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Research article

The flavonoid Sudachitin regulates glucose metabolism via PDE inhibition

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ABSTRACT

Sudachitin, a member of the flavonoid family, reportedly improves glucose metabolism after long-term administration, but details of the underlying mechanisms are unknown. We found that Sudachitin approximately doubles insulin secretion under high glucose concentrations in mouse pancreatic islets and MIN6 cells. When Sudachitin was orally administered to mice, early-phase insulin secretion was increased and a 30 % reduction in blood glucose levels was demonstrated 30 min after glucose loading. Insulin tolerance tests also showed Sudachitin to increase systemic insulin sensitivity. Additionally, we observed that Sudachitin raised intracellular cAMP levels in pancreatic islets. Phosphodiesterase (PDE) activity assays revealed Sudachitin to inhibit PDE activity and computer simulations predicted a high binding affinity between PDEs and Sudachitin. These findings suggest that Sudachitin enhances both insulin secretion and insulin sensitivity via an increase in intracellular cAMP resulting from PDE inhibition. These insights may facilitate understanding the mechanisms underlying the regulation of glucose metabolism by Sudachitin and other isoflavones.

1. Introduction

Diabetes mellitus (DM) markedly reduces healthy life expectancy and has major adverse impact on the global economy in terms of both treatment costs and workforce shortages. The prevalence of DM continues to rise globally, underscoring the pressing need for proactive prevention measures. In recent years, dietary flavonoids have attracted attention as potential treatments for preventing and managing diabetes. Numerous studies have demonstrated that dietary flavonoids enhance glucose uptake into skeletal muscle by inducing glucose transporter type 4 (GLUT4) translocation, inhibiting hepatic glycogenesis, and suppressing glucose absorption in the small intestine via AMPK activation, observations suggesting that multiple mechanisms contribute to glucose metabolism improvement [1–5].

Insulin is secreted by beta cells and is considered to be an important metabolic hormone, essential for glucose homeostasis [6].

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Insulin circulates in the blood and acts on skeletal myocytes and adipocytes to promote glucose uptake via membrane translocation of insulin-sensitive glucose transporter 4 (GLUT4) and stimulates fuel storage in the liver, fat and skeletal muscle [7]. Glucose-stimulated biphasic insulin secretion involves at least two signaling pathways, a KATP channel-dependent and a KATP channel-independent pathways. In the former, increased glucose metabolism increases the intracellular adenosine triphosphate/adenosine diphosphate (ATP/ADP) ratio, causing KATP channels to close and cells to depolarize. Activation of voltage-gated Ca channels increases Ca entry and stimulates insulin release [8]. In the KATP channel-independent pathway, insulin secretion is activated by Gs-coupled receptors such as GLP-1 to promote cAMP formation and amplify secretion via Epac2 and protein kinase A (PKA). Phosphodiesterases (PDEs) are enzymes that promote cAMP degradation and are also directly involved in the processes by which cAMP is degraded. Inhibition of PDEs in β-cells promotes insulin secretion, and indeed, drugs for treating diabetes that potentiate KATP channel-independent pathways, such as dipeptidyl peptidase-4 (DPP-4) inhibitors and glucagon-like peptide-1 (GLP-1) receptor agonists, are already widely used in clinical practice [9].

Dietary polyphenols, encompassing various compounds such as phenolic acids, flavonoids, and catechins, are abundant in a wide array of foods, including grains, fruits, and beverages like tea and wine. These polyphenols have garnered significant interest due to their potential health benefits, particularly in combating oxidative stress-related diseases like cancer, cardiovascular diseases, and diabetes. Epidemiological and clinical evidence suggests that regular consumption of polyphenol-rich diets may offer protection against chronic diseases by reducing oxidative cellular damage and inflammation. Furthermore, flavonoids, a subgroup of dietary polyphenols, have emerged as potential agents in preventing and managing diabetes by enhancing glucose metabolism through multiple mechanisms, including the induction of glucose transporter translocation and activation of AMPK [10–14].

Sudachitin, extracted from the peel of the Sudachi (Citrus sudachi) fruit, is a flavonoid characterized as a methoxyflavone with hydroxyl groups at positions 5, 7, and 4′ on the flavonoid skeleton, as well as methoxy groups at positions 6, 8, and 3'. Long-term administration of Sudachitin reportedly improves insulin sensitivity by increasing Sirt1 and PGC-1α expressions and also mitochondrial biogenesis in skeletal muscle [15]. Nobiletin, a methoxyflavone similar to Sudachitin, was reported to exert biological effects such as anti-inflammatory, antitumor, and neuroprotective effects [16–18]. Nobiletin was also reported to improve insulin resistance in obese diabetic mice [19], and similar effects are expected with Sudachitin. However, the short-term effects of Sudachitin and the associated underlying mechanisms have yet to be fully elucidated. Herein, we investigated the effects of a single administration of Sudachitin on glucose metabolism in C57BL/6J mice and MIN6 cells. Our findings revealed Sudachitin to enhance insulin secretion and increase systemic insulin sensitivity. Interestingly, we also discovered that Sudachitin inhibits PDEs, thereby possibly contributing to the mechanism underlying improved glucose tolerance.

