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Insulin-Releasing Properties of a Series of Cinnamic Acid Derivatives *in Vitro* and *in Vivo*

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Cinnamic acid derivatives are naturally occurring substances found in fruits, vegetables, and flowers and are consumed as dietary phenolic compounds. In the present study, cinnamic acid and its derivatives were evaluated for insulin secreting activity in perfused rat pancreas and pancreatic β-cells (INS-1) as well as an increase in $[Ca^{2+}]_i$ *in vitro*. The presence of *m*-hydroxy or *p*-methoxy residues on cinnamic acid was a significantly important substituent as an effective insulin releasing agent. The introduction of *p*-hydroxy and *m*-methoxy-substituted groups in cinnamic acid structure (ferulic acid) displayed the most potent insulin secreting agent among those of cinnamic acid derivatives. In particular, the stimulatory insulin secreting activities of test compounds were associated with a rise of $[Ca^{2+}]_i$ in INS-1. In perfused rat pancreas, *m*-hydroxycinnamic acid, *p*-methoxycinnamic acid, and ferulic acid (100 μM) significantly stimulated insulin secretion during 10 min of administration. The onset time of insulin secretion of those compounds was less than 1 min and reached its peak at 4 min that was about 2.8-, 3.3-, and 3.4-fold of the baseline level, respectively. Intravenous administration of *p*-methoxycinnamic acid and ferulic acid (5 mg/kg) significantly decreased plasma glucose and increased insulin concentration in normal rats and maintained its level for 15 min until the end of experiment. Meanwhile, *m*-hydroxycinnamic acid induced a significant lowering of plasma glucose after 6 min, but the effects were transient with plasma glucose concentration, rapidly returning to basal levels. Our findings suggested that *p*-methoxycinnamic acid and ferulic acid may be beneficial for the treatment of diabetes mellitus because they regulated blood glucose level by stimulating insulin secretion from pancreatic β-cells.

KEYWORDS: Cinnamic acid derivatives; *p*-methoxycinnamic acid; ferulic acid; *m*-hydroxycinnamic acid; insulin secretion

INTRODUCTION

Insulin is a primary hormone that regulates glucose homeostasis either by stimulating peripheral glucose uptake or by suppressing hepatic glucose production (*1*). An insufficient insulin secretion or loss of insulin action at target tissues causes impaired glucose and lipid metabolism, resulting in diabetes mellitus. Sulfonylureas are the compounds that directly stimulate insulin secretion from pancreatic β-cells (*2*). Currently, they are used to treat patients with type 2 diabetes. One of the adverse effects of using sulfonylurea-based drugs is that it can produce hypoglycemia and also generate secondary failure of insulin secretion (*3, 4*), which may be due to β-cell exhaustion resulting from overstimulation. Dietary intake from plant food and their ingredients could be a more effective strategy for the management of diabetes mellitus because of the likelihood of high compliance and because it is largely free from side effects. Hence, searching new chemical compounds from natural products have been explored for possibly safer antidiabetic agents.

Phenolic compounds have been of considerable interest to consumers and food manufacturers for several reasons. They are often characteristic of a plant species or even of a particular organ or tissue of that plant (*5, 6*). These useful compounds are widely distributed in the plant kingdom and

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are present in considerable amounts in fruits, vegetables, and beverages in the human diet (*7*). Recent reports have documented that the consumption of phenolic compounds is associated with the prevention and reduced risk of several degenerative diseases such as atherosclerosis, cardiovascular disease, and cancer (*8*). Cinnamic acid and its derivatives, also known as phenolic compounds, comprises one of the largest and most ubiquitous groups of plant metabolites with many structures already identified.

Cinnamic acid and its derivatives possess a variety of pharmacologic properties including hepatoprotective (*9*), antimalarial (*10*), antioxidant (*11*), and antityrosinase activities (*12*). Interestingly, they have been focused on their potential benefits in significant antihyperglycemic activities. For example, isoferulic acid (3-hydroxy-4-methoxycinnamic acid) exhibits significantly decreased levels of plasma glucose concentration in streptozotocin (STZ)-induced diabetic rats by suppression hepatic gluconeogenesis (*13*). Ferulic acid (4-hydro-3-methoxycinnamic acid), an isomer of isoferulic acid, has been reported to effectively suppress blood glucose levels in diabetic mice (*14*).

