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## RESEARCH ARTICLE



# The flavonoid resokaempferol improves insulin secretion from healthy and dysfunctional pancreatic $\beta$ -cells

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**Background and Purpose:** The pharmacology of flavonoids on  $\beta$ -cell function is largely undefined especially in the context of defective secretion of insulin. We sought to identify flavonoids that increased the insulin-secreting function of  $\beta$ -cells and to explore the underlying mechanisms.

**Experimental Approach:** INS-1  $\beta$ -cells in culture and islets of Langerhans isolated from control and diabetic male rats were used for insulin secretion experiments. Pharmacological and electrophysiological approaches were used for mechanistic studies.

**Key Results:** Among a set of flavonoids, exposure of INS-1  $\beta$ -cells to resokaempferol (ResoK) enhanced glucose-stimulated insulin secretion and therefore we further characterised its activity and its pharmacological mechanism. ResoK glucose-dependently enhanced insulin secretion in INS-1  $\beta$ -cells and pancreatic islets isolated from rats. Mechanistically, whole cell patch clamp recordings in INS-1 cells showed that ResoK rapidly and dose-dependently enhanced the L-type  $\text{Ca}^{2+}$  current whereas it was inactive towards T-type  $\text{Ca}^{2+}$  current. Accordingly, pharmacological inhibition of L-type  $\text{Ca}^{2+}$  current but not T-type  $\text{Ca}^{2+}$  current blocked the effects of ResoK on glucose-stimulated insulin secretion. ResoK was still active on dysfunctional  $\beta$ -cells as it ameliorated glucose-stimulated insulin secretion in glucotoxicity-induced dysfunctional INS-1 cells and in pancreatic islets isolated from diabetic rats.

**Conclusion and Implications:** ResoK is a glucose-dependent activator of insulin secretion. Our results indicated that the effects of ResoK on insulin secretion involved its capacity to stimulate L-type  $\text{Ca}^{2+}$  currents in cultured  $\beta$ -cells. As ResoK was also effective on dysfunctional  $\beta$ -cells, our work provides a new approach to stimulating insulin secretion, using compounds based on the structure of ResoK.

**Abbreviations:**  $\text{Ca}_v$ , voltage-gated  $\text{Ca}^{2+}$  channel;  $\text{EC}_{50}$ , half maximal effective concentration; Emax, efficacy; GK, Goto-Kakizaki;  $\text{K}_{\text{ATP}}$ , ATP-sensitive  $\text{K}^+$  channels; Kaemp, kaempferol; ResoK, resokaempferol;  $\text{V}_{0.5}$ , potential at half-maximal activation; Veh, vehicle.

Jérémie Neasta and Catherine Oiry contributed equally to this work and share last authorship.

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## KEYWORDS

diabetes, calcium channel, insulin secretion, natural products, polyphenol

## 1 | INTRODUCTION

Pancreatic  $\beta$ -cells are fundamental for glucose homeostasis by secreting insulin. This hormone maintains glycaemia within the physiological range mainly by promoting glucose uptake and utilisation by peripheral tissues. Therefore, together with worsening of insulin resistance, the progressive decline of  $\beta$ -cell capacity to secrete insulin in response to glucose is a cardinal feature of the pathogenesis and the evolution of Type 2 diabetes (Rorsman & Ashcroft, 2018). This relative insulin deficiency can be partly corrected by antidiabetic medications which improve glucose-induced insulin secretion (Perreault et al., 2021). In fact,  $\beta$ -cells respond to raised levels of blood glucose through the coupling of cytosolic and mitochondrial processes in which glucose undergoes a metabolic sequence leading to the inhibition of **ATP-sensitive  $K^+$  channels** ( $K_{ATP}$ ). This causes membrane depolarisation and a consequent opening of **voltage-gated  $Ca^{2+}$  channels**. The ensuing inward  $Ca^{2+}$  currents allow activation of the multiple-step exocytotic process that ultimately evokes insulin release (Gilon et al., 2014; Rorsman & Ashcroft, 2018). Not surprisingly, proteins involved in or regulating this complex series of events constitute current and potential therapeutic targets to boost the insulin-secreting activity of dysfunctional  $\beta$ -cells. For instance, sulphonylureas work by binding and mediating the closure of  $\beta$ -cell  $K_{ATP}$  channels which elicits insulin secretion, even in low concentrations of glucose. In contrast, other antidiabetic drugs including incretin analogues display glucose-dependent insulinotropic properties. The use of compounds with such pharmacological attributes is expected to have improved safety profiles, at least in terms of lower incidence of hypoglycaemia (Perreault et al., 2021). Therefore, considerable efforts are still being made to develop such compounds with the aim of providing alternative therapeutic options, as Type 2 diabetes is increasingly prevalent worldwide (Perreault et al., 2021).

In this context, compounds from plant sources and their derivatives are likely to be good candidates, as a number of them have been found to directly target  $\beta$ -cell function (Bermont et al., 2020; Ghorbani et al., 2019; Patyra et al., 2024; Zheng et al., 2022) and to improve metabolic health (Fu et al., 2010; Heikkilä et al., 2019). In particular, flavonoids are phytochemicals that exert pleiotropic health benefits through the regulation of protein targets and associated signalling pathways (Dall'Asta et al., 2015; Fusi et al., 2017; Howes et al., 2020; Man, Xia, et al., 2020). The opportunity of developing flavonoid-based therapeutics is strengthened by in vitro and in vivo studies showing that these compounds are bioactive towards different tissues affected by Type 2 diabetes (Cao et al., 2022; Fusi et al., 2020; Keyhani et al., 2023). However, flavonoids constitute a rich collection of naturally-occurring molecules with high structural and activity diversity whose pharmacological value against  $\beta$ -cell dysfunction is largely unexplored.

### What is already known?

- Pancreatic  $\beta$ -cells play a key role in glycaemic control by secreting insulin.
- Flavonoids display pharmacological activity on cellular protein targets.

### What does this study add?

- In cultures of INS  $\beta$ -cells, ResoK increases insulin secretion by stimulating L-type  $Ca^{2+}$  currents.
- ResoK improves insulin secretion in a glucose-dependent manner in  $\beta$ -cells from diabetic rats.

### What is the clinical significance?

- ResoK-based compounds may be used to ameliorate pancreatic  $\beta$ -cell function in Type 2 diabetes.

In this study, we describe an analysis of the insulin-secreting activity of different flavonoids, using a  $\beta$ -cell line in order to identify molecules improving glucose-induced insulin secretion. Next, the mechanism of action of one promising compound, namely resokaempferol (ResoK), was explored using pharmacological and electrophysiological approaches. Finally, the therapeutic potential of ResoK was examined by testing its capacity to enhance insulin secretion from dysfunctional  $\beta$ -cells.

## 2 | METHODS

### 2.1 | Animals

All animal care and experimental procedures complied with the Directive 2010/63/EU of the European Parliament and of the Council and were approved by the institutional Animal Care and Use Ethical Committees of the Languedoc-Roussillon and Université Paris Cité. All efforts were made to minimise any animal suffering. Animal studies are reported in compliance with the ARRIVE guidelines (Percie du Sert et al., 2020) and with the recommendations made by the *British Journal of Pharmacology* (Lilley et al., 2020). Male Wistar rats (Charles River, France) were housed in a conventional animal facility in Montpellier, France. Animals were housed in groups of three in transparent cages enriched with poplar heartwood

modules in a temperature and humidity-controlled animal facility (temperature =  $20 \pm 1^\circ\text{C}$ ; humidity = 60%) under a 12 h light:dark cycle (7 AM/7 PM) and had access to water and food ad libitum.

Male Goto-Kakizaki rats from our local colony (GK/Par) were bred and housed in the conventional Buffon animal facility- Université Paris Cité, Paris, France. Animals were housed in controlled environment (temperature =  $20 \pm 1^\circ\text{C}$ ; humidity = 60%) and 12 h light: dark cycle (8 AM/8 PM), with access to water and food ad libitum. Rats were housed two per ventilated cages and were furnished with standard enrichment (cardboard-house, craft paper as well as hood stick). After weaning, animals were fed with a standard chow diet.

## 2.2 | Insulin secretion experiments in INS-1 cells

INS-1 cells (RRID:CVCL\_0352; passages 33–50), a gift from Professor C. B. Wollheim, (Geneva, Switzerland), were cultured in RPMI-1640 medium (containing  $2\text{ g}\cdot\text{L}^{-1}$  glucose) supplemented with 10% (v/v) heat-inactivated fetal calf serum,  $100\text{ U}\cdot\text{ml}^{-1}$  penicillin,  $100\text{ }\mu\text{g}\cdot\text{ml}^{-1}$  streptomycin,  $2\text{ mmol}\cdot\text{L}^{-1}$  L-glutamine,  $10\text{ mmol}\cdot\text{L}^{-1}$  HEPES,  $1\text{ mmol}\cdot\text{L}^{-1}$  sodium pyruvate and  $50\text{ }\mu\text{mol}\cdot\text{L}^{-1}$  2-mercaptoethanol, in a humidified atmosphere (5%  $\text{CO}_2$ ,  $37^\circ\text{C}$ ). INS-1 cells were seeded in 24-well plates pre-coated with poly-L-lysine ( $4 \times 10^5$  cells per well) and cultured for 5 days before insulin secretion experiments. Glucotoxicity was induced by culturing cells for 48 h in RPMI-1640 medium containing  $25\text{ mmol}\cdot\text{L}^{-1}$  glucose. Cells were washed with buffer 1 ( $123\text{ mmol}\cdot\text{L}^{-1}$  NaCl,  $5.4\text{ mmol}\cdot\text{L}^{-1}$  KCl,  $1.3\text{ mmol}\cdot\text{L}^{-1}$   $\text{KH}_2\text{PO}_4$ ,  $1.4\text{ mmol}\cdot\text{L}^{-1}$   $\text{MgSO}_4$ ,  $2.9\text{ mmol}\cdot\text{L}^{-1}$   $\text{CaCl}_2$ ,  $5\text{ mmol}\cdot\text{L}^{-1}$   $\text{NaHCO}_3$  and  $20\text{ mmol}\cdot\text{L}^{-1}$  HEPES, pH 7.4) supplemented with  $1\text{ g}\cdot\text{L}^{-1}$  BSA. Then, cells were incubated for 1 h (5%  $\text{CO}_2$ ,  $37^\circ\text{C}$ ) in  $500\text{ }\mu\text{l}$  buffer 1/BSA containing the tested flavonoid in the absence (non-stimulating basal condition; i.e.,  $1.4\text{ mmol}\cdot\text{L}^{-1}$  glucose) or in the presence of secretagogues ( $8.3\text{ mmol}\cdot\text{L}^{-1}$  glucose;  $1.4\text{ mmol}\cdot\text{L}^{-1}$  glucose +  $10\text{ nmol}\cdot\text{L}^{-1}$  glibenclamide;  $1.4\text{ mmol}\cdot\text{L}^{-1}$  glucose +  $5\text{ mmol}\cdot\text{L}^{-1}$  monomethyl succinate). Exenatide (0.001; 0.01; 0.1; 1;  $10\text{ nmol}\cdot\text{L}^{-1}$ ) was tested in the same conditions in the presence of  $8.3\text{ mmol}\cdot\text{L}^{-1}$  glucose. When verapamil ( $20\text{ }\mu\text{mol}\cdot\text{L}^{-1}$ ), nifedipine ( $1\text{ }\mu\text{mol}\cdot\text{L}^{-1}$ ) or Z944 ( $2\text{ }\mu\text{mol}\cdot\text{L}^{-1}$ ) was used, INS-1 cells were pre-treated with these inhibitors for 15 min directly in the culture medium prior to proceeding with cell washes with buffer 1/BSA. Then, INS-1 cells were incubated for 1 h in  $500\text{ }\mu\text{l}$  buffer 1/BSA containing ResoK and the  $\text{Ca}^{2+}$  channel inhibitor at the same concentration that was used for the pre-treatment. Control treatments were always performed using the same amount of vehicle (DMSO) in each well. At the end of the 1 h incubation period, supernatants were collected and insulin was directly quantified with PerkinElmer Insulin assay kits.

For this assay, a standard curve ( $0.625$ ;  $1.25$ ;  $2.5$ ;  $5$ ;  $10$ ;  $16$ ;  $20\text{ ng}\cdot\text{ml}^{-1}$ ) was prepared by diluting the insulin stock solution in the following assay buffer:  $30\text{ mmol}\cdot\text{L}^{-1}$   $\text{Na}_2\text{HPO}_4$ ,  $20\text{ mmol}\cdot\text{L}^{-1}$   $\text{KH}_2\text{PO}_4$ , pH 7, 0.05% (v/v) tween-20. Samples containing insulin were diluted in the assay buffer so that measurements lay within the standard curve. Specifically, supernatants originating from INS-1 subjected to  $8.3\text{ mmol}\cdot\text{L}^{-1}$  glucose were diluted 30X, whereas the ones subjected

to  $1.4\text{ mmol}\cdot\text{L}^{-1}$  glucose were diluted 10X. Samples ( $8\text{ }\mu\text{l}$ ) of the diluted supernatants were dispensed in a 384-well microplate (Greiner #784075). Then  $4\text{ }\mu\text{l}$  of Insulin XL665 and  $4\text{ }\mu\text{l}$  of Insulin Eu Cryptate antibody were added. After an overnight incubation at room temperature in the dark, the signal was collected by the Tecan Spark 10 M reader at both 665 and 620 nm ( $\lambda_{\text{ex}} = 320\text{ nm}$ ) using the following settings: 75 flashes per well, lag time  $100\text{ }\mu\text{s}$ , integration time  $400\text{ }\mu\text{s}$ .