2. Materials and methods

2.1. Cell culture and islet culture

MIN6 cells were kindly provided by Dr. J. Miyazaki (Osaka University, Japan). The MIN6 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 25 mM glucose supplemented with 15 % fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C in a humidified atmosphere (5 % CO2). The MIN6 cells used had undergone 15 to 25 cell passages. Islets of Langerhans were isolated from C57BL/6J mice by ductal perfusion employing collagenase. The intact islets were handpicked and then maintained in RPMI medium supplemented with 10 % fetal calf serum, 100 U/ml of penicillin, and 100 mg/ml of streptomycin in humidified 5 % CO2 and 95 % air at 37 °C. All experiments on isolated islets were performed overnight (15 h) after isolation.

2.2. Animals

The Ethics of Animal Experimentation Committee at Yamaguchi University approved all experimental protocols involving mice (No. 25-022). The animals were housed in a temperature-controlled (22 °C ± 1 °C) room under a 12-h light and 12-h dark cycle. The mice were placed in clean cages and given ad libitum access to water and food. All of the experiments were performed employing 9- to 18-week-old C57BL/6J male mice.

2.3. Assays of insulin secretion from MIN6 cells and isolated islets

MIN6 cells were pre-incubated for 60 min at 37 °C in Krebs-Ringer Bicarbonate-HEPES (KRBH) containing 0.5 % bovine serum albumin (BSA) and 2.8 mM glucose. After pre-incubation, the MIN6 cells were incubated for 60 min at 37 °C in KRB buffer with addition of the specified stimulators containing either 2.8 mM or 20 mM glucose. After incubation, the secreted insulin was collected from the supernatant. The insulin including the remaining solution was then extracted by applying acid ethanol to determine the islet insulin content in each experiment. Pancreatic islets were obtained from C57BL/6J male mice at 8 or 10 weeks of age by collagenase digestion, as previously described in detail [20]. In brief, islets were cultured overnight after isolation and then pre-incubated for 60 min at 37 °C in KRBH buffer containing 0.5 % BSA and 2.8 mM glucose prior to all experiments. After pre-incubation, five size-matched islets were incubated for 60 min at 37 °C in KRBH containing either 2.8 mM or 20 mM glucose supplemented with/without each concentration of Sudachitin (Cat: 198–17741, FUJIFILM Wako, Osaka, Japan) in either the presence or the absence of MAY0132 (Sigma-Aldrich, St. Louis, USA), a selective inhibitor of the cAMP-binding protein EPAC2 subtype2. For the control treatment, DMSO was used at a concentration of 0.1 %, which was the highest concentration used under the present experimental conditions. The secreted insulin was collected from the supernatant. The insulin including the remaining solution was then extracted with acid ethanol

to determine islet insulin content in each of the experiments. Insulin was quantified using an ultra-sensitive mouse insulin ELISA kit (MORINAGA, Tokyo, Japan).

2.4. Oral glucose tolerance test (OGTT)

OGTTs were performed on 12-week-old male mice fasted for 16 h. Glucose was administered by gavage (2 g/kg). Wild-type mice (n = 20) were divided into two groups (n = 10/group), one treated with Sudachitin and a control group receiving DMSO alone (FUJIFILM Wako, Osaka, Japan). Sudachitin and DMSO were diluted in D-phosphate buffered saline (−) to 10 mg/kg and administered orally to the designated groups. Control DMSO was used at the same concentration and volume as that in which Sudachitin was dissolved. Briefly, 10 min after Sudachitin or DMSO administration, an oral glucose (2 g/kg) load was given to each mouse. Tail blood samples were collected at 0 (before glucose loading), 15, and 30 min, and blood glucose levels at different timepoints were determined employing ANTSENCE II (Horiba, Kyoto, Japan). Plasma insulin levels were measured with the aforementioned ultra-sensitive mouse insulin ELISA kit.