Moreover, the administration of ferulic acid also helps to enhance the antioxidant capacity of diabetic mice by reducing free radical formation, increasing activities of antioxidative enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (*15*). In addition, *p*-hydroxycinnamic acid markedly reduces plasma cholesterol and hepatic lipids in high cholesterol fed rats (*16*). This action promotes the great benefits to diabetic patients who are at the risk of developing chronic complications related to cerebrovascular, cardiovascular, and peripheral vascular diseases. Our earlier investigation revealed that the administration of *p*-methoxycinnamic acid markedly improved glucose metabolism in diabetic rats by increasing the activity of glycolytic enzymes and inhibiting the activity of gluconeogenic enzymes (*17*).

To the best of our knowledge, there have been many studies demonstrating the antidiabetic activity of cinnamic acid derivatives; however, the insulinotropic activity is yet to be identified. Therefore, it would be interesting to examine the structure−activity relationships of cinnamic acid and its derivatives as insulin secretagogues. To investigate this activity, the present study was divided into three parts. First, we examined the effect of cinnamic acid and its derivatives on insulin secretion and intracellular calcium $[Ca^{2+}]_i$ in the pancreatic β-cell line (INS-1). Second, the most effective compounds were elucidated for insulin-secreting activity by using pancreatic perfusion. Finally, the hypoglycemic effect of those compounds was performed in normal rats.

MATERIALS AND METHODS

Chemicals. *o*-Hydroxycinnamic acid, *m*-hydroxycinnamic acid, and *p*-hydroxycinnamic acid were purchased from Fluka (St. Louis, MO, USA.). *o*-Methoxycinnamic acid, *m*-methoxycinnamic acid, and *p*-methoxycinnamic acid were purchased from ACROS (Pittsburgh, PA, USA). Ferulic acid (3-methoxy-4-hydroxycinnamic acid) and isoferulic acid (4-methoxy-3-hydroxycinnamic acid) were purchased from Chromadex (Laguna Hills, CA, USA). Cinnamic acid, glucose oxidase commercial kit, and MTT (C,*N*-diphenyl-*N*′-4,5 dimethyl thiazol 2-yl tetrazolium bromide) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Pentobarbital sodium was purchased from Sanofi-Ceva (Bangkok, Thailand). The insulin radioimmunoassay (RIA) kit was purchased from Diagnostic Products Corporation (Los Angeles, CA, USA). Fura-2 acetoxymethylester (Fura-2AM) was purchased from Molecular Probes (Eugene, OR, USA). All other chemical reagents used in this study were of analytical grade.

Animals. Male Wistar rats were obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Thailand. Animal facilities and protocol were approved by the Laboratory Animal Care and Use Committee at Faculty of Veterinary Science, Chulalongkorn University, Thailand. Wistar rats were housed in individual stainless steel cages in a room maintained at 25 ± 1 °C on a 12:12-h light-dark cycle. They were fed standard laboratory chow with water ad libitum and fasted overnight before the experiments.

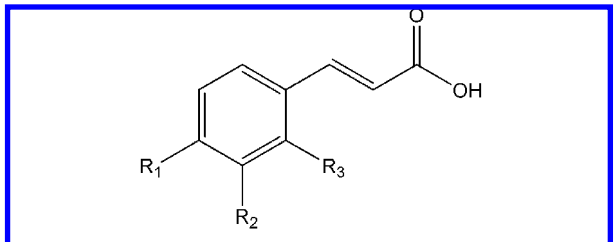
Cell Culture. INS-1 cells, an insulin-secreting cell line derived from rat pancreatic β-cells, were cultured in RPMI 1640 medium containing 11 mM glucose and supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, and 50 μM 2-mercaptoethanol in an atmosphere of 5% CO_2 in air at 37 °C.

Static Incubation for Insulin Secretion. INS-1 cells were plated onto 96-well plates at a density of 1×10^4 cells per well and grown for 48 h. For static secretion studies, cells were preincubated for 15 min in Krebs Ringer bicarbonate buffer (KRB) containing 4 mM glucose and 0.1% bovine serum albumin (BSA). Cells were then incubated for 30 min in KRB containing test compounds (100 μM). After incubation, the buffer was kept at 4 °C and subsequently assayed for insulin using radioimmunoassay (RIA). Test agents were dissolved in dimethyl sulfoxide (DMSO) to obtain desired concentrations. The final concentration of DMSO was 0.1%. After incubation with or without test compounds, cell viability was measured using C,*N*-diphenyl-*N*′-4,5 dimethyl thiazol 2-yl tetrazolium bromide (MTT) assay as described previously (*18*). Cells were washed twice with phosphate buffer solution (PBS) and further incubated with serum free RPMI medium containing 0.25 mg/mL MTT solution for 120 min at 37 °C. Isopropanol−HCl (200 μL) solution was added to dissolve intracellular purple formazan, and absorbance was read at 570 nm with a reference wavelength of 630 nm using a microplate reader.