## 2.3 | Cell viability assay

The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay was used to test cell viability following ResoK exposure. INS-1 cells were seeded in 96-well plates pre-coated with poly-L-lysine ( $10^5$  cells per well). After being cultured for 5 days in RPMI-1640 medium (containing  $2\text{ g}\cdot\text{L}^{-1}$  glucose) supplemented with 10% heat-inactivated fetal calf serum,  $100\text{ U}\cdot\text{ml}^{-1}$  penicillin,  $100\text{ }\mu\text{g}\cdot\text{ml}^{-1}$  streptomycin,  $2\text{ mmol}\cdot\text{L}^{-1}$  L-glutamine,  $10\text{ mmol}\cdot\text{L}^{-1}$  HEPES,  $1\text{ mmol}\cdot\text{L}^{-1}$  sodium pyruvate and  $50\text{ }\mu\text{mol}\cdot\text{L}^{-1}$  2-mercaptoethanol, cells were washed with buffer 1 supplemented with  $1\text{ g}\cdot\text{L}^{-1}$  BSA and incubated for 1 h (5%  $\text{CO}_2$ ,  $37^\circ\text{C}$ ) in buffer 1/BSA containing ResoK (0; 2; 5; 10; 20; 50 or  $100\text{ }\mu\text{mol}\cdot\text{L}^{-1}$ ) in the presence of  $8.3\text{ mmol}\cdot\text{L}^{-1}$  glucose. Then, cells were washed with buffer 1/BSA and incubated for 3 h (5%  $\text{CO}_2$ ,  $37^\circ\text{C}$ ) in buffer 1/BSA containing  $0.5\text{ g}\cdot\text{L}^{-1}$  MTT. Finally, cells were washed with phosphate buffered saline (without calcium or magnesium) and the formazan precipitate was solubilised in  $50\text{ }\mu\text{l}$  DMSO prior to recording absorbance at 570 nm using the Tecan Spark 10 M reader. Background absorbance was measured using wells in which no cells were seeded and was subtracted from assay readings.

## 2.4 | Insulin secretion experiments in rat pancreatic islets

As INS-1 cells constitute a rat-derived  $\beta$ -cell line, pancreatic islets were isolated from rats. Specifically, Wistar and GK rats were used to isolate non-diabetic and diabetic pancreatic islets respectively because GK rats were originally developed by selective breeding of glucose-intolerant Wistar rats (Goto et al., 1976; Portha et al., 2012).

Regarding the isolation of pancreatic islets from Wistar rats, the same protocol as previously described was used (Bayle et al., 2019). Two Wistar rats were killed to prepare pancreatic islets for each independent biological repeat ( $n = 5$  in Figure 3). Specifically, a total of 10 male Wistar rats of 8 to 9 week-old weighing 280–300 g at the time of the experiments were anaesthetised with isoflurane and killed by decapitation. This protocol was approved by the Institutional Animal Care and Use Committee Languedoc-Roussillon (ID #19002). The common bile duct was cannulated and the pancreas was filled by injection of collagenase V solution ( $10\text{ ml}$ ,  $1\text{ mg}\cdot\text{ml}^{-1}$ ), excised and digested at  $37^\circ\text{C}$  for 16 min. Islets were separated with Histopaque<sup>®</sup>1077 gradient and selected by hand-picking under a microscope.

Then, islets originating from the two rats were pooled and stabilised for 1 h (5% CO<sub>2</sub>, 37°C) in buffer 2 (120 mmol·L<sup>-1</sup> NaCl, 4.7 mmol·L<sup>-1</sup> KCl, 1.2 mmol·L<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>, 1.2 mmol·L<sup>-1</sup> MgSO<sub>4</sub>, 2.5 mmol·L<sup>-1</sup> CaCl<sub>2</sub>, 24 mmol·L<sup>-1</sup> NaHCO<sub>3</sub>, pH 7.4) supplemented with 1 g·L<sup>-1</sup> BSA and containing 2.8 mmol·L<sup>-1</sup> glucose. After this 1 h recovery period, batches of 5 islets chosen at random were incubated for 1 h at 37°C, in 1 ml buffer 2/BSA containing 2.8 mmol·L<sup>-1</sup> (basal insulin secretion) or 8.3 mmol·L<sup>-1</sup> glucose in the absence or presence of 20 μmol·L<sup>-1</sup> ResoK. We used glucose at 8.3 mmol·L<sup>-1</sup> to stimulate insulin secretion in pancreatic islets isolated from Wistar rats as we previously showed that this concentration led to an intermediate amount of insulin release (Bayle et al., 2022), which is appropriate to examine the potential stimulatory effect of a compound (Bayle et al., 2019). Control treatments were always performed using the same amount of vehicle (DMSO). At the end of the 1 h incubation period, supernatants were collected and stored at -20°C until insulin quantification with PerkinElmer Insulin assay kit. To do so, supernatants originating from pancreatic islets subjected to 8.3 mmol·L<sup>-1</sup> glucose were diluted 10X in the assay buffer, and those from islets subjected to 2.8 mmol·L<sup>-1</sup> glucose were diluted 2X. Then, the same protocol as the one described in the preceding section was used.

Glycaemia in GK rats was measured at 9 AM before humane killing (see below), using Accu-Check Glucometer (Roche, Boulogne-Billancourt, France) in blood samples collected at the tip of the tail. Glycaemia ranged from 1.86 to 3.45 g·L<sup>-1</sup> (Table S1). One GK rat was killed to prepare pancreatic islets for each independent biological repeat (n = 5 in Figures 7 and S4). Specifically, a total of 10 male GK rats of 8 to 10 week-old (340–360 g) were killed by lethal pentobarbital injection (182 mg·ml<sup>-1</sup>) and directly decapitated. This protocol was approved by the institutional Animal Care and Use Ethical Committee of the Université Paris Cité (ID #B-75-13-17). The pancreas of GK rats was digested with liberase at 37°C and islets were separated from exocrine tissue with Histopaque®1077 gradient and hand-picked under a stereomicroscope. Ten islets of equivalent size per rat and per condition were pre-incubated for 1 h in a Krebs-Ringer/bicarbonate/HEPES buffer 3 (115 mmol·L<sup>-1</sup> NaCl, 5 mmol·L<sup>-1</sup> KCl, 24 mmol·L<sup>-1</sup> NaHCO<sub>3</sub>, 10 mmol·L<sup>-1</sup> HEPES, 1 mmol·L<sup>-1</sup> CaCl<sub>2</sub>, 1 mmol·L<sup>-1</sup> MgCl<sub>2</sub>) containing 2 g·L<sup>-1</sup> fatty-acid-free BSA and 2.8 mmol·L<sup>-1</sup> glucose. After this 1 h recovery period, islets were incubated for 1 h with fresh buffer 3/BSA containing 2.8 or 16.7 mmol·L<sup>-1</sup> glucose, or 2.8 mmol·L<sup>-1</sup> glucose + 20 μmol·L<sup>-1</sup> ResoK, or 16.7 mmol·L<sup>-1</sup> glucose + 20 μmol·L<sup>-1</sup> ResoK, to measure insulin secretion. We used glucose at 16.7 mmol·L<sup>-1</sup> to stimulate insulin secretion in pancreatic islets isolated from GK rats to compensate for diabetic β-cell failure (Lutz, 2023; Mesto et al., 2022; Portha et al., 2012). When nifedipine (1 μmol·L<sup>-1</sup>) was used, 10 GK islets of equivalent size per rat and per condition were pre-incubated for 1 h in the same buffer 3/BSA. Fifteen minutes before the end of this pre-incubation period, nifedipine or the same volume of DMSO was directly added to the incubation buffer. After this 1 h recovery period, islets were incubated for 1 h with fresh buffer 3/BSA containing 2.8 or 16.7 mmol·L<sup>-1</sup> glucose, or 16.7 mmol·L<sup>-1</sup> glucose + 20 μmol·L<sup>-1</sup> ResoK, or 16.7 mmol·L<sup>-1</sup> glucose + 1 μmol·L<sup>-1</sup> nifedipine, or 16.7 mmol·L<sup>-1</sup> glucose + 20 μmol·L<sup>-1</sup> ResoK + 1 μmol·L<sup>-1</sup> nifedipine.

Control treatments were always performed using the same amount of vehicle (DMSO). Insulin released in the supernatant was measured by ELISA. The insulin concentrations used for the standard curve were 0.15; 0.4; 1; 3; 5.5 ng·ml<sup>-1</sup>. Samples originating from GK islets subjected to 16.7 mmol·L<sup>-1</sup> glucose were diluted 4X in the assay buffer, the ones subjected to 2.8 mmol·L<sup>-1</sup> glucose were not diluted. The absorbance was read at 450 nm using a microplate reader.

## 2.5 | Electrophysiological recordings

Voltage-gated Ca<sup>2+</sup> channel activities were recorded using the whole-cell patch-clamp technique with a Biologic RK400 amplifier (Biologic, France). Data acquisition and analysis were performed using the pCLAMP system (Axon Instruments, Union City, CA, USA). Currents were recorded with patch pipettes of 3–5 MΩ. Capacitive transients were electronically compensated. Residual capacitive transient and linear leakage currents were subtracted using a four sub-pulse protocol. The extracellular solution contained 130 mmol·L<sup>-1</sup> NaCl, 5.6 mmol·L<sup>-1</sup> KCl, 1 mmol·L<sup>-1</sup> MgCl<sub>2</sub>, 1 mmol·L<sup>-1</sup> glucose, 10 mmol·L<sup>-1</sup> HEPES, 5 mmol·L<sup>-1</sup> BaCl<sub>2</sub>, adjusted to pH 7.4 with NaOH. The pipette solution contained 130 mmol·L<sup>-1</sup> CsCl, 10 mmol·L<sup>-1</sup> EGTA, 5 mmol·L<sup>-1</sup> ATPNa<sub>2</sub>, 2 mmol·L<sup>-1</sup> MgCl<sub>2</sub>, 10 mmol·L<sup>-1</sup> HEPES, adjusted to pH 7.3 with CsOH. The holding potential was set at -80 or -50 mV, and depolarizing test pulses were applied as described. Compounds were applied to cells by pressure ejection from a glass pipette. The effect of ResoK was quantified as percentage of variation of the amplitude of the current compared to the amplitude of the basal current. All experiments were performed at room temperature.

## 2.6 | Comparative modelling and docking in the rat Ca<sub>v</sub>1.2 channel

The rat Ca<sub>v</sub>1.2 channel subunit α<sub>1C</sub> sequence (P22002) was recovered from UniProt (UniProt; <http://www.uniprot.org/uniprot/>). The search for homologous sequence and alignment were performed using @TOME-2 server (Pons & Labesse, 2009), and sequence of human Ca<sub>v</sub>1.2 channel subunit α<sub>1C</sub> (A0A0A0MSA1) was selected with a 97% of identity. Homology models of the rat subunit α<sub>1C</sub> were generated using the electron microscopy structure of human channel determined at 3.30 Å resolution as templates: Protein Data Bank accession no. 8HMB. Final models were built using Modeller 7.0 (Webb & Sali, 2017) and evaluated using the dynamic evolutionary trace as implemented in VITOUSING @TOME-2 comparative option. The files for the docked compounds (ResoK and benidipine) were generated with MarvinSketch 6.2.2 for the SMILES and with Frog2 server for the mol2 (Miteva et al., 2010). Docking simulation was performed using the server e-Drugs-3D (Pihan et al., 2012) and with Autodock-Vina (Eberhardt et al., 2021) with a box of 60 Å × 60 Å × 60 Å to cover all the protein with no constraint. The pictures shown in Figures S2 and S3 were generated using PyMOL.

## 2.7 | Randomisation and blinding

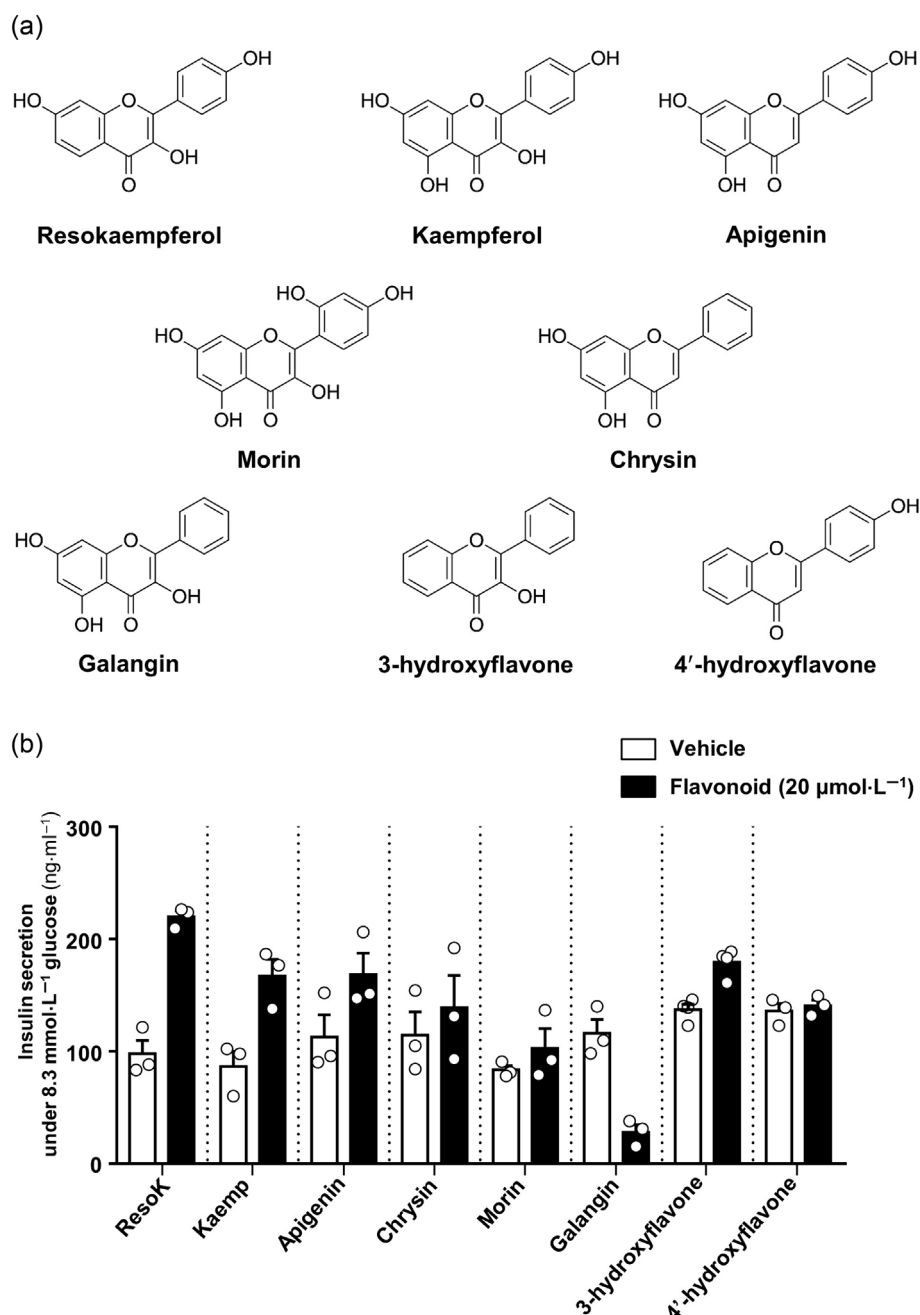
For insulin secretion experiments in INS-1 cells, treatment groups were randomly assigned to different wells within each 24-well plate. Pancreatic islets were isolated from fed, weight-matched Wistar or GK rats chosen at random. Then, pancreatic islets were randomly assigned to different treatments.