2.5. Assessment of Acute insulin secretion following Sudachitin administration

Wild-type mice at 15–18 weeks of age (n = 22) were fasted overnight (16 h) before treatment with Sudachitin or DMSO. They were then divided into two groups (n = 11/group): one given Sudachitin (10 mg/kg) dissolved in DMSO while the other group received the same volume as the control DMSO solution. Sudachitin was administered slowly over 1 min, with 0 min after administration. After 3 min, blood was collected from the tail veins of the mice. Blood was drawn from their tail veins over a period of 2 min. The entire process from the start of administration until the completion of blood collection took 6 min. Tail blood samples were collected at 0 (just prior to administration of Sudachitin or DMSO) and again at 3 min. Plasma insulin levels were measured with the ultra-sensitive mouse insulin ELISA kit.

2.6. Insulin tolerance tests (ITTs)

Male mice at 9–12 weeks of age were randomly selected for an ITT, in which insulin (0.75 units/kg) was injected intraperitoneally after a 3-h fast. Sudachitin (10 mg/kg) or DMSO (10 mg/kg) was administered orally immediately before the insulin injection. Blood glucose concentrations were determined at 0, 15, 30, 60, 90, and 120 min using ANTSENCE II.

2.7. Intracellular cAMP assay

Fifty Islets per group were pre-incubated for 45 min at 37 °C in 2.8 mM glucose KRBH buffer containing 0.5 % BSA. After this pre-incubation, the islets were incubated in KRB buffer supplemented with 20 mM glucose for 15 min in either the absence or the presence of Sudachitin (30 μM or 100 μM) or forskolin (10 μM), with the latter serving as a positive control. After incubation, the culture medium was removed, and the cells were washed with KRBH. The pancreatic islet pellet obtained was then adjusted to a volume of 200 μl in cell lysis buffer and subjected to ultrasonication. The cAMP concentration was measured employing a Cyclic AMP select ELISA Kit (Cayman Chemical, Ann Arbor, USA). The intracellular cAMP contents of the islets were calculated based on the measured concentration and the volume.

2.8. PDE activity assay

The PDE inhibitory activity of Sudachitin was determined employing the Spectrofluorimetric quantitation of Fluorescein-AMP (GMP)-IMAP method developed by Eurofins Panlabs Discovery Services (Taiwan). 100 nM FAM-cAMP (GMP) served as the substrate and each isoform of PDE was an active human recombinant expressed in baculovirus-infected insect cells. Enzyme solutions were pre-incubated with Sudachitin at 25 °C for 15 min, followed by the addition of GMP to initiate the reaction during another 30-min incubation at the same temperature, and terminated by the addition of IMAP binding solution. Fluorescence signals indicative of GMP degradation were quantified using a fluorescence plate reader. IC50 values were based on data from five concentrations (1, 3, 10, 30, and 100 μM).

2.9. Molecular docking

Molecular docking was conducted using AutoDock Vina to elucidate the mechanism of binding between PDEs and Sudachitin. Milrinone, Amrinone, Rolipram, and IBMX, which reportedly bind to specific PDEs, were used as positive control ligands. The structural information pertaining to the ligands including Sudachitin was obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/) by downloading the SDF file corresponding to each ligand, followed by conversion to a PDB file using Avogadro [21]. The crystal structure of PDEs was downloaded from the Protein Data Bank (PDB) (https://www.rcsb.org) database. For those PDEs registered as complexes with a ligand, the ligand was removed using UCSF Chimera. MGL AutoDock Tools were used to prepare the protein, including removing crystal water and adding polar hydrogen atoms. The PDB-structured proteins were transformed into the pdbqt format using AutoDock. Docking of the ligand and PDBs was achieved by employing AutoDock Vina's standard settings [22,23]. Visualization of docking was performed by PyMOL (The PyMOL Molecular Graphics System, Version 2.5.8, Schrödinger, LLC.). The

Biovia Discovery Studio was also applied to illustrate ligand-protein interactions [24–33].

2.10. Statistical analyses

Results are expressed as means ± SD. Statistical analyses were performed using Welch's *t*-test to compare experimental groups with the control group. One-way ANOVA was applied for comparisons among three or more groups to establish whether there were differences between any two or more of them. Two-way ANOVA was used for comparing the means of three or more independent groups that had been separated according to two variables. For PDE activity analysis, non-linear least squares regression analysis using MathIQTM (ID Business Solutions Ltd., UK) was applied to the data. A p-value <0.05 was considered to indicate a statistically significant difference, and the levels of significance were set at $p < 0.05$, indicated by *, and $p < 0.01$, indicated by **.