Measurement of $[Ca^{2+}]_i$ in Pancreatic B-Cell Line. INS-1 cells were grown in culture flasks for 3 days until 80−90% confluency had been reached. Thereafter, the cells were harvested by treatment with trypsin/EDTA and prepared for experiments. The $[Ca^{2+}]_i$ was determined using fura-2 dye as described previously (*19*). Cells were loaded with 2 μM fura-2AM in KRB containing 4 mM glucose for 30 min at 37 °C. The loaded cells were centrifuged at 300*g* for 2 min and resuspended with KRB. Measurement was performed in custom-made 35-mm culture dishes on the stage of an inverted fluorescence microscope (Carl Zeiss). Baseline of $[Ca^{2+}]_i$ was run for 60 s, then the various concentrations of test compounds (100 μM) were added to the cells. The 340/380nm fluorescence ratios were monitored using Attofluor Digital Fluorescence Imaging System (Atto Instruments, Rockville, MD, USA). Calibration was performed according to the procedure provided by Attofluor, using fura-2 penta K^+ as a standard.

In Situ Pancreatic Perfusion. Male Wistar rats (350−450 g) were fasted overnight before experiments with 4 rats in each group. The rats were anesthetized with pentobarbital sodium (60 mg/kg, intraperitoneally) and were maintained at 37 °C on a hot plate during the experiment. In situ pancreatic perfusion experiments were performed as previously described (*20*). Briefly, after cannulation of the celiac artery, the rat pancreas was immediately perfused with oxygenated (95% O_2−5% CO_2) KRB solution supplemented with 20 mM HEPES, 5.5 mM glucose, 1% dextran, and 0.2% BSA at pH 7.4 as the basal medium. The perfusion rate was maintained at 1 mL/min, and the effluent was collected from the cannulated portal vein. After the baseline period of 10 min, the perfusate containing test compounds (100 μM) was administered for 10 min followed by a washout period with the basal medium for 10 min. The perfusate containing glucose (10 mM) was administered as a positive control for 10 min at the end of the experiments. The effluent fractions were kept at 4 °C and subsequently assayed for insulin using RIA kit.

Measurement of Plasma Glucose and Insulin Levels. The fed rats (200−250 g) were separated into several groups containing 6 animals. All animals were anaesthetized with sodium pentobarbital 60 mg/kg intraperitoneally. Fifteen minutes later, a catheter was inserted into the jugular vein for test compounds or saline administration, and a second short catheter was introduced into the femoral vein to obtain blood samples. After 5 min of equilibration, during which the metabolic changes induced by anesthesia and surgical stress vanished (*21*), a blood



Compound	R_1	R_2	R_3	Chemical name
1	H	H	H	Cinnamic acid
2	H	H	OH	*o*-hydroxycinnamic acid
3	II	OII	II	*m*-hydroxycinnamic acid
4	OH	H	H	*p*-hydroxycinnamic acid
5	H	H	OCH_3	*o*-methoxycinnamic acid
6	II	$OCII_3$	II	*m*-methoxycinnamic acid
7	OCH_3	H	H	*p*-methoxycinnamic acid
8	OII	$OCII_3$	II	Ferulic acid
9	OCH_3	OH	H	Isoferulic acid

Figure 1. Structure of cinnamic acid (*1*) and its cinnamic acid derivatives (*2–9*).

sample (250 μL) was withdrawn from the femoral vein to measure plasma glucose and insulin concentrations for the baseline level. Test compounds were then injected, and the blood sample was collected before and 3, 6, 10, 15, and 20 min after test compound administration. Heparin-containing blood samples were immediately centrifuged (2,500*g*), and the plasma was separated and frozen at − 20 °C until analyzed for glucose and insulin concentrations. The plasma glucose concentrations were determined using the glucose oxidase method with the absorbance at the wavelength of 450 nm of a spectrophotometer (Sunrise, Austria). The plasma insulin concentrations were determined using RIA kits.

Statistical Analysis. Data are expressed as the means ± SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA). The least significant difference test was used for mean comparisons and $P < 0.05$ was considered to be statistically significant.