Except for the exploratory data reported in Figure 1, experiments were not carried out under blind conditions. Blinding was not practically feasible owing to the slightly yellow colour of ResoK solutions. Nevertheless, during the course of this study, insulin secretion experiments in INS-1 cells were performed and replicated by different operators and yielded the same conclusion. In addition, insulin secretion experiments using pancreatic islets from Wistar rats were conducted

in Montpellier (France) whereas the ones using pancreatic islets from GK rats were independently conducted in Paris (France) by different operators and both set of experiments showed a pharmacological effect of ResoK. Finally, statistical analyses were undertaken by another operator who was blinded for treatment assignment. All procedures were previously standardised (Bayle et al., 2019; Mesto et al., 2022; Youl et al., 2010) to improve reproducibility and lower the risk of technical bias.

## 2.8 | Data and statistical analyses

The data and statistical analyses comply with the recommendations on experimental design and analysis in pharmacology (Curtis

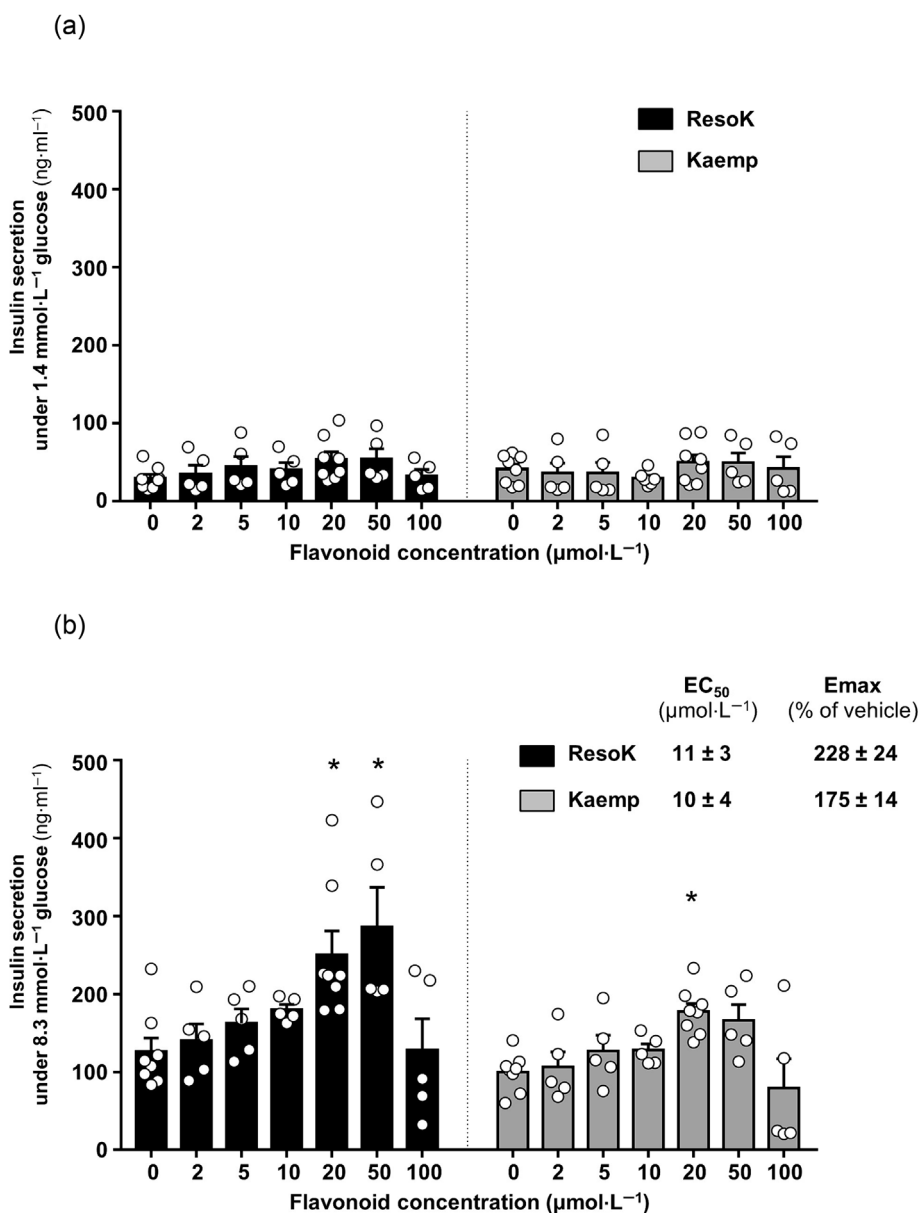


**FIGURE 1** Exploratory data reporting the effect of a set of flavonoids on glucose-induced insulin secretion in INS-1 cells. Eight flavonoids whose structures are provided in (a) were tested for their capacity to enhance glucose-induced insulin secretion. (b) INS-1 cells were incubated for 1 h with 8.3 mmol·L<sup>-1</sup> glucose in the absence (vehicle) or presence of 20 μmol·L<sup>-1</sup> of each flavonoid. After 1 h of incubation, the extracellular medium was collected and insulin concentration was quantified. The histogram shows the insulin secretion (ng·ml<sup>-1</sup>), as means ± SEM from n = 3 experiments. Kaemp, kaempferol; ResoK, resokaempferol.

et al., 2022). All the data were analysed using GraphPad Prism v10 software (Graphpad Software, San Diego, USA). Calculations of  $EC_{50}$  and  $E_{max}$  were computed using non-linear regression curve fit (variable slope - four parameters). All averaged data are expressed as mean  $\pm$  SEM. The declared group size (n) is the number of independent values and therefore always represents the number of experimental independent repeats (but not technical replicates). Statistical analysis was carried out using these independent values. Each experimental independent repeat was carried out with distinct biological preparations. Group sizes suitable for each set of experiments were estimated to ensure adequate power and to detect a pre-specified effect based on the published data, on pilot studies and on our previous reports (Bayle et al., 2019; Péraldi-Roux et al., 2022; Toubal et al., 2020). Studies were designed to generate groups of equal size. However, in Figure 2, group sizes are not equal (n = 8 or n = 5) because the control concentrations (i.e., [flavonoid] = 0 and 20  $\mu\text{mol}\cdot\text{L}^{-1}$ ) were

systematically tested to certify the validity of each independent repeat within each 24-well plate. To ensure the reliability of single values, technical triplicate and quintuplicate were used for insulin secretion experiments in INS-1 cells and Wistar pancreatic islets respectively. For insulin secretion experiments using GK pancreatic islets, each condition was run in duplicate. For electrophysiological studies, each independent experimental repeat (n) was carried out with distinct biological preparations using different INS-1 cells cultivated in different cell culture dishes. Specifically, currents from one cell were recorded in each independent experimental repeat.

Data emanating from insulin secretion experiments using rat pancreatic islets were normalised to the assay-matched basal group (i.e., insulin secretion under 2.8  $\text{mmol}\cdot\text{L}^{-1}$  glucose) to control for unwanted sources of variation and thus plotted as fold matched control values. This is necessary due to the inherent interassay variability which could distort the interpretation of the averaged data originating



**FIGURE 2** Effects of resokaempferol (ResoK) and kaempferol (Kaemp) on basal and glucose-induced insulin secretion in INS-1 cells. The effects of increasing concentrations (2–100  $\mu\text{mol}\cdot\text{L}^{-1}$ ) of ResoK and Kaemp in INS-1 cells were determined under (a) basal (1.4  $\text{mmol}\cdot\text{L}^{-1}$  glucose) or (b) 8.3  $\text{mmol}\cdot\text{L}^{-1}$  glucose-stimulated conditions. After 1 h of incubation, the extracellular medium was collected and insulin concentration ( $\text{ng}\cdot\text{ml}^{-1}$ ) was quantified. Data shown are individual values with means  $\pm$  SEM.  $E_{max}$  values are given as percentage of vehicle response under 8.3  $\text{mmol}\cdot\text{L}^{-1}$  glucose. Under basal condition (a), one-way ANOVA detected no effect of either flavonoid. Under 8.3  $\text{mmol}\cdot\text{L}^{-1}$  glucose-stimulated condition (b), there was an effect of the treatment for ResoK (Kruskal-Wallis followed by Dunn's test) and Kaemp (one-way ANOVA followed by Holm-Sidak's test). \* $P < 0.05$ , significantly different from 0  $\mu\text{mol}\cdot\text{L}^{-1}$  of flavonoid; n = 8 when [flavonoid] = 0 and 20  $\mu\text{mol}\cdot\text{L}^{-1}$ , for the other concentrations n = 5.

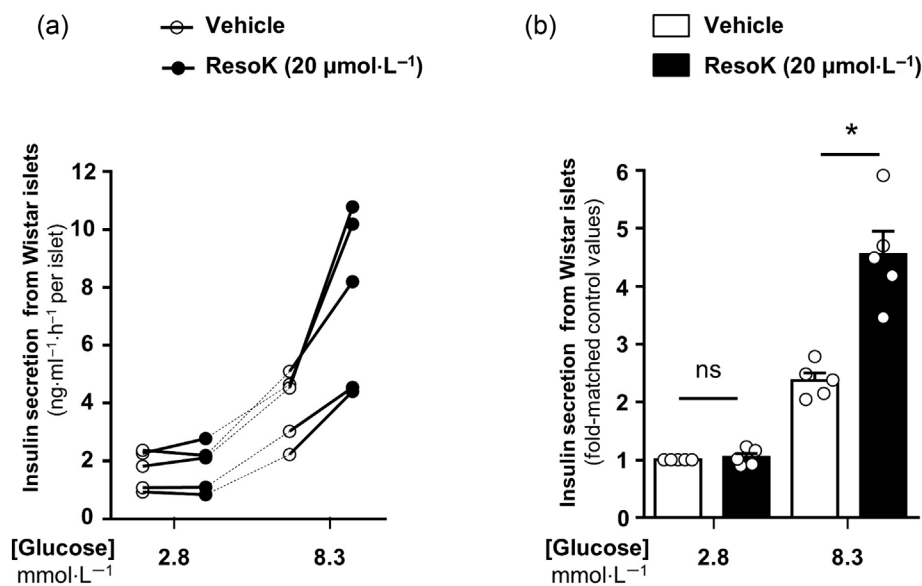
from such a biological system (e.g., islets were isolated from different animals for each independent experimental repeat; size of pancreatic islets between animals can differ so does the number of  $\beta$ -cells within each individual islet). In this case, non-parametric analyses were carried out to compare data that were not normally distributed. Nevertheless for better transparency, the data of insulin secretion (in  $\text{ng}\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$  per islet) are also provided (Figures 3a, 7c and S4). Similarly, electrophysiological data showing the stimulating effect of ResoK on L-type and T-type currents were normalised within each cell to the assay-matched vehicle treatment to control for unwanted sources of variation. This is necessary because the measured currents can largely vary between cells depending of their size for instance. This within cell normalisation allowed us to adequately quantify the average effect of ResoK on currents. Therefore, these data sets were expressed and plotted as fold matched control values (Curtis et al., 2022).

Unbiased approaches for identifying outliers could not be used because  $n < 12$  (Curtis et al., 2022). Statistical analyses were run only on group size of  $n$  independent values  $\geq 5$ , and as follows. First, each data set was tested for normality (Shapiro–Wilk test,  $P > 0.05 = \text{passed}$ ) and for variance homogeneity (Brown–Forsythe test,  $P > 0.05 = \text{passed}$ ). Parametric statistical tests ( $t$  test and ANOVAs) were used only if the data were normally distributed and there was no inhomogeneity of variance. If only one data set failed the Shapiro–Wilk test, nonparametric analyses were performed using Mann–Whitney or Kruskal–Wallis tests. Comparisons between two sets of data were performed by unpaired two-tailed  $t$  test or unpaired two-tailed Mann–Whitney test. Comparisons between multiple sets of data were performed by one-way ANOVA, two-way

ANOVA or Kruskal–Wallis tests with no matching. Holm–Sidak's or Dunn's post hoc comparisons were conducted only if tested ANOVA or Kruskal–Wallis respectively achieved statistical significance ( $P < 0.05 = \text{passed}$ ). The limit of statistical significance throughout the study was set at  $P < 0.05$ . [Correction added on 4 October 2024, after first online publication: ‘Kruskall-Wallis’ has been changed to ‘Kruskal-Wallis’ in this version.]

## 2.9 | Materials

Standard chow diet (diet D113), poplar heartwood modules for cage enrichment and Lignocel® Select Fine for bedding were from Safe (Augy, France). RPMI-1640 medium (#BE12-167F) was purchased from Lonza (Levallois Perret, France) and from CAPRICORN Scientific (#RPMI-XA). Fetal calf serum was purchased from Eurobio (Les Ulis, France). Chemical reagents used for buffer preparation in electrophysiological and insulin secretion studies, glucose (#G7528), collagenase V (#C9263), histopaque®1077 (#10771) glibenclamide (#PHR1287), monomethyl succinate (#M81101), bovine serum albumin (BSA, #A8022), DMSO (#D8418), trypsin, penicillin, streptomycin, poly-L-lysine, L-glutamine, nifedipine (#N7634), verapamil (#V4629), Z944 (#SML2635) were obtained from Merck-Sigma-Aldrich (St. Louis, MO, USA). Fatty-acid-free BSA (#10775835001) and liberase (#05401020001) for GK islet isolation were from Roche (Boulogne-Billancourt, France). Exenatide was from MedChemExpress (#HY-13443). MTT was from Euromedex (#4022). Pure flavonoids were purchased from Extrasynthese (Genay, France): ResoK (#1251, purity  $\geq 95\%$ ), kaempferol (#11245, purity  $\geq 99\%$ ), apigenin (#11025,



**FIGURE 3** Effect of resokaempferol (ResoK) on basal and glucose-induced insulin secretion from non-diabetic pancreatic islets. Pancreatic islets from Wistar rats were incubated for 1 h in the absence (vehicle) or in the presence of  $20 \mu\text{mol}\cdot\text{L}^{-1}$  ResoK under basal ( $2.8 \text{ mmol}\cdot\text{L}^{-1}$  glucose) or  $8.3 \text{ mmol}\cdot\text{L}^{-1}$  glucose-stimulated conditions and insulin secretion was quantified. The data expressed in  $\text{ng}\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$  per islet are provided in (a) where matched experiments are connected with lines. In (b), the data shown are individual values with the means  $\pm$  SEM of insulin secretion of the same data expressed as fold matched control values (i.e.,  $2.8 \text{ mmol}\cdot\text{L}^{-1}$  glucose). \* $P < 0.05$ , significantly different as indicated; ns, not significant: Mann–Whitney test,  $n = 5$ .



purity  $\geq 99\%$ ), **morin** (#1344S, purity  $\geq 99\%$ ), **chrysin** (#1362S, purity  $\geq 99\%$ ), **galangin** (#1114S, purity  $\geq 99\%$ ) and from abcr GmbH (Karlsruhe, Germany) 3-hydroxyflavone (#AB117256, purity = 98%), 4'-hydroxyflavone (#AB304389, purity = 98%). Insulin quantification was determined using PerkinElmer Insulin assay kit (#62IN2PEH, Cisbio International, Bagnols-sur-Cèze, France). Insulin quantification from GK pancreatic islets was performed by ELISA (Alpco Diagnostics, Salem, MA, USA, #80-INSRTU-E01). Stock solutions of flavonoids were prepared at 20 mmol·L<sup>-1</sup> in DMSO and stored at  $-20^{\circ}\text{C}$ . The final concentration of DMSO did not exceed 0.1% (v/v) except when ResoK was used at 50 and 100  $\mu\text{mol}\cdot\text{L}^{-1}$ . Nevertheless, all the treatments were always performed using the same amount of vehicle (DMSO) and did not affect any functional assay.

