicate that voltage-gated Ca\(^{2+}\) channels are involved in the GLP-1 secretion. GLP-1 secretion by compound A was also inhibited by verapamil and diltiazem (Fig. 4A), strongly suggesting that a membrane depolarization-coupled opening of voltage-gated Ca\(^{2+}\) channels accounts for the GLP-1 secretion by compound A. Since K\(^+\) ion flux is one of the mechanisms to maintain membrane potential, the effect of a K\(^+\) ionophore, valinomycin, on compound A-induced [Ca\(^{2+}\)]\(_i\) increases was examined. As shown in Fig. 4B, compound A-induced elevation of [Ca\(^{2+}\)]\(_i\) was abolished by pretreatment with valinomycin. Taken together, compound A may not directly open voltage-gated Ca\(^{2+}\) channels, but modulate K\(^+\) permeability that secondarily influences Ca\(^{2+}\) influx.

ATP-sensitive potassium (K\(_{\text{ATP}}\)) channels play a key role in insulin secretion from pancreatic β-cells. The STC-1 and GLUTag cells reportedly also express K\(_{\text{ATP}}\) channels (Mangel et al. 1994, Reimann & Gribble 2002). We confirmed a specific binding of [\(^3\)H]-glibenclamide in the plasma membrane fraction of STC-1 cells. The IC\(_{50}\) value for glibenclamide was 0.55 nmol/l, indicative of the presence of high-affinity sulfonylurea receptor, sulfonylurea receptor-1 in the cells (Aguilar-Bryan et al. 1995). However, compound A did not inhibit [\(^3\)H]-glibenclamide binding to the STC-1 membrane fraction even at 50 μmol/l (data not shown). Together with our finding that compound A did not induce insulin secretion from rat islets, K\(_{\text{ATP}}\) channels do not account for the mechanism of action of compound A in GLP-1-secreting cells.
membrane potential to of spontaneous action potentials and depolarized resting Compound A at 300 nmol/l initially increased the frequency exposure to test agents. mide at 10 membrane depolarization even in the presence of glibenclamide other hand, compound A at 300 nmol/l was able to induce cause membrane depolarization in the cells (Fig. 5B). On the affinity glibenclamide binding sites, but the perforated-patch described earlier, we found that the STC-1 cells have high- compound A showed reversal potential of reversible manner. In the voltage-ramp experiments, compound A induced membrane depolarization in a Fig. 5A). The effect of compound A disappeared upon removal from bathing solution. The data clearly indicate that compound A induced membrane depolarization in a reversible manner. In the voltage-ramp experiments, compound A showed reversal potential of \( \sim 0 \, \text{mV} \). As described earlier, we found that the STC-1 cells have high-affinity glibenclamide binding sites, but the perforated-patch clamp study showed that glibenclamide at 10 \( \text{mM} \) did not cause membrane depolarization in the cells (Fig. 5B). On the other hand, compound A at 300 \( \text{nmol/l} \) was able to induce membrane depolarization even in the presence of glibenclamide at 10 \( \text{mM} \) in the STC-1 cells (Fig. 5B), supporting the fact that compound A depolarizes the cells via mechanisms not involving \( \text{K}_{\text{ATP}} \) channels. In separate experiments, we confirmed that glibenclamide at 10 \( \text{mM} \), a concentration sufficient to occupy glibenclamide binding sites, had no effect on GLP-1 secretion in STC-1 cells (data not shown). The data support that \( \text{K}_{\text{ATP}} \) channel is not involved in the excitation–secretion pathway mediated by compound A in STC-1 cells.

Discussion

We report here a discovery of novel small molecule that induces GLP-1 secretion from cells having a phenotype of enteroendocrine L-cells. A lack of hormone release from other endocrine cell types by compound A suggests that the underlying mechanism is unique to GLP-1-secreting cell type. Compound A induced membrane depolarization–coupled opening of voltage-gated \( \text{Ca}^{2+} \) channels that leads to GLP-1 secretion from STC-1 cells. Current results can rule out the involvement of \( \text{G}_{\alpha q} \)-coupled receptors and \( \text{K}_{\text{ATP}} \) channels as a target of compound A. We may also exclude the potential of \( \text{G}_{\alpha q} \)-coupled receptors due to no \( \text{[Ca}^{2+}]_{\text{i}} \) induction in the absence of extracellular \( \text{Ca}^{2+} \). Although the mechanism by which compound A depolarizes membrane is yet to be identified, a novel mechanism should be involved in the GLP-1 secretion.

It is reported that STC–1 cells secrete not only GLP-1 but also other enteroendocrine hormones such as peptide YY, cholecystokinin, GIP, and secretin. Thus, STC–1 cells do not conserve intact L-cell’s nature, but have mixed enteroendocrine phenotypes. In addition, FRIC is mixed culture of proximal/distal gut cells, and therefore could show multiple enteroendocrine functions. Thus, there is a potential that compound A induces secretion of not only GLP-1 but also other enteroendocrine hormones from those cells. However, since compound A fails to induce hormone secretion from islets, pituitary, and PC–12 cells, the action of compound A is at least selective to enteroendocrine cell types over other tested endocrine cell types. Further exploration of action mechanisms of compound A will reveal whether or not the action of compound A is selective for GLP–1 secretion.

Recently, the GLP–1-based therapies, peptidase resistant GLP–1 analogs, and DPP4 inhibitors, have been available in clinic for treatment of type 2 diabetes. Selective GLP–1 secretagogue will become a third approach for GLP–1-based therapies. Particularly, GLP–1 secretagogue is expected to produce a greater effect when combined with DPP4 inhibitors. Thus, further understanding of the stimulus–secretion coupling in L-cells would bring a new approach for treatment of type 2 diabetes and possibly obesity. Compound A may also provide a useful research tool for investigating the physiology of enteroendocrine L-cells.

Declaration of interest

Following authors are employees of Banyu Pharmaceutical Co., Ltd: Jun-ichi Eiki; Kaori Sasaki; Noriharu Nagano; Tomoharu Iino; Mari Yonemoto; Yoko Takayenoki-Iino; Satoru Ito; Teruyuki Nishimura; Yoshityuki Sato; Makoto Bamba; Hitomi Watanabe; Kaori Sasaki; Sumika Ohyama; Akio Kanatani; and Yoshio Nagase. All authors do not have any conflict of interest.

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