RESULTS

Effect of Cinnamic Acid and Its Derivatives on Insulin Secretion in INS-1. Nine compounds of cinnamic acid and its derivatives (**Figure 1**) were investigated for insulin secreting activity in INS-1 cells. The result in **Figure 2A** shows the percentage insulin secretion of test agents at a concentration of 100 μM. Cinnamic acid (*1*) was found to be inactive. Upon the introduction of a hydroxy group to cinnamic acid, it was found that the insulin secreting activity increased in the order of **3** > **4** > **2**. Replacement of the hydroxy residue substituted in the cinnamic acid by a methoxy residue markedly improved the activity of insulin secretion, except when the substituent was at the meta-position. The insulin-secreting activity of the methoxy residue in cinnamic acid was **7** > **6** = **5**. Meanwhile, the induction of *meta*-methoxy and *para*-hydroxy residues on cinnamic acid and ferulic acid (*8*) significantly increased insulin secretion about 2.0-fold, resulting in ferulic acid being the most effective insulin-secreting agent among the cinnamic acid derivatives. In contrast, isoferulic acid (*9*), an isomer of ferulic acid, had no effect on insulin secretion. Glibenclamide (Gli) was used as a positive control in this experiment, and it stimulated insulin secretion about 1.65-fold. Cytotoxicity testing by MTT assay demonstrated that test compounds (100 μM) did not show any effect on cell viability of INS-1 cells, suggesting that they were not toxic to the pancreatic β-cell lines (data not shown).

Effect of Cinnamic Acid and Its Derivatives on $[Ca^{2+}]_i$ in INS-1. Since Ca^{2+} is an important intracellular signal, and it mediates the effect of many insulin secretagogues, we next

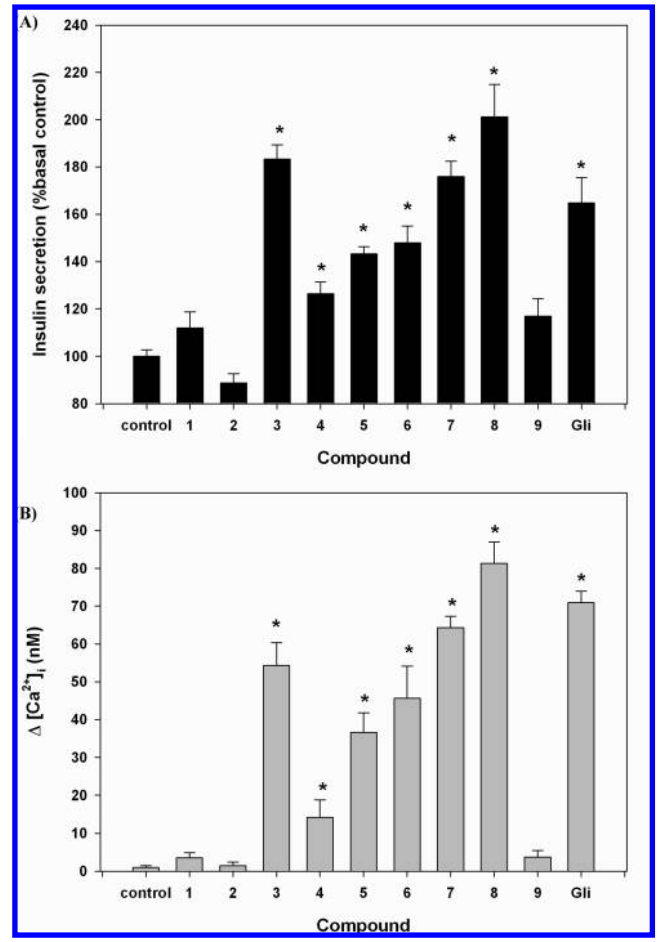


Figure 2. (**A**) Effects of compounds (*1–9*) and glibenclamide (Gli) on insulin secretion in INS-1 cells. The insulin concentration in the control group was 13.92 ± 0.38 ng/well/30 min. Data were expressed as the mean ± SE; $n = 3$ independent experiments with quadruplicates in each experiment. (**B**) Effects of compounds (*1–9*) and glibenclamide (Gli) on $\Delta[Ca^{2+}]_i$ increase in INS-1 cells. Data shown are representative of 4 independent experiments with 20 cells/experiment. *$P < 0.05$ compared with control.

studied the effect of cinnamic acid and its derivatives on $[Ca^{2+}]_i$ in INS-1 cells. The results of these experiments are shown in **Figure 2B**. Compounds **3**−**8** significantly increased $[Ca^{2+}]_i$ in INS-1 cells. The order of these compounds on $[Ca^{2+}]_i$ increase was consistent with those obtained from insulin secretion in INS-1 cells.