## 2.10 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <https://www.guidetopharmacology.org> and are permanently archived in the Concise Guide to PHARMACOLOGY 2021/22 (Alexander et al., 2023).

## 3 | RESULTS

### 3.1 | Identification of ResoK as an activator of glucose-induced insulin secretion

Because flavonoids can behave as pharmacological agents, their activity is strongly related to their molecular structure. However, several thousands of flavonoids have been described so far (Fusi et al., 2020). Therefore, we first decided to focus on a small set of compounds that had been shown to modulate vascular tone by acting on ion channels which are also key regulators of  $\beta$ -cell function (Carullo et al., 2021; Rorsman & Ashcroft, 2018; Saponara et al., 2011) (Figure 1a). To evaluate their effect on the insulin-secreting function of  $\beta$ -cells, INS-1 cells were exposed to these flavonoids for 1 h under a stimulating glucose concentration (8.3 mmol·L<sup>-1</sup>) and then insulin secretion was quantified. This intermediate stimulating concentration of glucose was chosen because it avoids any saturation of the system that could conceal a pharmacological action (Bayle et al., 2019). Flavonoids were used at 20  $\mu\text{mol}\cdot\text{L}^{-1}$  since this concentration approximates the EC<sub>50</sub> values that were reported in different functional assays (Fusi et al., 2020; Saponara et al., 2011; Schonhofer et al., 2021). Among the eight flavonoids tested, our exploratory data suggested that ResoK and Kaemp had the capacity to increase glucose-induced insulin secretion when used at 20  $\mu\text{mol}\cdot\text{L}^{-1}$  (Figure 1b). Hence, we next sought to determine the potency and efficacy of these two compounds on insulin secretion.

Concentration-response relationships of ResoK and Kaemp (2, 5, 10, 20, 50 and 100  $\mu\text{mol}\cdot\text{L}^{-1}$ ) were executed in INS-1 cells exposed to basal (1.4 mmol·L<sup>-1</sup> glucose) or 8.3 mmol·L<sup>-1</sup> glucose-stimulated conditions. Under basal condition none of these compounds

modulated insulin secretion (Figure 2a). In contrast, under 8.3 mmol·L<sup>-1</sup> glucose-stimulated condition, both ResoK and Kaemp enhanced insulin secretion when used at 20  $\mu\text{mol}\cdot\text{L}^{-1}$  and ResoK was also effective at 50  $\mu\text{mol}\cdot\text{L}^{-1}$  (Figure 2b). This effect declined for the two flavonoids when used at 100  $\mu\text{mol}\cdot\text{L}^{-1}$ . While ResoK and Kaemp exhibited similar EC<sub>50</sub>, our data also suggested that the efficacy (Emax) of the former was higher (Figure 2b). Under the same experimental conditions, the clinically effective antidiabetic drug exenatide displayed an EC<sub>50</sub> = 31  $\pm$  3 pmol·L<sup>-1</sup> and an Emax = 194  $\pm$  3% (in percentage of vehicle response under 8.3 mmol·L<sup>-1</sup> glucose, n = 7). In addition, during the course of the present study, Bermont et al also reported that Kaemp stimulated glucose-induced insulin secretion in INS-1E cells and in human pseudo-islets (Bermont et al., 2020) which reinforced the relevance of our data. For all these reasons, the rest of our study was focused on ResoK whose activity on the insulin-secreting function of  $\beta$ -cells was unknown. We first tested whether ResoK affected INS-1 cell viability in the same experimental settings that were used to profile its pharmacological activity on glucose-induced insulin secretion. Thus, INS-1 cells were incubated for 1 h with increasing concentrations of ResoK (2, 5, 10, 20, 50 and 100  $\mu\text{mol}\cdot\text{L}^{-1}$ ) in the presence of 8.3 mmol·L<sup>-1</sup> glucose and the cell viability was determined using MTT assay. We observed that up to 100  $\mu\text{mol}\cdot\text{L}^{-1}$ , ResoK did not compromise INS-1 cell viability (Figure S1).

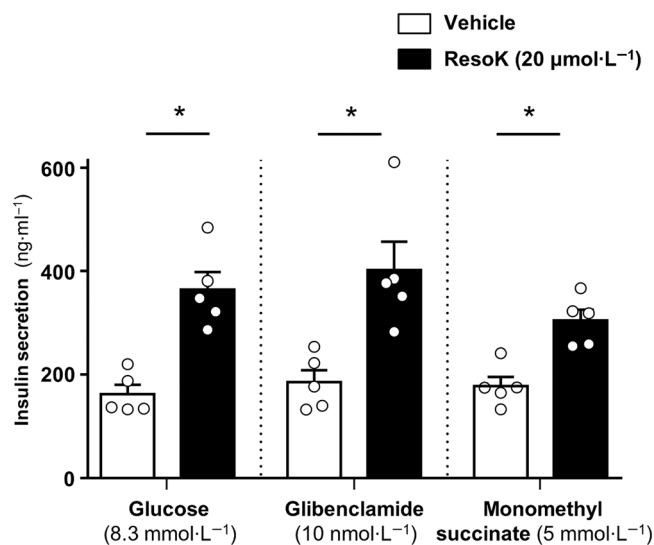
Next, we asked whether the activity of ResoK on INS-1 cells could be expanded to native  $\beta$ -cells clustered into islets of Langerhans. Therefore, pancreatic islets were isolated from non-diabetic male Wistar rats and incubated for 1 h with ResoK (20  $\mu\text{mol}\cdot\text{L}^{-1}$ ) in the presence of either a basal (2.8 mmol·L<sup>-1</sup>) or a stimulating (8.3 mmol·L<sup>-1</sup>) glucose concentration (Figures 3a,b). In line with what we observed using INS-1 cells, we found that ResoK enhanced insulin secretion in the presence of a stimulating, but not a basal, concentration of glucose.

### 3.2 | Exploration of the pharmacological action of ResoK

As we observed that the pharmacological behaviour of ResoK on insulin secretion was similar in both rat pancreatic islets and INS-1 cells, we decided to use this cell line to address the mechanism of action of this flavonoid. First, we assessed whether ResoK also modulated insulin secretion induced by two other secretagogues acting through distinct mechanisms compared to glucose. We opted for the sulfonyl-urea glibenclamide and the mitochondrial substrate monomethyl succinate. Glibenclamide stimulates insulin secretion by directly inducing the closure of K<sub>ATP</sub> and thus it does not require any metabolic signal (Perreault et al., 2021). In contrast to glibenclamide, the action of succinate derivatives such as monomethyl succinate relies on mitochondrial activation but through a mechanism that bypasses glycolysis (De Marchi et al., 2017; MacDonald et al., 1989). INS-1 cells were exposed to glibenclamide (10 nmol·L<sup>-1</sup>) and monomethyl succinate (5 mmol·L<sup>-1</sup>) using concentrations that induced release of insulin,

similar to that induced by  $8.3 \text{ mmol}\cdot\text{L}^{-1}$  glucose. As shown in Figure 4, ResoK ( $20 \text{ }\mu\text{mol}\cdot\text{L}^{-1}$ ) enhanced both glibenclamide- and monomethyl succinate-stimulated insulin secretion. These results suggested that ResoK acted on a common cellular process that is involved in the insulin secreting activity of glucose, glibenclamide and monomethyl succinate.

Because the pharmacological action of these three secretagogues depends on  $\text{Ca}^{2+}$  influx (Panten et al., 1989; Zawlich et al., 1993) and voltage-gated  $\text{Ca}^{2+}$  channels are activated only upon cellular stimulation, we next tested the ability of ResoK to modulate their activity. Therefore, we studied the electrophysiological properties of whole-cell  $\text{Ca}^{2+}$  currents in INS-1-cells with the patch-clamp method using  $\text{Ba}^{2+}$  as the charge carrier (Figure 5). We observed that ResoK ( $20 \text{ }\mu\text{mol}\cdot\text{L}^{-1}$ ) increased inward currents at potentials for which L-type current is predominant (Figure 5a). Accordingly, ResoK enhanced L-type current at test potential of  $-10 \text{ mV}$  (Figure 5b and Table S2) whereas it was ineffective on T-type current measured at  $-40 \text{ mV}$  (Figure 5c). The effect of  $20 \text{ }\mu\text{mol}\cdot\text{L}^{-1}$  ResoK on L-type current evolved rapidly, peaking within 50 s following the perfusion of the flavonoid and gradually reversed after its removal (Figure 5d). The response induced by ResoK was concentration-dependent and detected within the same micromolar range as its action on insulin secretion (Figure 5e). Finally, the current-voltage relationship normalised to  $-1$  for the maximum inward current enabled us to observe that the rise of the L-type current generated by ResoK was dependent on depolarisations (Figure 5f). Indeed, ResoK-enhanced L-type current



**FIGURE 4** Effects of resokaempferol (ResoK) on insulin secretion promoted by glucose, glibenclamide and monomethyl succinate in INS-1 cells. INS-1 cells were incubated with  $20 \text{ }\mu\text{mol}\cdot\text{L}^{-1}$  ResoK in the presence of  $8.3 \text{ mmol}\cdot\text{L}^{-1}$  glucose or  $1.4 \text{ mmol}\cdot\text{L}^{-1}$  glucose +  $10 \text{ nmol}\cdot\text{L}^{-1}$  glibenclamide or  $1.4 \text{ mmol}\cdot\text{L}^{-1}$  glucose +  $5 \text{ mmol}\cdot\text{L}^{-1}$  monomethyl succinate. After 1 h of incubation, the extracellular medium was collected and insulin concentration was quantified. The data shown in the histogram are individual values with the means  $\pm$  SEM of insulin secretion ( $\text{ng}\cdot\text{ml}^{-1}$ ). \* $P < 0.05$ , significantly different as indicated; two-tailed unpaired  $t$  test,  $n = 5$ .

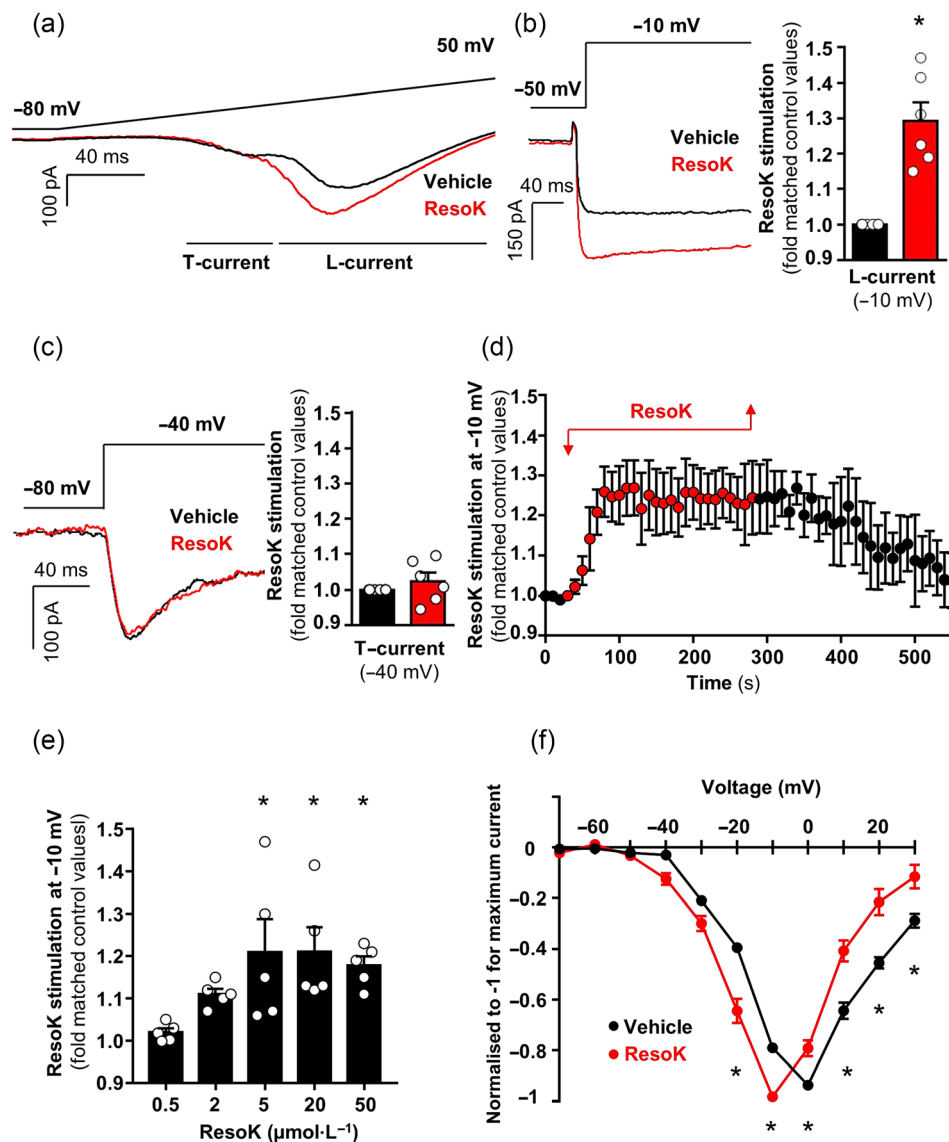
was greater for voltage stimulation around  $-20$  and  $-10 \text{ mV}$ , for which L-type current is prevailing. However, ResoK did not significantly decrease the potential at half-maximal activation ( $V_{0.5}$ ) of the L-type current (from  $-20.0 \pm 0.9 \text{ mV}$  to  $-24.8 \pm 2.3 \text{ mV}$ , for vehicle vs. ResoK respectively;  $n = 6$ ,  $P = 0.08$ ;  $t$  test).