3. Results

3.1. Sudachitin enhances insulin secretion in isolated pancreatic islets and β cells

To analyze the impact of Sudachitin on insulin secretion, we performed an insulin secretion assay using MIN6 cells under 20 mM glucose conditions. A 2.4-fold ($p < 0.01$) and a 3.7-fold ($p < 0.01$) increase were observed with 30 μM Sudachitin and 100 μM Sudachitin, respectively, suggesting a Sudachitin concentration-dependent enhancement of insulin secretion in the presence of 20 mM glucose (Fig. 1A). On the other hand, no enhancement of insulin secretion with Sudachitin was observed at the 2.8 mM glucose concentration. Islets isolated from C57BL/6J mice were subjected to glucose-stimulated insulin secretion. In isolated islets, insulin secretion rose approximately 1.6-fold in response to 10 μM Sudachitin under 20 mM glucose conditions but this change did not reach statistical significance ($p > 0.05$) as compared to the control group. In contrast, a 3.5-fold enhancement ($p < 0.01$) was observed with 30 μM Sudachitin, indicating a concentration-dependent effect similar to that observed in MIN6 cells. At the 2.8 mM glucose concentration, Sudachitin treatment did not increase insulin secretion (Fig. 1B). These results suggest that Sudachitin is involved in the amplification pathway of glucose-dependent insulin secretion.

3.2. Sudachitin improves glucose tolerance in mice

To assess the effect of Sudachitin on systemic glucose metabolism in vivo, an OGTT was performed after a single oral administration of Sudachitin to C57BL/6J mice. Sudachitin reduced blood glucose levels at 15 and 30 min after glucose loading (Fig. 2A). Insulin concentrations measured during the OGTT were significantly lower in the Sudachitin-treated than in the control group at both 15 and 30 min (Fig. 2B). In contrast, insulin secretion 3 min after Sudachitin was approximately 2.0-fold higher in the Sudachitin-treated than in the control group ($p < 0.05$) (Fig. 2C). To further assess the effect of Sudachitin on glucose metabolism in vivo, ITTs were performed to identify whether insulin sensitivity was impacted. The ITTs demonstrated blood glucose levels to be significantly lower in Sudachitin-treated than in control group mice, suggesting that the former also improves insulin sensitivity (Fig. 2D). Taken together, these results indicate that Sudachitin may improve glucose tolerance in vivo via multiple mechanisms based on both insulin secretion and insulin sensitivity.

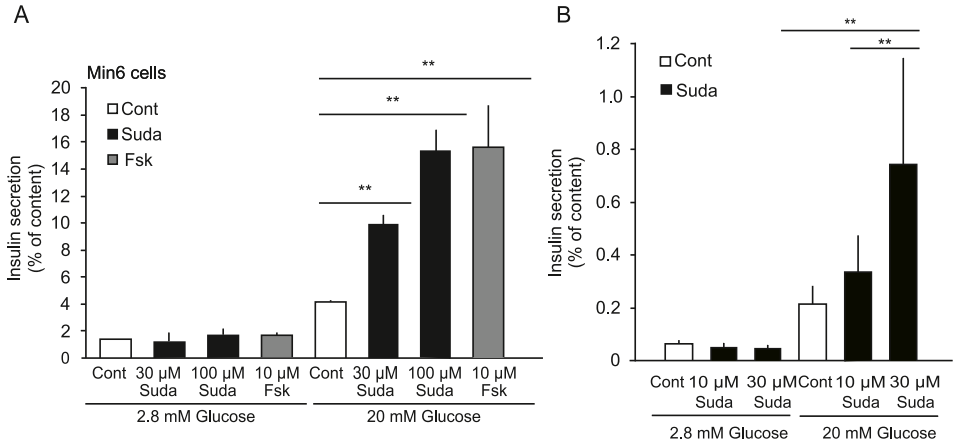

Fig. 1. Sudachitin enhanced insulin secretion in vitro at high glucose concentrations (A) MIN6 cells were incubated for 60 min with 2.8 mM or 20 mM supplemental glucose with or without Sudachitin or forskolin at the indicated concentrations. The experiments were performed 48 h after 300,000 MIN6 cells had been seeded per well onto a 24-well plate (n = 3). (B) Five size-matched mouse islets were placed in each batch and incubated for 60 min with 2.8 mM or 20 mM supplemental glucose with or without Sudachitin at the indicated concentrations (n = 6–9). The data obtained was statistically analyzed. Values are means ± SD (*$P < 0.05$; **$P < 0.01$).Cont; Control, Suda; Sudachitin, Fsk; Forskolin.