Dose-Dependent Effect of Compounds 3, 7, and 8 in INS-1 Cells. As the results mentioned above, **3**, **7**, and **8** were effective promoters of insulin secretion. Thus, we further examined whether these compounds could stimulate insulin secretion in a dose-dependent manner in INS-1 cells. The result in **Figure 3** shows the dose-dependent effects of **3**, **7**, and **8**-stimulated insulin secretion at various concentrations in INS-1 cells. Compound **8** (1−100 μM) significantly stimulated insulin secretion in a concentration-dependent manner. In addition, **3** and **7** (100 μM) also significantly increased insulin secretion, whereas 10 μM of these compounds did not exert any significant effects on stimulatory insulin secretion.

Effect of Compounds 3, 7, and 8 in Perfused Rat Pancreas. The results in **Figure 4** show the profile of **3**, **7**, and **8** on insulin secretion from perfused rat pancreas. DMSO, which was used as a solvent of test compounds, did not affect the basal insulin concentrations when compared to those in the group perfused with KRB alone (**Figure 4A**). Compounds **3**, **7**, and **8** (100 μM)

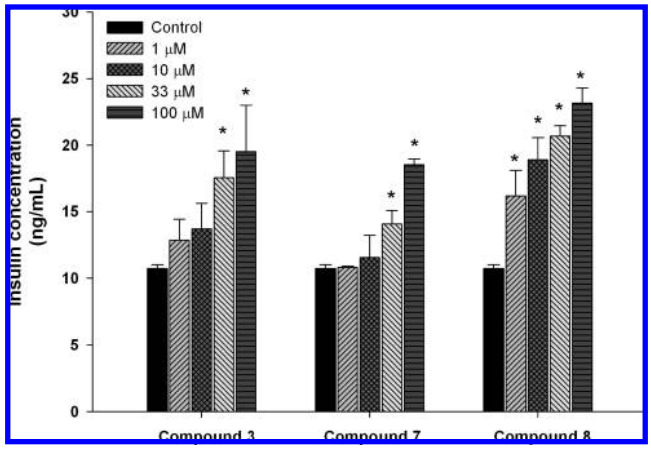


Figure 3. Dose dependent effects of compound **3**, **7**, and **8** on insulin secretion in INS-1 cells. Data were expressed as the mean ± SE; $n = 3$ independent experiments with quadruplicates in each experiment. *$P < 0.05$ compared with control.

significantly increased insulin secretion during 10 min of administration (**Figure 4B−D**). The onset time of insulin secretion stimulated by these compounds was less than 1 min and reached the peak at 4 min that was about 2.8-, 3.3-, and 3.4-fold of the baseline level, respectively. The effluent insulin concentration returned to the baseline during 10-min of the washout period, and increased to 5-fold of the baseline level upon administration of 10 mM glucose as a positive control at the end of experiment.

Intravenous Administration of Compounds 3, 7, and 8 in Normal Rats. The effects of **3**, **7**, and **8** on fasting plasma glucose concentrations in normal rats are shown in **Table 1**. Intravenous administration of **7** and **8** (5 mg/kg) to normal rats produced a marked plasma glucose lowering effect within 6 min of injection and was maintained for 15 min. Compound **3** significantly demonstrated plasma glucose lowering effect at 6 min and returned to basal plasma glucose concentration within 10 min.

The effects of **3**, **7**, and **8** on fasting plasma insulin concentrations in normal rats are shown in **Table 2**. A significant increase in plasma insulin concentrations in normal rats treated with **7** and **8** were observed at 6 min after single administration, which exhibited the maximal increase of plasma insulin concentrations about 1.37- and 1.49-fold of the baseline level, respectively, and maintained its level for 15 min during the experiment. Also, **3** increased insulin secretion at 6 min after administration, and markedly increased plasma insulin secretion about 1.20-fold over the basal level and returned to baseline within 10 min after administration.

DISCUSSION

This is the first study to investigate the structure−activity relationships of cinnamic acid and its derivatives on insulin secretion. Our findings indicated that cinnamic acid had no insulin-releasing activity. When a comparison of insulin-releasing activities between hydroxy and methoxy residues on cinnamic acid was investigated, it revealed that the presence of a methoxy substituent at the para-position was more active than that of the hydroxy substituent. However, the presence of a *meta*-methoxy residue on cinnamic acid was dramatically less potent than that of the hydroxy residue. The above findings indicated that the introduction of *meta*-hydroxy or *para*-methoxy groups on cinnamic acid structure were likely to enhance an increase in insulin secretion. We hypothesized whether the presence of both *meta*-hydroxy and *para*-methoxy residues on cinnamic acid may play a key role in exhibiting the most potent insulinotropic agent.