A molecular docking study was carried out to determine whether ResoK was able to bind in the L-type  $\text{Ca}^{2+}$  channel  $\text{Ca}_v1.2$ . To this end, a homology model of the central pore of the rat  $\alpha_{1C}$  subunit was constructed using the three-dimensional cryo-EM map of the human  $\alpha_{1C}$  subunit of  $\text{Ca}_v1.2$ , which shares 97% sequence identity, in complex with benidipine (Wei et al., 2024). To validate the model, we compared the docking site of benidipine with the resolved structure of the benidipine-bound  $\text{Ca}_v1.2$  complex obtained by cryo-EM. We found that the docking site of benidipine largely overlapped its binding site identified in the experimental structure of the  $\text{Ca}_v1.2$ -benidipine complex (Figure S2). The docked pose of ResoK involves low-energy bonds between its hydroxyl groups and several amino acids of the channel located in a region that includes domain III (T1142) and domain IV (Y1489) which accommodate the dihydropyridine binding site (Figure S3).

Next, we hypothesized that the insulin-secreting activity of ResoK relied, at least in part, on its capacity to enhance L-type  $\text{Ca}^{2+}$  currents. We tested this possibility by determining the effect of ResoK on glucose-induced insulin secretion in the presence of  $\text{Ca}^{2+}$  channel inhibitors (Figure 6). First, we used two structurally and mechanistically unrelated L-type  $\text{Ca}^{2+}$  current inhibitors, namely verapamil and nifedipine (Alexander et al., 2023). Thus, INS-1 cells were incubated with ResoK ( $20 \text{ }\mu\text{mol}\cdot\text{L}^{-1}$ ) in the presence of verapamil or nifedipine and then insulin release was quantified under  $8.3 \text{ mmol}\cdot\text{L}^{-1}$  glucose-stimulated condition. Verapamil and nifedipine were used at 20 and  $1 \text{ }\mu\text{mol}\cdot\text{L}^{-1}$  respectively to ensure efficient inhibition of L-type  $\text{Ca}^{2+}$  currents in accordance with their published  $\text{pIC}_{50}$  (Alexander et al., 2023). As shown above, ResoK enhanced glucose-induced insulin secretion but this effect was not observed in the presence of verapamil (Figure 6a) or nifedipine (Figure 6b). Besides, we also evaluated the involvement of T-type  $\text{Ca}^{2+}$  currents on the insulin-secreting activity of ResoK in INS-1 cells. To do so, we used a selective inhibitor of T-type  $\text{Ca}^{2+}$  currents, namely Z944, at  $2 \text{ }\mu\text{mol}\cdot\text{L}^{-1}$  in accordance with its  $\text{pIC}_{50}$  (Alexander et al., 2023) and a previous report (Harding et al., 2021). In contrast to what we observed with L-type  $\text{Ca}^{2+}$  current inhibitors, the stimulating activity of ResoK on glucose-induced insulin secretion was preserved, after exposure to Z944 (Figure 6c).

### 3.3 | Effects of ResoK on insulin secretion in dysfunctional $\beta$ -cells

We assessed the capacity of ResoK to ameliorate insulin secretion in  $\beta$ -cells whose responsiveness to glucose was impaired (Figure 7). First, dysfunction was promoted by culturing INS-1 cells under glucotoxic conditions ( $25 \text{ mmol}\cdot\text{L}^{-1}$  glucose) for 48 h (Bayle et al., 2019). As expected, glucotoxicity compromised glucose-induced insulin secretion which attested to the dysfunction of  $\beta$ -cells (Figure 7a).

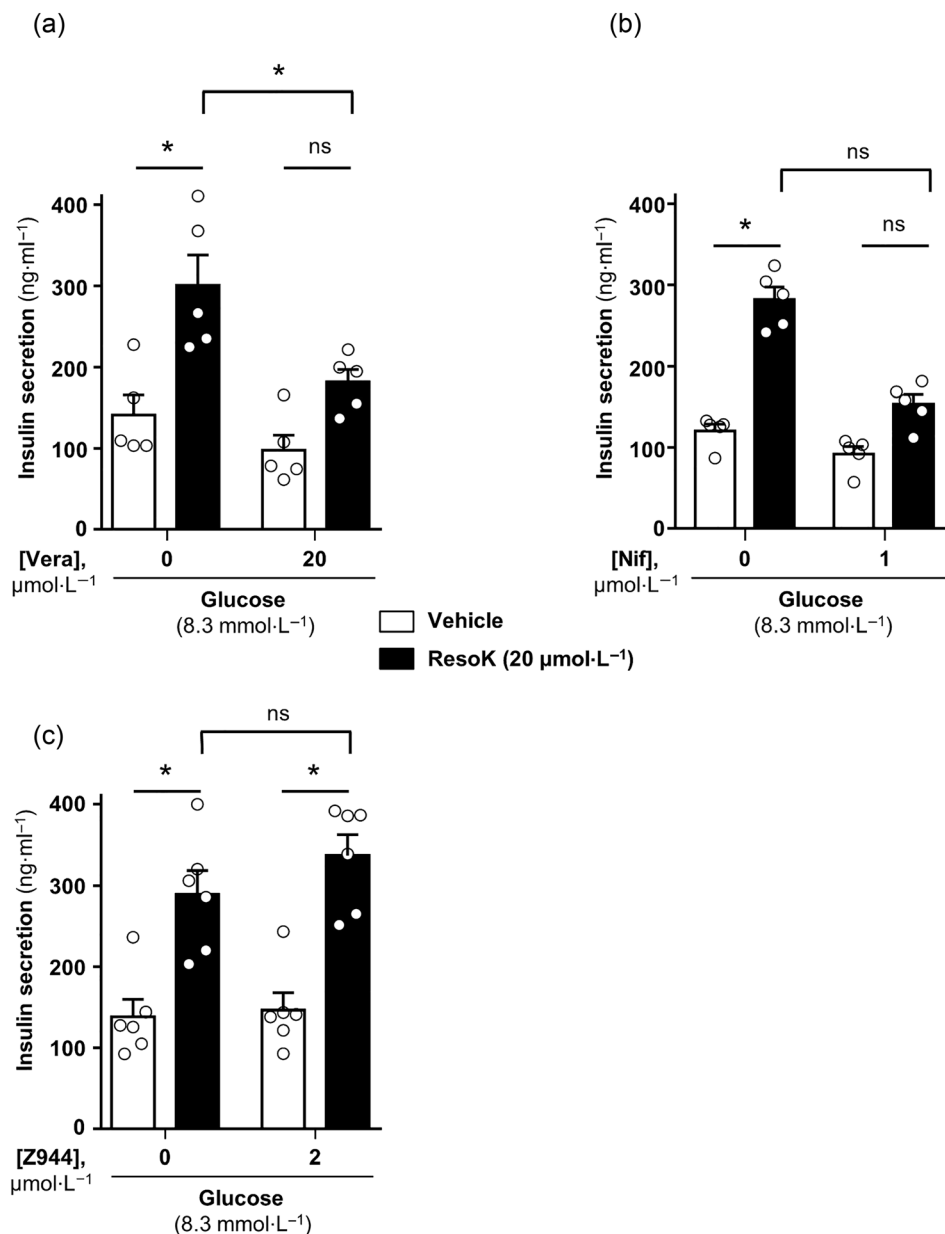


**FIGURE 5** Effects of resokaempferol (ResoK) on L- and T-type Ca<sup>2+</sup> channel currents carried by Ba<sup>2+</sup> in INS-1 cells. Typical traces of inward currents stimulated (a) by a ramp depolarisation (from a holding potential  $-80$  mV to record T and L-type currents), or (b) by a depolarisation at  $-10$  mV (from a holding potential at  $-50$  mV to record slow inactivated L-type current) or (c) by a depolarisation at  $-40$  mV (from a holding potential at  $-80$  mV to record transient T-type current) in the absence (vehicle) or presence of  $20 \mu\text{mol}\cdot\text{L}^{-1}$  ResoK. In (b) and (c), the data shown are the means  $\pm$  SEM of ResoK stimulation,  $*P < 0.05$ , significantly different from control; Mann-Whitney test,  $n = 6$ . (d) Time course of the effect of  $20 \mu\text{mol}\cdot\text{L}^{-1}$  ResoK on L-type current. ResoK was added at  $t = 30$  s and then washed out at  $t = 300$  s. (e) Concentration-response of ResoK on the L-type current. The data shown are individual values with the means  $\pm$  SEM of ResoK stimulation. One-way ANOVA showed an effect of the treatment.  $*P < 0.05$ , significantly different from  $0.5 \mu\text{mol}\cdot\text{L}^{-1}$  ResoK with Holm-Sidak's test,  $n = 5$ . (f) Effect of  $20 \mu\text{mol}\cdot\text{L}^{-1}$  ResoK on the current-to-voltage (I/V) relationship of L-type current. Both curves were normalised to  $-1$  (maximum inward current). Two-way ANOVA showed an interaction between treatment and depolarisation,  $*P < 0.05$  significantly different from vehicle with Holm-Sidak's test,  $n = 6$ .

Therefore, glucotoxicity-induced dysfunctional INS-1 cells were exposed to ResoK ( $20 \mu\text{mol}\cdot\text{L}^{-1}$ ) for 1 h under  $8.3 \text{mmol}\cdot\text{L}^{-1}$  glucose-stimulated condition and insulin release was quantified. Interestingly, we observed that ResoK was still active on defective cells as it restored glucose-induced insulin secretion in INS-1 cells that underwent glucotoxic insult (Figure 7a). In addition, using whole-cell patch clamp recording, we found that ResoK kept its capacity to enhance L-type current from INS-1 cells previously subjected to glucotoxicity (Figure 7b and Table S2).

Next, we tested the ability of ResoK to improve insulin secretion from diabetic  $\beta$ -cells within islets of Langerhans. To do so, pancreatic islets were isolated from inbred Goto-Kakizaki (GK) male rats. This strain is a lean model of spontaneous Type 2 diabetes (Goto et al., 1976; Lutz, 2023) and as a result,  $\beta$ -cells from diabetic GK rats are poorly responsive to glucose (Mesto et al., 2022; Portha et al., 2012). Therefore, GK pancreatic islets were incubated with a higher glucose concentration than the one used to stimulate Wistar pancreatic islets since our data suggested that a stimulating

**FIGURE 6** Effects of  $\text{Ca}^{2+}$  channel inhibitors on resokaempferol (ResoK)-stimulated insulin secretion in INS-1 cells. INS-1 cells were incubated for 1 h with  $8.3 \text{ mmol}\cdot\text{L}^{-1}$  glucose in the absence (vehicle) or presence of ResoK at  $20 \text{ }\mu\text{mol}\cdot\text{L}^{-1}$  and (a)  $20 \text{ }\mu\text{mol}\cdot\text{L}^{-1}$  of verapamil (Vera) or (b)  $1 \text{ }\mu\text{mol}\cdot\text{L}^{-1}$  of nifedipine (Nif) or (c)  $2 \text{ }\mu\text{mol}\cdot\text{L}^{-1}$  of Z944 and then insulin secretion was quantified. The data shown are individual values with the means  $\pm$  SEM of insulin secretion ( $\text{ng}\cdot\text{mL}^{-1}$ ). In (a), Two-way ANOVA showed an effect of both ResoK and verapamil,  $*P < 0.05$  with Holm–Sidak's test,  $n = 5$ . In (b), Kruskal–Wallis showed an effect of the treatment,  $*P < 0.05$  with Dunn's test,  $n = 5$ . In (c),  $*P < 0.05$ , two-way ANOVA showed an effect of ResoK only, with Holm–Sidak's test,  $n = 6$ .