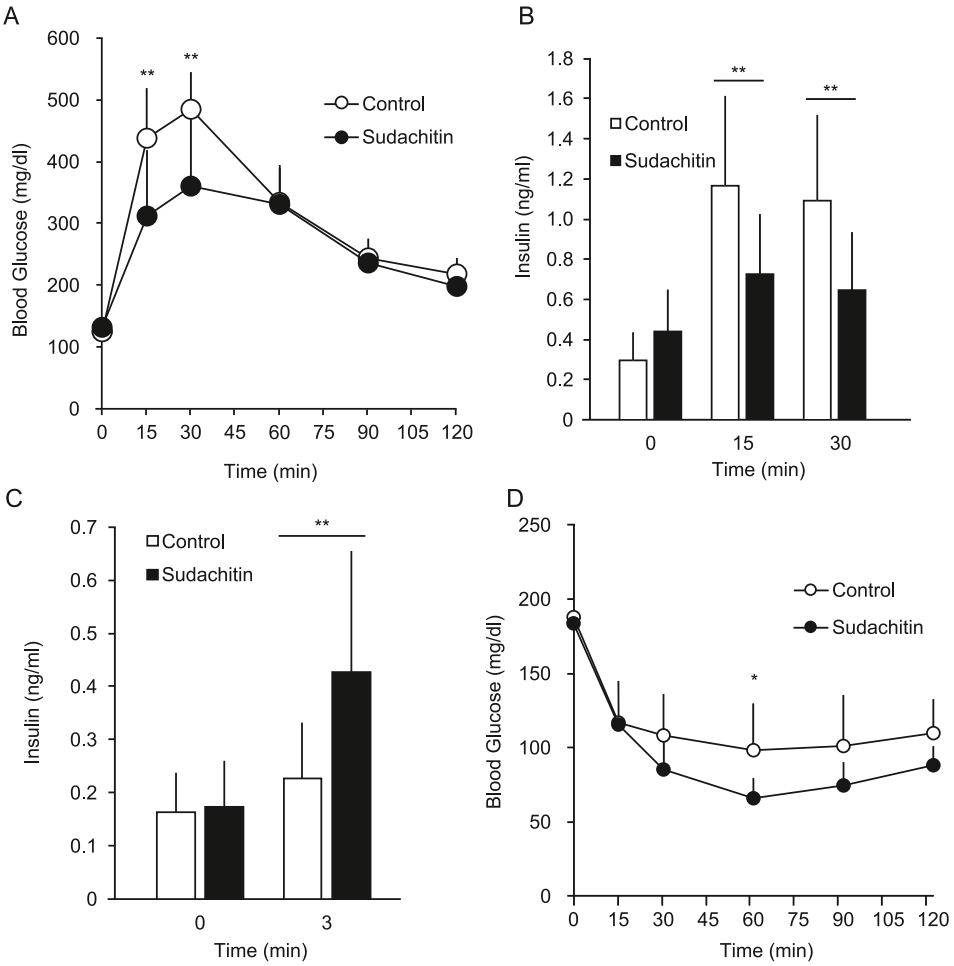

Fig. 2. Sudachitin increased early phase insulin secretion as well as systemic insulin sensitivity (A,B) Blood glucose and plasma insulin in wild-type mice during 75 g oral glucose tolerance tests (OGTT) with either 10 mg/kg Sudachitin (n = 9) or control (DMSO, n = 9). (C) Effect of Sudachitin on the early phase of insulin secretion in vivo. Wild-type mice were administered either 10 mg/kg Sudachitin (n = 11) or DMSO only (n = 11). Plasma insulin levels were measured at 0 min (just before administration) and 3 min. (D) Blood glucose during insulin tolerance test (ITT). Wild-type mice were treated with either 10 mg/kg Sudachitin (n = 7) or only DMSO (n = 7) and immediately thereafter intraperitoneally injected with insulin (0.75 units/kg). The data obtained was statistically analyzed. Values are means ± SD. (*P < 0.05).

3.3. Sudachitin increases intracellular cAMP via PDE inhibition

Given that Sudachitin increases glucose-dependent insulin secretion, as noted above, Sudachitin might be involved in an amplification pathway acting in insulin secretion in pancreatic β-cells. In the analysis employing mouse pancreatic islets, Sudachitin treatment significantly increased cAMP in a concentration-dependent manner with an approximately five-fold increase at 100 μM Sudachitin ($p < 0.01$) (Fig. 3A). We further analyzed insulin secretion using MAY 0132, an inhibitor of EPACs which serve in the terminal step of the amplification pathway. The results obtained showed that MAY0132 concentration-dependently inhibited the insulin secretion induced by Sudachitin (Fig. 3B).