To confirm this hypothesis, isoferulic acid and its isomer, ferulic acid, were examined for their activities. It appeared that isoferulic acid was found to be inactive, whereas ferulic acid exhibited the most effective agent among the cinnamic acid derivatives. This is surprising given the differences from the hypothesis that cinnamic acid containing *meta*-methoxy and *para*-hydroxy residues plays an important role in the augmentation of insulinotropic activity. One explanation of such findings is that using X-ray crystallography and computer modeling to evaluate the binding activity is needed for further investigation.

Moreover, previous reports demonstrated that isoferulic acid, mainly found in the rhizoma of *Cimicifuga dahurica*, decreased plasma glucose in STZ-diabetic rats by enhancing glucose uptake and suppressing hepatic gluconeogenesis (*22*). Liu et al. proposed a potential mechanism responsible for antihyperglycemic activity that isoferulic acid activates α_1-adrenoceptors to enhance the secretion of β-endorphin, which stimulates the opioid μ-receptors to increase glucose utilization and/or reduce hepatic gluconeogenesis (*23*). One of the most unique mechanisms of controlling blood glucose is the release of insulin hormone from pancreatic β-cells that activates specific insulin receptors, which leads to increased glucose uptake in muscle and adipose tissues (*24*). The present study clearly supports the results from Liu's findings that the mechanism by which isoferulic acid exerts its antihyperglycemic effect is only activation of α_1-adrenoceptors without any effect on the stimulation of insulin secretion from pancreatic β-cells.

In $[Ca^{2+}]_i$ experiments, the results suggested that compounds **3**−**8**-stimulated insulin secretion was associated with a rise of $[Ca^{2+}]_i$. In general, closure of the ATP-sensitive K^+ channels (K_{ATP} channels) in the β-cells evokes membrane depolarization and subsequently activates and opens the voltage-dependent Ca^{2+} channels (VDCCs), which increases in $[Ca^{2+}]_i$, then stimulates insulin release from the vesicles (*25*). Interestingly, it was found that *p*-methoxycinnamic acid stimulated insulin secretion from pancreatic β-cells by increasing Ca^{2+} influx via the L-type Ca^{2+} channels, but not through the closure of ATP-sensitive K^+ channels and also may increase cyclic AMP content by inhibiting phosphodiesterase (*26*).

According to the results from the static incubation, the insulinotropic activity of *m*-hydroxycinnamic acid, *p*-methoxycinnamic acid, and ferulic acid was next confirmed by *in situ* pancreatic perfusion, which provides evidence of a direct pancreatic action and excellent support of their effects on normal β-cells. Our findings suggest that these compounds directly stimulated insulin secretion from normal pancreatic β-cells. From animal models, *m*-hydroxycinnamic acid was a rapid onset and short duration of action, suggesting that it would not be a useful agent for controlling postprandial hyperglycemia in diabetic patients. Meanwhile, *p*-methoxycinnamic acid and ferulic acid were also of rapid onset action, but produced the more prolonged action that maintained the lowering plasma glucose by increasing plasma insulin concentrations until the end of the experiment.

As discussed previously, Woo studied the rate of decline of serum *p*-methoxycinnamic acid concentration in rabbits following a single dose of sodium *p*-methoxycinnamic acid by intravenous administration (*27*). The results revealed that it disappeared very rapidly from serum with a half-life 0.4 h when injected intravenously. In the meantime, oral administration of *p*-methoxycinnamic acid salt form to rabbit was rapidly absorbed