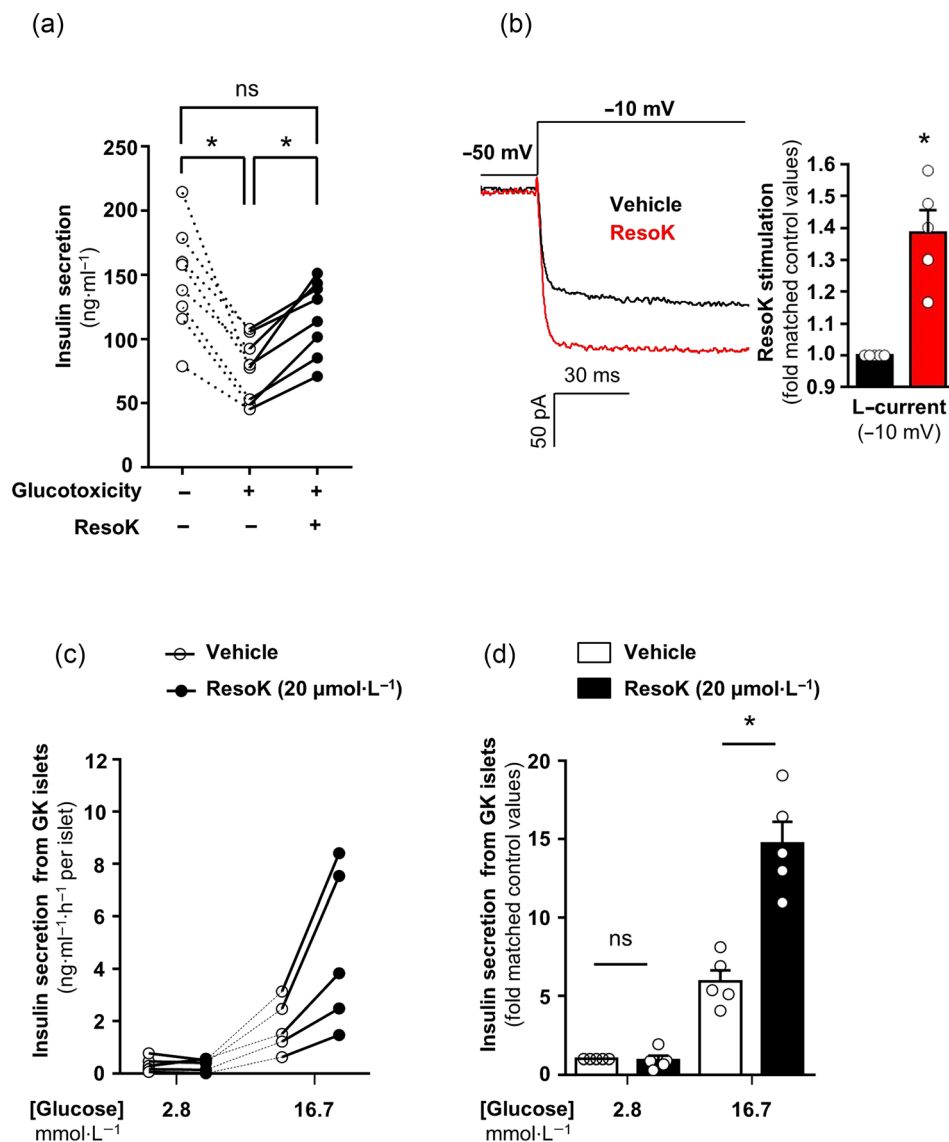


concentration of glucose was necessary to reveal the pharmacological effect of ResoK (Figures 2 and 3). Specifically, pancreatic islets from GK rats were incubated with either a basal ( $2.8 \text{ mmol}\cdot\text{L}^{-1}$ ) or a stimulating ( $16.7 \text{ mmol}\cdot\text{L}^{-1}$ ) glucose concentration in the presence or absence of ResoK ( $20 \text{ }\mu\text{mol}\cdot\text{L}^{-1}$ ) (Figure 7c,d). Importantly, we observed that ResoK retained its capacity to sustain insulin secretion from diabetic GK pancreatic islets only when they were subjected to glucose stimulation. Finally, we asked whether this pharmacological effect was sensitive to L-type  $\text{Ca}^{2+}$  current inhibition as it was the case in INS-1 cells. Therefore, GK pancreatic islets were incubated with ResoK ( $20 \text{ }\mu\text{mol}\cdot\text{L}^{-1}$ ) in the presence of  $1 \text{ }\mu\text{mol}\cdot\text{L}^{-1}$  nifedipine and then insulin release was quantified following stimulation with  $16.7 \text{ mmol}\cdot\text{L}^{-1}$  glucose (Figure S4). We observed that the stimulatory activity of ResoK on glucose-induced insulin secretion from diabetic GK pancreatic islets was inhibited by nifedipine.

## 4 | DISCUSSION

In the present study, we first analysed the activity of a small set of flavonoids on  $\beta$ -cell function with the final aim of identifying compounds capable of ameliorating glucose-induced insulin secretion from dysfunctional pancreatic  $\beta$ -cells. From this initial analysis, ResoK was selected and its pharmacological properties on  $\beta$ -cells were further characterised.

ResoK belongs to the flavonol subclass of flavonoids and is also known as 3,7,4'-trihydroxyflavone or 5-deoxykaempferol. ResoK is thus closely-related to Kaemp but, in contrast to the latter, it does not seem to occur widely throughout the plant kingdom (Del Rio et al., 2013). Although Pubchem and the LOTUS databases indicate that ResoK is a natural product found in plants such as *Pterocarpus marsupium*, *Anthyllis vulneraria* or *Lens culinaris* (Rutz et al., 2022), it is



**FIGURE 7** Effect of resokaempferol (ResoK) on insulin secretion in dysfunctional  $\beta$ -cells. INS-1 cells were cultured in a control medium or in a medium containing 25 mmol·L<sup>-1</sup> glucose for 48 h (glucotoxicity). Then, in (a) cells were incubated with 8.3 mmol·L<sup>-1</sup> glucose for 1 h, in the absence (vehicle) or in the presence of 20  $\mu$ mol·L<sup>-1</sup> ResoK and insulin secretion was assayed. Matched data are connected with lines. One-way ANOVA showed an effect of the treatment. \* $P < 0.05$ , significantly different as indicated; ns, not significant, with Holm–Sidak’s test,  $n = 8$ . Panel (b) shows typical traces of L-type Ba<sup>2+</sup> currents at test potential of  $-10$  mV in the absence (vehicle) or presence of 20  $\mu$ mol·L<sup>-1</sup> ResoK recorded from INS-1 cells beforehand subjected to glucotoxicity. The histogram shows the individual values with mean  $\pm$  SEM of ResoK stimulation; \* $P < 0.05$ , significantly different from vehicle; Mann–Whitney test,  $n = 5$ . In (c) and (d) diabetic pancreatic islets from GK rats were incubated for 1 h in the absence (vehicle) or in the presence of 20  $\mu$ mol·L<sup>-1</sup> ResoK under basal (2.8 mmol·L<sup>-1</sup> glucose) or 16.7 mmol·L<sup>-1</sup> glucose-stimulated conditions and insulin secretion was assayed. The data expressed in ng·ml<sup>-1</sup>·h<sup>-1</sup> per islet are provided in (c) where matched data are connected with lines. In (d), the data shown are the means  $\pm$  SEM of insulin secretion of the same data expressed as fold matched control values (i.e., 2.8 mmol·L<sup>-1</sup> glucose). Mann–Whitney test,  $n = 5$ .

likely to be a minor dietary component and accordingly this flavonoid has been the subject of only few pharmacological studies (Correia da Silva et al., 2022; Lee et al., 2010; Saponara et al., 2011; Yu et al., 2016). Historically, most studies investigating the biological activities of flavonoids have primarily considered compounds such as Kaemp, quercetin or catechins, or their derivatives, that commonly occur in foodstuffs (Del Rio et al., 2013). Here, we have not opted for a nutritionally-based approach. Instead, we prioritised the favourable

pharmacological actions elicited by flavonoids on  $\beta$ -cell function by measuring their capacity to enhance glucose-induced insulin secretion because  $\beta$ -cell physiology and signalling are finely and essentially tuned in a way to properly secrete insulin upon glycaemic demand (Noguchi & Huisang, 2019). This framework prompted us to focus on ResoK which we found to be particularly active in enhancing insulin secretion in response to several secretagogues and whose pharmacological activity on  $\beta$ -cell function had never been explored. The

diverse pharmacological landscape of the flavonoids tested here on  $\beta$ -cell function indicates that their activity is highly dependent on their molecular structures (Fusi et al., 2017; Yokoyama et al., 2015). This is in line with previous studies showing that these closely-related molecules are not merely antioxidant compounds but rather behave as pharmacological agents and thus modulate cell processes through protein-mediated mechanisms following selective binding to targets (Anghel et al., 2022; Fusi et al., 2020; Lin et al., 2022; Saponara et al., 2011; Walker et al., 2000; Zheng et al., 2022). In this respect, common dietary flavonoids such as quercetin or catechins were reported to protect or sustain  $\beta$ -cell survival and function through the modulation of distinct signalling pathways (Dall'Asta et al., 2015; Ghorbani et al., 2019).

Here, we report that micromolar concentrations of ResoK were necessary to generate a pharmacological action on insulin secretion. Specifically, the  $EC_{50}$  of ResoK was approximately  $10 \mu\text{mol}\cdot\text{L}^{-1}$  which falls within the same range of potencies that were published for other flavonoids in various biological systems (Anghel et al., 2022; Biagioli et al., 2021; Jing et al., 2021; Salehi et al., 2020; Saponara et al., 2011). Such concentrations are unlikely to be reached in the bloodstream following consumption of food as, in this case,  $C_{\text{max}}$  values of flavonoids and their metabolites are generally lower than  $1 \mu\text{mol}\cdot\text{L}^{-1}$  (Fusi et al., 2020; Williamson et al., 2018). From a therapeutic perspective, ongoing improvements of drug delivery aimed at increasing flavonoid bioavailability and pharmacokinetics studies indicate that micromolar concentration of flavonoids used in vitro are achievable in the bloodstream following oral administration (Bilia et al., 2017; Fusi et al., 2020; Ghorbani et al., 2019; Penalva et al., 2017). Evidence of safety of ResoK when used at such non-dietary dose is lacking. Nevertheless, its closely-related analogue, Kaemp, failed to demonstrate any safety concerns in healthy adults when administered orally at a dose five times higher than the estimated human dietary intake (Akiyama et al., 2023).

Our present results showed that ResoK improved the insulin secreting function of INS-1 cells and also of  $\beta$ -cells clustered in their native context that is to say within pancreatic islets. Importantly, we observed that, in both cases, ResoK effect was evoked only upon exposure of cells to a stimulating concentration of glucose. These findings indicated that the insulinotropic activity of ResoK is glucose-dependent, in contrast to current sulfonylurea antidiabetic drugs. Such a pharmacological feature is essential to leveraging the insulinotropic effect of a compound while minimising side-effect liabilities and in particular the likelihood of experiencing hypoglycaemia (Perreault et al., 2021). The glucose dependency effect of ResoK also implied that it targeted a glucose-dependent regulator of insulin secretion and/or was unable to modulate its activity in the absence of any stimulus. Because voltage-gated  $\text{Ca}^{2+}$  channels ( $\text{Ca}_v$ ) are key regulators of insulin secretion and are activated only when cellular depolarisation exceeds a threshold (Gilon et al., 2014; Rorsman & Ashcroft, 2018), we investigated their involvement in the pharmacological action of ResoK. The use of two mechanistically distinct inhibitors of  $\text{Ca}_v1$  channels, namely verapamil and nifedipine (Alexander et al., 2023), pinpointed their contribution in the pharmacological effect of ResoK

in INS-1 cells. This finding suggested that ResoK behaved as an activator of L-type currents which are conducted by  $\text{Ca}_v1$  channels. This pharmacological behaviour is, indeed, expected to translate into an enhancement of insulin secretion from  $\beta$ -cells as  $\text{Ca}^{2+}$  influx through  $\text{Ca}_v1$  channels plays a major role over the other subtypes of  $\text{Ca}_v$  to trigger insulin exocytosis (Yang & Berggren, 2006). In contrast, inhibition of  $\text{Ca}_v3$  channels, which are also expressed in  $\beta$ -cells (Yang & Berggren, 2006), did not alter ResoK effect indicating that this flavonoid was unable to modulate T-type currents. Patch-clamp studies which allowed us to individually record L-type and T-type currents in the presence of ResoK further substantiated its relative stimulatory selectivity towards  $\text{Ca}_v1$  over  $\text{Ca}_v3$  channels. A similar selectivity has been previously described for quercetin when tested on rat arterial smooth muscle cells and INS-1 cells (Bardy et al., 2013; Saponara et al., 2002). However, quercetin also stimulated insulin secretion in INS-1 cells submitted to a non-stimulatory glucose concentration ( $1.4 \text{mmol}\cdot\text{L}^{-1}$ ) (Bardy et al., 2013) in contrast to what we report here for ResoK. Surprisingly, seminal work by Saponara et al identified ResoK as an inhibitor of L-type current in rat arterial myocytes (Saponara et al., 2011). Even though, we have not undertaken an in-depth analysis of this discrepancy, such a pleiotropic activity has already been described for certain dihydropyridines whose activity can shift from agonism to antagonism depending on the experimental conditions (Hockerman et al., 1997; Lacinová, 2005).

Electrophysiological studies also indicated that ResoK stimulated L-type currents in a concentration- and voltage-dependent fashion. This effect arose at membrane potentials above depolarisation threshold and culminated around  $-20$  and  $-10$  mV. This pharmacological behaviour on L-type current is predicted to enhance  $\text{Ca}^{2+}$  influx in the presence of ResoK when any stimulus depolarised cells sufficiently to move the resting potential towards  $-20$  mV. Thus, this could explain why ResoK also stimulated insulin secretion induced by two other unrelated compounds, namely glibenclamide and monomethyl succinate, that cause membrane depolarisation yet through two dissimilar mechanisms (Heart et al., 2007; Perreault et al., 2021). Besides, ResoK did not stimulate L-type current at resting potential or low depolarising stimuli and, unlike quercetin, it did not modify  $V_{0.5}$  (Bardy et al., 2013). These data corroborated the finding that ResoK was unable to stimulate insulin secretion from resting cells, in particular under a non-stimulating glucose concentration by contrast to quercetin. Time-course studies indicated that the stimulatory effect of ResoK on L-type currents developed within a few seconds, raising the possibility it directly targeted  $\text{Ca}_v1$  channels. This hypothesis is supported by previously reported docking simulations and our own, which predict that  $\text{Ca}_v1$ , at least the rat  $\text{Ca}_v1.2$  subtype, can accommodate flavonoid templates including ResoK through the formation of an interaction network with several amino acids located in the same binding pocket of the  $\alpha_{1C}$  subunit (Carullo et al., 2021; Fusi et al., 2017; Trezza et al., 2022).

The pharmacological properties of ResoK shed light on its potential to compensate for  $\beta$ -cell dysfunction, mainly by supporting insulin secretion when glucose concentration exceeds threshold values. We therefore ensured that the glucose-dependent activity of ResoK on

insulin secretion was preserved in cellular models in which  $\beta$ -cell function was spontaneously or intentionally deteriorated. For this purpose, the use of pancreatic islets prepared from GK rats constituted a model of choice as this rodent strain spontaneously develops Type 2 diabetes-like phenotypes, owing to the loss of  $\beta$ -cell mass and defective insulin secretion, rather than insulin resistance (Lutz, 2023; Portha et al., 2012). We used GK/Par rats 8 to 10 weeks-old, as at this age,  $\beta$ -cell secretory dysfunction is already apparent along with hyperglycaemia (Portha et al., 2012). In our experimental setting, ResoK also improved insulin release from GK islets only when they were subjected to a stimulatory glucose concentration ( $16.7 \text{ mmol}\cdot\text{L}^{-1}$ ) and this pharmacological effect was inhibited by nifedipine. Together, these data provided further evidence that the pharmacological activity of ResoK was glucose-dependent and relied, at least in part, on functional  $\text{Ca}_v1$  channels in diabetic GK pancreatic islets. In line with these findings, we observed that ResoK was still able to stimulate L-type current in dysfunctional INS-1 cells raising the hypothesis that  $\text{Ca}_v1$  channels may constitute a glucose-dependent druggable target aimed at improving the ability of glucose to stimulate insulin secretion. It should be noted that the activity of ResoK was explored without considering sex-related biological factors that can also influence islet cell function (Gannon et al., 2018). In particular, the effect of ResoK remains to be evaluated in pancreatic islets prepared from female rats in order to determine whether its pharmacological profile is preserved regardless of sex differences.