Finally, we speculated that PDE inhibition might be involved in the Sudachitin-induced cAMP increase in β-cells based on the observation that Sudachitin inhibits PDE isoform in macrophages, a phenomenon not previously reported in the context of its insulinotropic effect [34]. Therefore, we performed an in vitro PDE inhibition assay using fluorescence spectrophotometry to measure GMP. The activities of PDE3, PDE8 and PDE10 family members, previously reported to promote insulin secretion, were inhibited by Sudachitin [35–37]. (Fig. 3C) (Table 1).

Finally, the binding between Sudachitin and PDE was investigated through computer simulations using AutoDock Vina [38]. We analyzed the binding energies of Sudachitin and known PDE inhibitors at PDE3A, PDE3B, PDE4C, and PDE10A, which are known to be important for insulin secretion (Table 2) [35,39–41]. Examining PDE3B revealed Sudachitin to share binding sites with other positive control compounds, exhibiting a binding energy of −9.1 kcal/mol (Table 2, Fig. 3D). The binding energies of selective PDE3B inhibitors, Milrinone and Amrinone, were −8.4 kcal/mol and −7.4 kcal/mol, respectively. Notably, Sudachitin showed lower binding energies than Milrinone and Amrinone, suggesting stronger binding and interactions with Sudachitin in the simulations (Fig. 3D). The