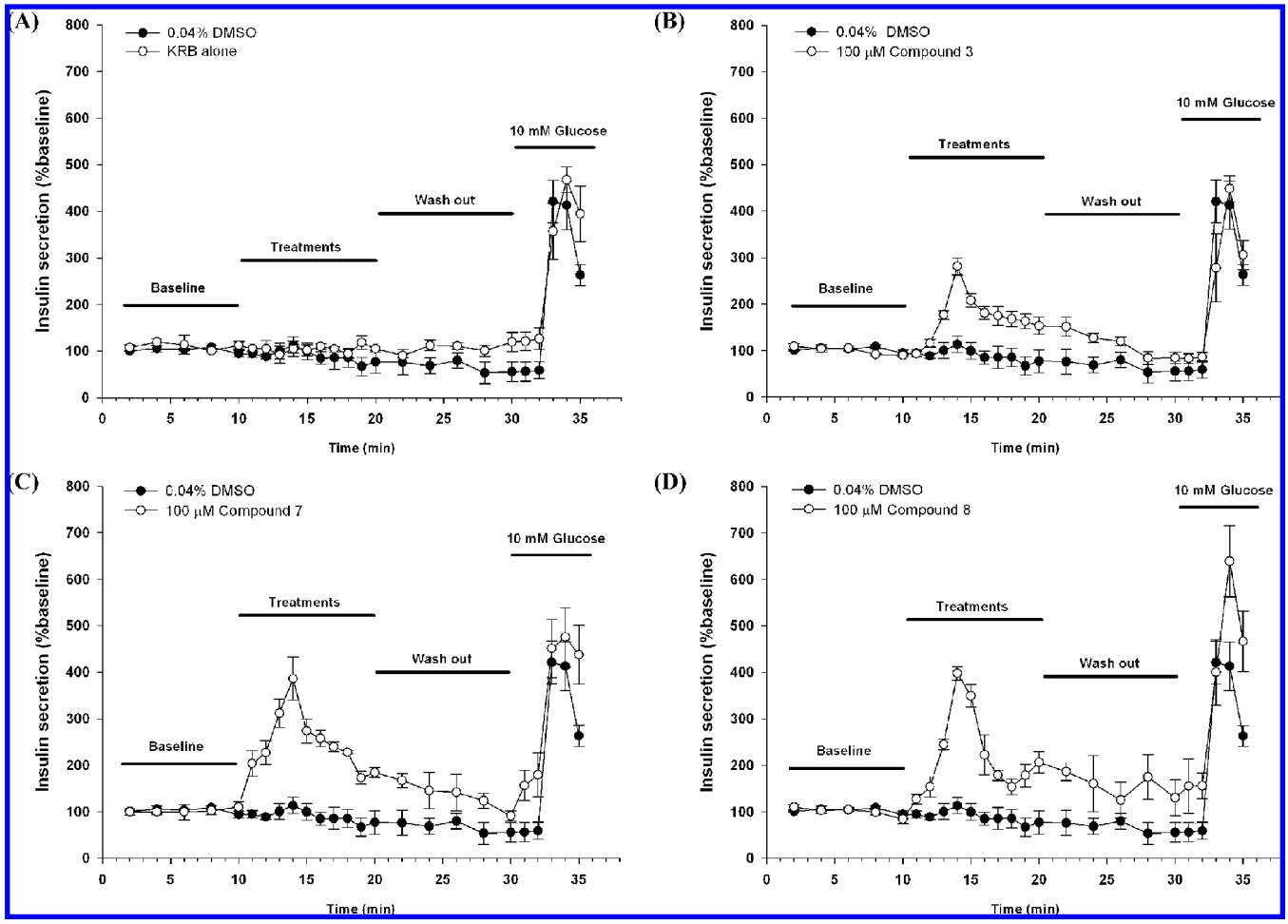


Figure 4. (**A**) Effects of DMSO (0.04%), (**B**) compound **3**, (**C**) compound **7**, and (**D**) compound **8** (100 μM) on insulin secretion in perfused rat pancreata. In these experiments, a 20-min equilibration period preceded time 0. Test compounds were administered for 10 min. Data were expressed as the mean ± SE; $n = 4$. Range of baseline insulin concentrations of effluents was 1.80−5.13 ng/mL.

Table 1. Effects of Compound **3**, **7**, and **8** on Plasma Glucose Concentration in Normal Rats[a]

	plasma glucose (mg/dL)						
groups	− 5 min	0 min	3 min	6 min	10 min	15 min	20 min
control	92.9 ± 4.2	98.8 ± 1.7	94.9 ± 4.8	97.3 ± 4.6	105.3 ± 2.9	112.2 ± 4.2	99.9 ± 3.0
compound **3** (5 mg/kg)	87.1 ± 6.4	90.9 ± 6.6	94.9 ± 11.9	77.1 ± 5.8*	97.3 ± 9.2	107.1 ± 12.0	111.9 ± 12.5
compound **7** (5 mg/kg)	91.3 ± 6.1	98.3 ± 8.7	102.4 ± 115	78.0 ± 2.7*	87.5 ± 7.1*	85.9 ± 6.8*	87.3 ± 9.7
compound **8** (5 mg/kg)	91.2 ± 7.2	95.3 ± 2.1	107.9 ± 9.1	80.9 ± 3.2*	75.6 ± 5.1*	87.9 ± 6.3*	90.9 ± 6.6

[a] Results were expressed as the means ± S.E.M.; $n = 6$. *$P < 0.05$ compared with control.