In the setting of metabolic disorders, the multi-target nature of flavonoids (Carullo et al., 2021; Fusi et al., 2020) could endow ResoK with other exploitable health activities beyond improving  $\beta$ -cell function, as other cell types and tissues involved in diabetes-related complications are sensitive to glycaemic dysregulation. Accordingly, the search of unimolecular multifunctional compounds is currently a matter of extensive research in order to provide new options tailored to different groups of patients according to their comorbidities (Bailey et al., 2023). In particular, a number of flavonoids and their derivatives elicit cardiovascular benefits in different biological systems (Fusi et al., 2020; Man, Xia, et al., 2020; Man, Zhou, et al., 2020) which could spur interest for future evaluation of ResoK activity in animal models of cardiometabolic disorders. Such follow-up studies may potentially make ResoK a chemical scaffold for the design of more valuable compounds. Nevertheless and in this perspective, careful determination of the structural determinants responsible for the pharmacological activity of ResoK is a prerequisite as subtle modifications in the chemical structure of flavonoids can alter their activity (Saponara et al., 2011).

In conclusion, by focusing on the underappreciated bioactivity of ResoK, this work further documents the pharmacological effects attributed to flavonoid compounds. Specifically, our findings showed that ResoK exerted action on  $\beta$ -cell function and importantly this action was glucose-dependent and was conserved in diabetic  $\beta$ -cells which led to an improvement of glucose-stimulated insulin secretion. Through the implementation of pharmacological and electrophysiological approaches, we also proposed a mechanistic insight that implies a selective stimulation of L-type  $\text{Ca}^{2+}$  current by ResoK. Altogether, our

data constitute a preliminary phase towards the development of ResoK-based compounds to sustain glucose-stimulated insulin secretion from dysfunctional  $\beta$ -cells.

## AUTHOR CONTRIBUTIONS

**Guillaume Gautheron:** Data curation; formal analysis; investigation; methodology; resources; validation. **Sylvie Péraldi-Roux:** Data curation; formal analysis; investigation; methodology. **Justine Vaillé:** Data curation; formal analysis; investigation. **Andrzej Patyra:** Formal analysis; investigation. **Morgane Bayle:** Formal analysis; investigation. **Estelle Youl:** Formal analysis; investigation. **Soufiyan Omhmed:** Formal analysis; investigation. **G rard Cros:** Conceptualization; funding acquisition; project administration. **Benjamin Uzan:** Conceptualization; data curation; formal analysis; investigation; validation. **Jamileh Movassat:** Conceptualization; data curation; formal analysis; investigation; validation. **Jean-Fran ois Quignard:** Conceptualization; data curation; formal analysis; investigation; methodology; validation. **J r mie Neasta:** Conceptualization; investigation; methodology; project administration; supervision; validation; writing—original draft; writing—review and editing. **Catherine Oiry:** Conceptualization; funding acquisition; investigation; methodology; project administration; supervision; validation; writing—original draft; writing—review and editing.

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## CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to disclose.

## DATA AVAILABILITY STATEMENT

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

## DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the BJP guidelines for [Design and Analysis](#) and [Animal Experimentation](#), and as recommended by funding agencies, publishers and other organisations engaged with supporting research.

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## REFERENCES

- Akiyama, M., Mizokami, T., Ito, H., & Ikeda, Y. (2023). A randomized, placebo-controlled trial evaluating the safety of excessive administration of kaempferol aglycone. *Food Science & Nutrition*, 11, 5427–5437. <https://doi.org/10.1002/fsn3.3499>

- Alexander, S. P. H., Mathie, A. A., Peters, J. A., Veale, E. L., Striessnig, J., Kelly, E., Armstrong, J. F., Faccenda, E., Harding, S. D., Davies, J. A., Aldrich, R. W., Attali, B., Baggetta, A. M., Becirovic, E., Biel, M., Bill, R. M., Caceres, A. I., Catterall, W. A., Conner, A. C., ... Zhu, M. (2023). The Concise Guide to PHARMACOLOGY 2023/24: Ion channels. *British Journal of Pharmacology*, 180(Suppl 2), S145–S222. <https://doi.org/10.1111/bph.16181>
- Anghel, S. A., Badea, R. A., Chiritoiu, G., Patriche, D. S., Alexandru, P. R., & Pena, F. (2022). Novel luciferase-based glucagon-like peptide 1 reporter assay reveals naturally occurring secretagogues. *British Journal of Pharmacology*, 179, 4738–4753. <https://doi.org/10.1111/bph.15896>
- Bailey, C. J., Flatt, P. R., & Conlon, J. M. (2023). An update on peptide-based therapies for type 2 diabetes and obesity. *Peptides*, 161, 170939. <https://doi.org/10.1016/j.peptides.2023.170939>
- Bardy, G., Virsolvy, A., Quignard, J. F., Ravier, M. A., Bertrand, G., Dalle, S., Cros, G., Magous, R., Richard, S., & Oiry, C. (2013). Quercetin induces insulin secretion by direct activation of L-type calcium channels in pancreatic beta cells. *British Journal of Pharmacology*, 169, 1102–1113. <https://doi.org/10.1111/bph.12194>
- Bayle, M., Neasta, J., Dall'Asta, M., Gautheron, G., Virsolvy, A., Quignard, J. F., Youl, E., Magous, R., Guichou, J. F., Crozier, A., del Rio, D., Cros, G., & Oiry, C. (2019). The ellagitannin metabolite urolithin C is a glucose-dependent regulator of insulin secretion through activation of L-type calcium channels. *British Journal of Pharmacology*, 176, 4065–4078. <https://doi.org/10.1111/bph.14821>
- Bayle, M., Péraldi-Roux, S., Gautheron, G., Cros, G., Oiry, C., & Neasta, J. (2022). Liver-expressed antimicrobial peptide 2 antagonizes the insulinostatic effect of ghrelin in rat isolated pancreatic islets. *Fundamental & Clinical Pharmacology*, 36, 375–377. <https://doi.org/10.1111/fcp.12722>
- Bermont, F., Hermant, A., Benninga, R., Chabert, C., Jacot, G., Santo-Domingo, J., Kraus, M. R. C., Feige, J. N., & de Marchi, U. (2020). Targeting mitochondrial calcium uptake with the natural Flavonol Kaempferol, to promote metabolism/secretion coupling in pancreatic  $\beta$ -cells. *Nutrients*, 12, 538. <https://doi.org/10.3390/nu12020538>
- Biagioli, M., Marchianò, S., Roselli, R., di Giorgio, C., Bellini, R., Bordini, M., Gidari, A., Sabbatini, S., Francisci, D., Fiorillo, B., Catalanotti, B., Distrutti, E., Carino, A., Zampella, A., Costantino, G., & Fiorucci, S. (2021). Discovery of a AHR pelargonidin agonist that counter-regulates Ace2 expression and attenuates ACE2-SARS-CoV-2 interaction. *Biochemical Pharmacology*, 188, 114564. <https://doi.org/10.1016/j.bcp.2021.114564>
- Bilia, A., Piazzini, V., Guccione, C., Risaliti, L., Asprea, M., Capocchi, G., & Bergonzi, M. C. (2017). Improving on nature: The role of nanomedicine in the development of clinical natural drugs. *Planta Medica*, 83, 366–381. <https://doi.org/10.1055/s-0043-102949>
- Cao, Y.-L., Lin, J.-H., Hammes, H.-P., & Zhang, C. (2022). Flavonoids in treatment of chronic kidney disease. *Molecules*, 27, 2365. <https://doi.org/10.3390/molecules27072365>
- Carullo, G., Ahmed, A., Trezza, A., Spiga, O., Brizzi, A., Saponara, S., Fusi, F., & Aiello, F. (2021). A multitarget semi-synthetic derivative of the flavonoid morin with improved in vitro vasorelaxant activity: Role of CaV1.2 and KCa1.1 channels. *Biochemical Pharmacology*, 185, 114429. <https://doi.org/10.1016/j.bcp.2021.114429>
- Correia da Silva, D., Valentão, P., Andrade, P. B., & Pereira, D. M. (2022). A pipeline for natural small molecule inhibitors of endoplasmic reticulum stress. *Frontiers in Pharmacology*, 13, 956154. <https://doi.org/10.3389/fphar.2022.956154>
- Curtis, M. J., Alexander, S. P. H., Cirino, G., George, C. H., Kendall, D. A., Insel, P. A., Izzo, A. A., Ji, Y., Panettieri, R. A., Patel, H. H., Sobey, C. G., Stanford, S. C., Stanley, P., Stefanska, B., Stephens, G. J., Teixeira, M. M., Vergnolle, N., & Ahluwalia, A. (2022). Planning experiments: Updated guidance on experimental design and analysis and their reporting III. *British Journal of Pharmacology*, 179, 3907–3913. <https://doi.org/10.1111/bph.15868>
- Dall'Asta, M., Bayle, M., Neasta, J., Scazzino, F., Bruni, R., Cros, G., Del Rio, D., & Oiry, C. (2015). Protection of pancreatic  $\beta$ -cell function by dietary polyphenols. *Phytochemistry Reviews*, 14, 933–959.
- De Marchi, U., Hermant, A., Thevenet, J., Ratinaud, Y., Santo-Domingo, J., Barron, D., & Wiederkehr, A. (2017). A novel ATP-synthase-independent mechanism coupling mitochondrial activation to exocytosis in insulin-secreting cells. *Journal of Cell Science*, 130, 1929–1939. <https://doi.org/10.1242/jcs.200741>
- Del Rio, D., Rodriguez-Mateos, A., Spencer, J. P. E., Tognolini, M., Borges, G., & Crozier, A. (2013). Dietary (poly)phenolics in human health: Structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxidants & Redox Signaling*, 18, 1818–1892. <https://doi.org/10.1089/ars.2012.4581>
- Eberhardt, J., Santos-Martins, D., Tillack, A. F., & Forli, S. (2021). AutoDock Vina 1.2.0: New docking Methods, expanded force field, and python bindings. *Journal of Chemical Information and Modeling*, 61, 3891–3898. <https://doi.org/10.1021/acs.jcim.1c00203>
- Fu, Z., Zhang, W., Zhen, W., Lum, H., Nadler, J., Bassaganya-Riera, J., Jia, Z., Wang, Y., Misra, H., & Liu, D. (2010). Genistein induces pancreatic beta-cell proliferation through activation of multiple signaling pathways and prevents insulin-deficient diabetes in mice. *Endocrinology*, 151, 3026–3037. <https://doi.org/10.1210/en.2009-1294>
- Fusi, F., Spiga, O., Trezza, A., Sgaragli, G., & Saponara, S. (2017). The surge of flavonoids as novel, fine regulators of cardiovascular Cav channels. *European Journal of Pharmacology*, 796, 158–174. <https://doi.org/10.1016/j.ejphar.2016.12.033>
- Fusi, F., Trezza, A., Tramaglino, M., Sgaragli, G., Saponara, S., & Spiga, O. (2020). The beneficial health effects of flavonoids on the cardiovascular system: Focus on K<sup>+</sup> channels. *Pharmacological Research*, 152, 104625. <https://doi.org/10.1016/j.phrs.2019.104625>
- Gannon, M., Kulkarni, R. N., Tse, H. M., & Mauvais-Jarvis, F. (2018). Sex differences underlying pancreatic islet biology and its dysfunction. *Molecular Metabolism*, 15, 82–91. <https://doi.org/10.1016/j.molmet.2018.05.017>
- Ghorbani, A., Rashidi, R., & Shafiee-Nick, R. (2019). Flavonoids for preserving pancreatic beta cell survival and function: A mechanistic review. *Biomedicine & Pharmacotherapy*, 111, 947–957. <https://doi.org/10.1016/j.biopha.2018.12.127>
- Gilon, P., Chae, H.-Y., Rutter, G. A., & Ravier, M. A. (2014). Calcium signaling in pancreatic  $\beta$ -cells in health and in type 2 diabetes. *Cell Calcium*, 56, 340–361. <https://doi.org/10.1016/j.ceca.2014.09.001>
- Goto, Y., Kakizaki, M., & Masaki, N. (1976). Production of spontaneous diabetic rats by repetition of selective breeding. *The Tohoku Journal of Experimental Medicine*, 119, 85–90. <https://doi.org/10.1620/tjem.119.85>
- Harding, E. K., Dedek, A., Bonin, R. P., Salter, M. W., Snutch, T. P., & Hildebrand, M. E. (2021). The T-type calcium channel antagonist, Z944, reduces spinal excitability and pain hypersensitivity. *British Journal of Pharmacology*, 178, 3517–3532. <https://doi.org/10.1111/bph.15498>
- Heart, E., Yaney, G. C., Corkey, R. F., Schultz, V., Luc, E., Liu, L., Deeney, J. T., Shirihai, O., Tornheim, K., Smith, P. J., & Corkey, B. E. (2007). Ca<sup>2+</sup>, NAD(P)H and membrane potential changes in pancreatic  $\beta$ -cells by methyl succinate: Comparison with glucose. *The Biochemical Journal*, 403, 197–205.
- Heikkilä, E., Hermant, A., Thevenet, J., Bermont, F., Kulkarni, S. S., Ratajczak, J., Santo-Domingo, J., Dioum, E. H., Canto, C., Barron, D., Wiederkehr, A., & de Marchi, U. (2019). The plant product quinic acid activates Ca<sup>2+</sup>-dependent mitochondrial function and promotes insulin secretion from pancreatic beta cells. *British Journal of Pharmacology*, 176, 3250–3263. <https://doi.org/10.1111/bph.14757>