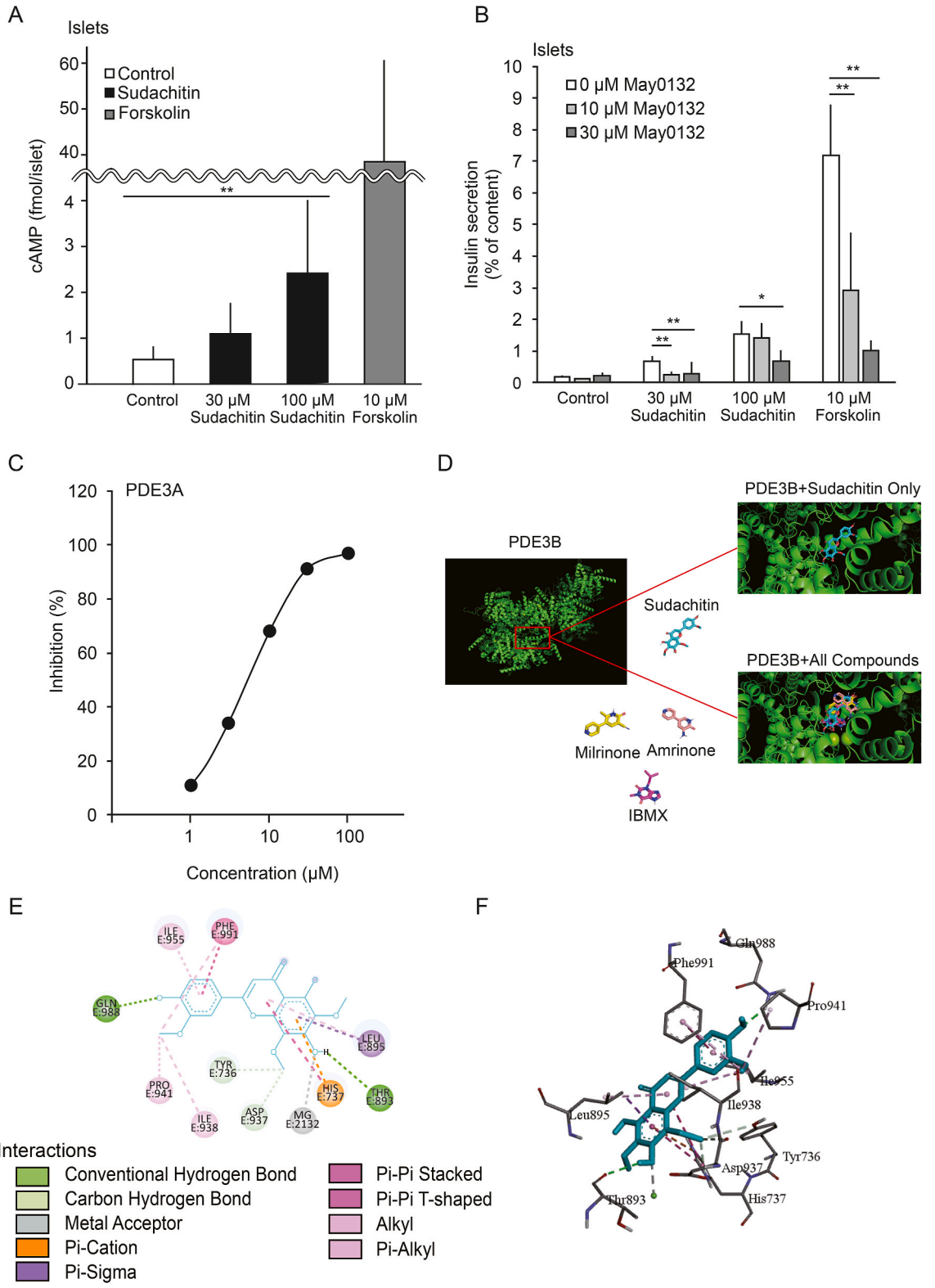

Fig. 3. Sudachitin increased intracellular cAMP concentration via inhibition of phosphodiesterase (PDE) (A) cAMP in wild-type mouse islets. Fifty islets were incubated with KRBB supplemented with 20 mM glucose and DMSO (n = 10), Sudachitin (10 μM n = 6, 100 μM n = 6) or forskolin (n = 10) for 15 min. The forskolin group was excluded from the statistical analysis due to its disproportionate impact on numerical values. (B) Effect of EPAC2 inhibition on Sudachitin-induced insulin secretion. Islets were incubated for 60 min with 20 mM glucose with or without Sudachitin or

forskolin in the presence or absence of May0132, a selective inhibitor of the cAMP-binding protein EPAC2 subtype 2 (n = 3–6). (C) Inhibition Curve of PDE with Sudachitin. The graph illustrates the inhibition profile of Sudachitin on PDE3A at five concentrations (1, 3, 10, 30, and 100 μM). The vertical axis represents the percentage inhibition of PDE3 activity, the horizontal axis the concentration of added Sudachitin. The values were determined in duplicate. (D) Molecular docking predictions for Sudachitin and known inhibitors binding to PDE3B using AutoDock Vina and PyMOL (E,F) Molecular docking interaction of Sudachitin against PDB3B in 2D (Fig. 3E) and D (Fig. 3F) representations.

Table 1
Inhibitory actions of Sudachitin on PDE isoforms. All IC50 values were determined in duplicate.

PDEs	Concentration (μM)	Inhibition (%)	IC50 (μM)
PDE3A	100	97	5.19
	30	91	
	10	68	
	3	34	
	1	11	
PDE5A	100	91	6.54
	30	79	
	10	62	
	3	35	
	1	10	
PDE8A1	100	85	26.3
	30	51	
	10	23	
	3	13	
	1	5	
PDE10A2	100	95	9.99
	30	82	
	10	50	
	3	18	
	1	2	

Table 2
Molecular docking predictions for Sudachitin and known inhibitors binding to PDE isoforms using AutoDock Vina. Numerical values represent the respective binding energies, with lower values indicating stronger binding.

	Sudachitin	IBMX nonspecific inhibitor	Selective inhibitor	Protein Data Bank ID
PDE3A	−7.8	−6.3	Not Available	7L27
PDE3B	−9.1	−6.1	−8.4 (Milrinone), −7.4 (Amrinone)	1SOJ
PDE4C	−7.9	−5.9	−6.4 (Rolipram)	2QYM
PDE10A	−8.5	−6.1	Not Available	5SJH

binding interaction of Sudachitin with PDE3B was stabilized by hydrogen bonding with Thr893 and Gln988 (Fig. 3E and F). Other interactions were involved such as the Carbon Hydrogen Bond (Tyr736, Asp937), Pi-Sigma (Leu895), Pi-Cation (His737), Pi-Pi T-shaped (Phe991), and Pi-Alkyl (Ile955, Pro941, Ile 938). These interactions are presented in Fig. 3E and F. The findings suggest that Sudachitin promotes insulin secretion by directly inhibiting PDE, which appears to be essential for insulin secretion.

4. Discussion

A single administration of Sudachitin lowered blood glucose levels via stimulation of insulin secretion and the modulation of insulin sensitivity. Previous studies have shown oral administration of Sudachitin (5 mg/kg), over a 12-week period, to improve glucose tolerance by activating the SIRT1-PGC1α pathway in skeletal muscle. This activation subsequently enhances mitochondrial synthesis and increases energy expenditure, leading to improved insulin resistance [15]. SIRT1 plays an essential role in insulin secretion, as shown by studies of impaired insulin secretion using SIRT1 knockout models [42]. Resveratrol, an activator of SIRT, also reportedly promotes insulin secretion in a glucose-dependent manner via a SIRT1-dependent mechanism. However, this requires pre-culture lasting at least 24 h. Furthermore, there are no reports focusing on short-duration stimulation such as in this study, including investigations of other SIRT activators. Therefore, the insulin secretion-promoting effects of Sudachitin seen in the SIRT1 knockout model await further detailed investigation [43].