Table 2. Effects of Compound **3**, **7**, and **8** on Plasma Insulin Concentration in Normal Rats[a]

	plasma insulin (pg/mL)						
groups	− 5 min	0 min	3 min	6 min	10 min	15 min	20 min
control	602.5 ± 10.9	603.2 ± 25.4	577.7 ± 29.2	643.0 ± 22.3	659.2 ± 67.6	690.2 ± 31.2	642.8 ± 34.0
compound **3** (5 mg/kg)	561.4 ± 30.1	613.4 ± 37.9	689.2 ± 31.9	774.3 ± 38.0*	665.8 ± 15.7	620.5 ± 54.2	630.2 ± 36.9
compound **7** (5 mg/kg)	539.4 ± 31.6	617.3 ± 14.9	723.3 ± 75.0	883.0 ± 90.9*	800.4 ± 43.1*	840.6 ± 94.5*	780.0 ± 93.3
compound **8** (5 mg/kg)	507.5 ± 35.7	545.3 ± 19.1	664.5 ± 37.3	960.0 ± 74.9*	793.8 ± 70.0*	806.5± 81.4*	710.8 ± 54.8

[a] Results were expressed as the means ± S.E.M.; $n = 6$. *$P < 0.05$ compared with control.

into blood stream, and blood levels peaked within 1 h, and the apparent half-life was more than 2 times that administered intravenously. Our previous studies reported that oral administration of *p*-methoxycinnamic acid exhibited decreased fasting plasma glucose concentrations and increased fasting plasma insulin concentrations at doses of 40 mg/kg or higher in both normal and diabetic rats after 30 min of administration without causing serious hypoglycemia (*28*). In addition, it also enhanced the glucose-induced increase in plasma insulin concentrations in diabetic rats after 30 min of glucose administration. Furthermore, the daily administration of *p*-methoxycinnamic acid suppresses the activity of hepatic gluconeogenic enzyme, glucose-6-phosphatase, and increases the activities of three glycolytic enzymes, hexokinase, glucokinase, and phosphofructokinase, in the liver of diabetic rats (*17*). We suggest that *p*-methoxycinnamic acid was rapidly absorbed, had a short

apparent half-life, and improved glucose tolerance without hypoglycemia, which may be beneficial to patients with diabetes mellitus who have defects in the response of insulin secretion to glucose stimulation.

Ferulic acid is commonly found in fruits and vegetables such as potato (*29*), sweet corn (*30*), and rice bran (*31*). The daily intake of consumers with a regular intake of cereal products can be up to 100 mg of ferulic acid (*32*). To date, studies showed that ferulic acid is absorbed from the stomach with high bioavailability and transported into the portal vein. It is mainly metabolized in the liver and is found in the free form of ferulic acid and its conjugated forms, ferulic acid sulfate and glucuronides, in both plasma and urine (*33*). Zhao et al. (*33*) reported on the bioavailability of ferulic acid in rats, that it was detected that the proportion of free ferulic acid in the portal vein was about 49%. However, it declined to 6.2% after blood reached the arteries.

Recent studies have reported that oral administration of ferulic acid (50 mg/kg) or the ethyl acetate fraction of rice bran (200 mg/kg) significantly decreases blood glucose levels and increases plasma insulin levels in type 2 diabetic mice by elevating hepatic glycogen synthesis and glucokinase activity (*33*). From phytochemical analysis, ethyl acetate fraction of rice bran contains a large quantity of phenolic acids (526 mg%), of which ferulic acid is the most abundant (303 mg%). Moreover, it has been reported that oral administration of rice bran (20 g/day for 8-week period) decreases the levels of glycosylated hemoglobin, fasting glucose, and increases serum insulin concentration in type 2 diabetic patients (*34*). It is possible that an intake of rice bran-enriched ferulic acid decreases plasma glucose and increases plasma insulin concentrations in both diabetic rats and patients because of its insulin-releasing activity. Further comprehensive pharmacological investigations of ferulic acid are required to evaluate its toxicity and clinical efficacy for potential application in the prevention and treatment of diabetes mellitus.

With the overall results of the present study, we have demonstrated here that *p*-methoxycinnamic acid and ferulic acid appear to potentially stimulate insulin secretion from pancreatic β-cells. From this point of view, dietary intake of these cinnamic acid derivative-enriched products is a feasible therapeutic strategy for the prevention and treatment of patients with type 2 diabetes mellitus.

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