- Hockerman, G. H., Peterson, B. Z., Johnson, B. D., & Catterall, W. A. (1997). Molecular determinants of drug binding and action on L-type calcium channels. *Annual Review of Pharmacology and Toxicology*, 37, 361–396. <https://doi.org/10.1146/annurev.pharmtox.37.1.361>
- Howes, M.-J. R., Perry, N. S. L., Vázquez-Londoño, C., & Perry, E. K. (2020). Role of phytochemicals as nutraceuticals for cognitive functions affected in ageing. *British Journal of Pharmacology*, 177, 1294–1315. <https://doi.org/10.1111/bph.14898>
- Jing, S., Wang, L., Wang, T., Fan, L., Chen, L., Xiang, H., Shi, Y., & Wang, D. (2021). Myricetin protects mice against MRSA-related lethal pneumonia by targeting ClpP. *Biochemical Pharmacology*, 192, 114753. <https://doi.org/10.1016/j.bcp.2021.114753>
- Keylani, K., Arbab Mojeni, F., Khalaji, A., Rasouli, A., Aminzade, D., Karimi, M. A., Sanaye, P. M., Khajevand, N., Nemayandeh, N., Poudineh, M., Azizabadi Farahani, M., Esfandiari, M. A., Haghshoar, S., Kheirandish, A., Amouei, E., Abdi, A., Azizinezhad, A., Khani, A., & Deravi, N. (2023). Endoplasmic reticulum as a target in cardiovascular diseases: Is there a role for flavonoids? *Frontiers in Pharmacology*, 13, 1027633. <https://doi.org/10.3389/fphar.2022.1027633>
- Lacinová, L. (2005). Voltage-dependent calcium channels. *General Physiology and Biophysics*, 24(Suppl 1), 1–78.
- Lee, K. M., Lee, K. W., Byun, S., Jung, S. K., Seo, S. K., Heo, Y.-S., Bode, A. M., Lee, H. J., & Dong, Z. (2010). 5-deoxykaempferol plays a potential therapeutic role by targeting multiple signaling pathways in skin cancer. *Cancer Prevention Research (Philadelphia, Pa.)*, 3, 454–465. <https://doi.org/10.1158/1940-6207.CAPR-09-0137>
- Lilley, E., Stanford, S. C., Kendall, D. E., Alexander, S. P. H., Cirino, G., Docherty, J. R., George, C. H., Insel, P. A., Izzo, A. A., Ji, Y., Panettieri, R. A., Sobey, C. G., Stefanska, B., Stephens, G., Teixeira, M., & Ahluwalia, A. (2020). ARRIVE 2.0 and the British Journal of pharmacology: Updated guidance for 2020. *British Journal of Pharmacology*, 177, 3611–3616. <https://doi.org/10.1111/bph.15178>
- Lin, Y.-T., Li, Y., Hsu, H.-C., Tsai, J.-Y., Lee, J.-H., Tai, C.-J., Wu, M. J., & Wu, C. C. (2022). Discovery of 7, 4'-dimethoxy-3-hydroxyflavone as a protease-activated receptor 4 antagonist with antithrombotic activity and less bleeding tendency in mice. *Biochemical Pharmacology*, 202, 115152. <https://doi.org/10.1016/j.bcp.2022.115152>
- Lutz, T. A. (2023). Mammalian models of diabetes mellitus, with a focus on type 2 diabetes mellitus. *Nature Reviews. Endocrinology*, 19, 350–360. <https://doi.org/10.1038/s41574-023-00818-3>
- MacDonald, M. J., Fahien, L. A., Mertz, R. J., & Rana, R. S. (1989). Effect of esters of succinic acid and other citric acid cycle intermediates on insulin release and inositol phosphate formation by pancreatic islets. *Archives of Biochemistry and Biophysics*, 269, 400–406. [https://doi.org/10.1016/0003-9861\(89\)90123-9](https://doi.org/10.1016/0003-9861(89)90123-9)
- Man, A. W. C., Xia, N., Daiber, A., & Li, H. (2020). The roles of gut microbiota and circadian rhythm in the cardiovascular protective effects of polyphenols. *British Journal of Pharmacology*, 177, 1278–1293. <https://doi.org/10.1111/bph.14850>
- Man, A. W. C., Zhou, Y., Xia, N., & Li, H. (2020). Involvement of gut microbiota, microbial metabolites and interaction with polyphenol in host immunometabolism. *Nutrients*, 12, 3054. <https://doi.org/10.3390/nu12103054>
- Mesto, N., Bailbe, D., Eskandar, M., Pommier, G., Gil, S., Tolu, S., Movassat, J., & Turrel-Cuzin, C. (2022). Involvement of P2Y signaling in the restoration of glucose-induced insulin exocytosis in pancreatic  $\beta$  cells exposed to glucotoxicity. *Journal of Cellular Physiology*, 237, 881–896. <https://doi.org/10.1002/jcp.30564>
- Miteva, M. A., Guyon, F., & Tufféry, P. (2010). Frog2: Efficient 3D conformation ensemble generator for small compounds. *Nucleic Acids Research*, 38, W622–W627. <https://doi.org/10.1093/nar/gkq325>
- Noguchi, G. M., & Huising, M. O. (2019). Integrating the inputs that shape pancreatic islet hormone release. *Nature Metabolism*, 1, 1189–1201. <https://doi.org/10.1038/s42255-019-0148-2>
- Panten, U., Burgfeld, J., Goerke, F., Rennie, M., Schwanstecher, M., Wallasch, A., Zütkler, B. J., & Lenzen, S. (1989). Control of insulin secretion by sulfonylureas, meglitinide and diazoxide in relation to their binding to the sulfonylurea receptor in pancreatic islets. *Biochemical Pharmacology*, 38, 1217–1229. [https://doi.org/10.1016/0006-2952\(89\)90327-4](https://doi.org/10.1016/0006-2952(89)90327-4)
- Patyra, A., Vaillé, J., Omhmed, S., Dudek, M. K., Neasta, J., Kiss, A. K., & Oiry, C. (2024). Pharmacological and phytochemical insights on the pancreatic  $\beta$ -cell modulation by Angelica L. roots. *Journal of Ethnopharmacology*, 329, 118133. <https://doi.org/10.1016/j.jep.2024.118133>
- Penalva, R., González-Navarro, C. J., Gamazo, C., Esparza, I., & Irache, J. M. (2017). Zein nanoparticles for oral delivery of quercetin: Pharmacokinetic studies and preventive anti-inflammatory effects in a mouse model of endotoxemia. *Nanomedicine: Nanotechnology, Biology and Medicine*, 13, 103–110.
- Péraldi-Roux, S., Bayle, M., M'Kadmi, C., Damian, M., Vaillé, J., Fernandez, G., Cornejo, M. P., Marie, J., Banères, J. L., Ben Haj Salah, K., Fehrentz, J. A., Cantel, S., Perello, M., Denoyelle, S., Oiry, C., & Neasta, J. (2022). Design and characterization of a triazole-based growth hormone secretagogue receptor modulator inhibiting the glucoregulatory and feeding actions of ghrelin. *Biochemical Pharmacology*, 202, 115114. <https://doi.org/10.1016/j.bcp.2022.115114>
- Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M. T., Baker, M., Browne, W. J., Clark, A., Cuthill, I. C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S. T., Howells, D. W., Karp, N. A., Lasic, S. E., Lidster, K., MacCallum, C. J., Macleod, M., ... Würbel, H. (2020). The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *British Journal of Pharmacology*, 177, 3617–3624. <https://doi.org/10.1111/bph.15193>
- Perreault, L., Skyler, J. S., & Rosenstock, J. (2021). Novel therapies with precision mechanisms for type 2 diabetes mellitus. *Nature Reviews. Endocrinology*, 17, 364–377. <https://doi.org/10.1038/s41574-021-00489-y>
- Pihan, E., Colliandre, L., Guichou, J.-F., & Douguet, D. (2012). E-Drug3D: 3D structure collections dedicated to drug repurposing and fragment-based drug design. *Bioinformatics*, 28, 1540–1541. <https://doi.org/10.1093/bioinformatics/bts186>
- Pons, J.-L., & Labesse, G. (2009). @TOME-2: A new pipeline for comparative modeling of protein-ligand complexes. *Nucleic Acids Research*, 37, W485–W491. <https://doi.org/10.1093/nar/gkp368>
- Portha, B., Giroix, M.-H., Turrel-Cuzin, C., Le-Stunff, H., & Movassat, J. (2012). The GK Rat: A Prototype for the Study of Non-overweight Type 2 Diabetes. In H.-G. Joost, H. Al-Hasani, & A. Schürmann (Eds.), *Animal models in diabetes research* (pp. 125–159). Humana Press. [https://doi.org/10.1007/978-1-62703-068-7\\_9](https://doi.org/10.1007/978-1-62703-068-7_9)
- Rorsman, P., & Ashcroft, F. M. (2018). Pancreatic  $\beta$ -cell electrical activity and insulin secretion: Of mice and men. *Physiological Reviews*, 98, 117–214. <https://doi.org/10.1152/physrev.00008.2017>
- Rutz, A., Sorokina, M., Galgonek, J., Mietchen, D., Willighagen, E., Gaudry, A., Graham, J. G., Stephan, R., Page, R., Vondrášek, J., Steinbeck, C., Pauli, G. F., Wolfender, J. L., Bisson, J., & Allard, P. M. (2022). The LOTUS initiative for open knowledge management in natural products research. *eLife*, 11, e70780. <https://doi.org/10.7554/eLife.70780>
- Salehi, B., Machin, L., Monzote, L., Sharifi-Rad, J., Ezzat, S. M., Salem, M. A., Merghany, R. M., el Mahdy, N. M., Kılıç, C. S., Sytar, O., Sharifi-Rad, M., Sharopov, F., Martins, N., Martorell, M., & Cho, W. C. (2020). Therapeutic potential of quercetin: New insights and perspectives for human health. *ACS Omega*, 5, 11849–11872. <https://doi.org/10.1021/acsomega.0c01818>
- Saponara, S., Carosati, E., Mugnai, P., Sgaragli, G., & Fusi, F. (2011). The flavonoid scaffold as a template for the design of modulators of the vascular  $Ca^{2+}$  channels. *British Journal of Pharmacology*, 164, 1684–1697. <https://doi.org/10.1111/j.1476-5381.2011.01476.x>

- Saponara, S., Sgaragli, G., & Fusi, F. (2002). Quercetin as a novel activator of L-type  $Ca^{2+}$  channels in rat tail artery smooth muscle cells. *British Journal of Pharmacology*, 135, 1819–1827. <https://doi.org/10.1038/sj.bjp.0704631>
- Schonhofer, C., Yi, J., Sciorillo, A., Andrae-Marobela, K., Cochrane, A., Harris, M., Brumme, Z. L., Brockman, M. A., Mounzer, K., Hart, C., Gyampoh, K., Yuan, Z., Montaner, L. J., & Tietjen, I. (2021). Flavonoid-based inhibition of cyclin-dependent kinase 9 without concomitant inhibition of histone deacetylases durably reinforces HIV latency. *Biochemical Pharmacology*, 186, 114462. <https://doi.org/10.1016/j.bcp.2021.114462>
- Toubal, S., Oiry, C., Bayle, M., Cros, G., & Neasta, J. (2020). Urolithin C increases glucose-induced ERK activation which contributes to insulin secretion. *Fundamental & Clinical Pharmacology*, 34, 571–580. <https://doi.org/10.1111/fcp.12551>
- Trezza, A., Spiga, O., Mugnai, P., Saponara, S., Sgaragli, G., & Fusi, F. (2022). Functional, electrophysiology, and molecular dynamics analysis of quercetin-induced contraction of rat vascular musculature. *European Journal of Pharmacology*, 918, 174778. <https://doi.org/10.1016/j.ejphar.2022.174778>
- Walker, E. H., Pacold, M. E., Perisic, O., Stephens, L., Hawkins, P. T., Wymann, M. P., & Williams, R. L. (2000). Structural determinants of phosphoinositide 3-kinase inhibition by wortmannin, LY294002, quercetin, myricetin, and staurosporine. *Molecular Cell*, 6, 909–919. [https://doi.org/10.1016/S1097-2765\(05\)00089-4](https://doi.org/10.1016/S1097-2765(05)00089-4)
- Webb, B., & Sali, A. (2017). Protein structure modeling with MODELLER. *Methods in Molecular Biology*, 1654, 39–54. [https://doi.org/10.1007/978-1-4939-7231-9\\_4](https://doi.org/10.1007/978-1-4939-7231-9_4)
- Wei, Y., Yu, Z., Wang, L., Li, X., Li, N., Bai, Q., Wang, Y., Li, R., Meng, Y., Xu, H., Wang, X., Dong, Y., Huang, Z., Zhang, X. C., & Zhao, Y. (2024). Structural bases of inhibitory mechanism of CaV1.2 channel inhibitors. *Nature Communications*, 15, 2772. <https://doi.org/10.1038/s41467-024-47116-8>
- Williamson, G., Kay, C. D., & Crozier, A. (2018). The bioavailability, transport, and bioactivity of dietary flavonoids: A review from a historical perspective. *Comprehensive Reviews in Food Science and Food Safety*, 17, 1054–1112. <https://doi.org/10.1111/1541-4337.12351>
- Yang, S.-N., & Berggren, P.-O. (2006). The role of voltage-gated calcium channels in pancreatic  $\beta$ -cell physiology and pathophysiology. *Endocrine Reviews*, 27, 621–676. <https://doi.org/10.1210/er.2005-0888>
- Yokoyama, T., Kosaka, Y., & Mizuguchi, M. (2015). Structural insight into the interactions between death-associated protein kinase 1 and natural flavonoids. *Journal of Medicinal Chemistry*, 58, 7400–7408. <https://doi.org/10.1021/acs.jmedchem.5b00893>
- Youl, E., Bardy, G., Magous, R., Cros, G., Sejalon, F., Virsolvy, A., Richard, S., Quignard, J. F., Gross, R., Petit, P., Bataille, D., & Oiry, C. (2010). Quercetin potentiates insulin secretion and protects INS-1 pancreatic  $\beta$ -cells against oxidative damage via the ERK1/2 pathway. *British Journal of Pharmacology*, 161, 799–814. <https://doi.org/10.1111/j.1476-5381.2010.00910.x>
- Yu, Q., Zeng, K., Ma, X., Song, F., Jiang, Y., Tu, P., & Wang, X. M. (2016). Resokaempferol-mediated anti-inflammatory effects on activated macrophages via the inhibition of JAK2/STAT3, NF- $\kappa$ B and JNK/p38 MAPK signaling pathways. *International Immunopharmacology*, 38, 104–114. <https://doi.org/10.1016/j.intimp.2016.05.010>
- Zawalich, W. S., Zawalich, K. C., Cline, G., Shulman, G., & Rasmussen, H. (1993). Comparative effects of monomethylsuccinate and glucose on insulin secretion from perfused rat islets. *Diabetes*, 42, 843–850. <https://doi.org/10.2337/diab.42.6.843>
- Zheng, W., Li, L., & Li, H. (2022). Phytochemicals modulate pancreatic islet  $\beta$  cell function through glucagon-like peptide-1-related mechanisms. *Biochemical Pharmacology*, 197, 114817. <https://doi.org/10.1016/j.bcp.2021.114817>

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