In the in vivo study, the promotion of insulin secretion was confirmed 3 min after Sudachitin administration. However, given the very short duration of its effect after oral administration, it is possible that the blood Sudachitin concentration did not reach the levels observed to promote insulin secretion in vitro. Previous studies have shown intestinal enteroendocrine L-cells to enhance GLP-1 secretion via PDE inhibition [44]. Thus, the insulin secretion-enhancing effect of Sudachitin observed in this study may not occur only due to a direct impact on β-cells but also via stimulation of L-cells in the gastrointestinal tract.

Furthermore, like Sudachitin, nobiletin reportedly enhances insulin secretion with a short duration of action. However, the mechanism underlying nobiletin's insulin secretion-enhancing effects differs from that of Sudachitin, suggesting potential variability in flavonoid-mediated insulin secretion pathways. Additionally, while PDEs have been implicated in insulin secretion processes, the specific involvements of individual PDE subtypes in this mechanism remain unclear.PDEs are a family consisting of enzymes encoded by 11 genes, of which PDE1, 3, 4, 8, 10, and 11 are reportedly expressed in islets and involved in insulin secretion, but the details of the mechanisms of action of individual PDE subtypes in the insulin secretion process have yet to be fully clarified [9,35–37].

Another interesting observation from our present study is the improvement of insulin resistance after a single dose of Sudachitin. A single dose of a cGMP PDE inhibitor (zaprinast) was shown to enhance microvascular perfusion and increase insulin-mediated muscle glucose uptake [45]. Since cGMP is a substrate of PDE5 and Sudachitin also inhibits PDE5 activity (Table 1) [22], it is reasonable to speculate that this pathway is involved in enhancing insulin action. To test this speculation, it is essential to measure cGMP concentrations in muscle tissue. Conversely, PDE4 inhibitors were recently reported to induce a decrease in skeletal muscle glycogen and inhibit glucose uptake into muscle tissue [46]. Different isoforms of PDE are expected to differ in the mechanisms by which they regulate insulin sensitivity, warranting further examination in future studies.

In addition, while Sagara et al. provided valuable insights on the effects of Sudachitin on insulin secretion and PDE inhibition, our study significantly extends these findings [47]. We performed in vivo experiments employing mice, thereby shedding light on physiological effects that were not fully elucidated in previous studies. Importantly, we performed a thorough examination of the mechanism of action of Sudachitin, including a comprehensive evaluation of PDE inhibition, isoform specificity, and computer simulations of molecular interactions. These additional analyses provided a better understanding of Sudachitin's mechanism of action and its potential physiological relevance, as well as augmenting the existing body of knowledge on this topic.

5. Conclusion

Our findings suggest that Sudachitin, derived from natural sources, holds promise as a therapeutic agent for diabetes management and prevention. Through its insulin secretagogue effect mediated by PDE inhibition, Sudachitin appears to improves glucose regulation. Our study highlights the need for further research to elucidate the specific mechanisms underlying Sudachitin's effects on insulin secretion and sensitivity, as well as its effectiveness in comparison with those of other flavonoids. Despite these areas requiring further exploration, the present study underscores the potential of Sudachitin as a valuable addition to the armamentarium of diabetes therapeutics.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics declarations

This study was reviewed and approved by Animal Experimentation Committee at Yamaguchi University School of Medicine, with the approval number: J22003.

CRediT authorship contribution statement

Ryoko Hatanaka: Visualization, Investigation. **Akihiko Taguchi:** Writing – review & editing, Writing – original draft, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Yuko Nagao:** Visualization, Investigation. **Kaito Yorimoto:** Investigation. **Akari Takesato:** Investigation. **Konosuke Masuda:** Investigation. **Takao Ono:** Investigation. **Yoshishige Samukawa:** Conceptualization. **Yukio Tanizawa:** Writing – review & editing, Supervision, Funding acquisition. **Yasuharu Ohta:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Akihiko Taguchi reports financial support was provided by Taisho Pharmaceutical Co., Ltd. Akihiko Taguchi reports financial support was provided by the Japan Society for the Promotion of Science. Akihiko Taguchi reports financial support was provided by Mishima Kaiun Memorial Foundation. Yasuharu Ohta reports financial support was provided by the Japan Society for the Promotion of Science. Yukio Tanizawa reports financial support was provided by the Japan Society for the Promotion of Science. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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