Nutrition&Metabolism

RESEARCH Open Access

Allantoin activates imidazoline I-3 receptors to enhance insulin secretion in pancreatic β-cells

Cheng-Chia Tsai[1], Li-Jen Chen[2,3], Ho-Shan Niu[3], Kun-Ming Chung[4], Juei-Tang Cheng[5*] and Kao-Chang Lin[4,6]

Abstract

Background: Imidazoline I_3 receptors (I-3R) can regulate insulin secretion in pancreatic β-cells. It has been indicated that allantoin ameliorates hyperglycemia by activating imidazoline I_2 receptors (I-2R). Thus, the effect of allantoin on I-3R is identified in the present study.

Methods: We used male Wistar rats to screen allantoin's ability for lowering of blood glucose and stimulation of insulin secretion. Chinese hamster ovary-K1 cells transfected with imidazoline receptors (NISCH-CHO-K1 cells) were also applied to characterize the direct effect of allantoin on this receptor. Additionally, KU14R as specific antagonist was treated to block I-3R in rats and in the cultured pancreatic β-cells named Min 6 cells.

Results: In rats, allantoin decreased blood sugar with an increase in plasma insulin. Also, allantoin enhanced calcium influx into NISCH-CHO-K1 cells in a way similar to agmatine, an I-R agonist. Moreover, KU14R dose-dependently blocked allantoin-induced insulin secretion both in Min 6 cells and in Wistar rats.

Conclusion: Allantoin can activate I-3R to enhance insulin secretion for lowering of blood sugar in Wistar rats. Thus, allantoin may provide beneficial effects as a supplement for diabetic patients after clinical trials.

Keywords: Allantoin, Insulin secretion, Imidazoline I_3 receptor, CHO-K1, NISCH

Background

Allantoin is one of the active principles contained in yam (Dioscorea spp.) [1]. Yam is widely used in the drug industry, and *Dioscorea rhizome* contains ureides, including allantoin, that prevent inflammation and ulcers [2]. Herbs from Dioscoreaceae have been used to improve diabetic disorders [3]. In Chinese traditional medicine, Shan-Yaw (*Dioscorea opposita*) improves insulin resistance [4], and the effect also observed in animals [5]. Allantoin is contained in this herb [6], and we have identified the plasma glucose- lowering action of allantoin in diabetic rats [7].

The antihyperglycemic action of allantoin in type-1-like diabetic rats involves its activation of imidazoline I-2 receptors (I-2Rs) [7]. Imidazoline receptors (I-Rs) have many functions [8]. Due to the presence of agmatine as endogenous ligand, the functions of I-Rs subtypes were widely investigated: I-1 receptor is known to regulate blood pressure [9]; I-3R mediates insulin secretion [10]; and I-2R is mentioned to reduce blood glucose [11,12]. The I-R expressed on pancreatic β-cells has been classified as I-3 site [13] showing different properties from the I-1 and/or I-2 sites [14,15]. The insulin-secreting action of efaroxan, an I-3R ligand, is mediated by calcium influx through the closure of ATP-sensitive K^+ (K_{ATP}) channel [16]. However, the effect of allantoin on I-3R for insulin secretion is still unknown.

Compounds with guanidine-like structures, including metformin [17], can bind to I-Rs [8]. I-R activation promotes glycemic control [18-20]. An increase in insulin secretion via the activation of I-3R located in pancreatic β-cells also seems helpful for glycemic control [21,22]. Allantoin has the ability to activate I-2R [7], but the effect of allantoin on I-3R remained obscure. Thus, the present study investigated whether allantoin can bind I-R and explored its effects on I-3R both in vivo and in vitro.

Methods

Animals

The male Wistar rats weighing from 280 to 330 g were purchased from the Animal Center of National Cheng

* Correspondence: jtcheng5503@yahoo.com.tw
[5]Department of Medical Research, Chi-Mei Medical Center, Yong Kang City, Tainan County 73101, Taiwan
Full list of author information is available at the end of the article

Kung University Medical College. The rats were free access to food and water in the house under a 12-h light/dark cycle. The animal experiments were approved and conducted in accordance with local institutional guidelines for the care and use of laboratory animals. All experiments conformed to the Guide for the Care and Use of Laboratory Animals as well as the guidelines of the Animal Welfare Act.

The effect of allantoin on postprandial blood glucose was performed as described previously [23]. The rats were separated into three groups and eight animals in each group. After fasting for 12 h, blood samples obtained from tail vein were used to determine the plasma glucose level at the basal level (0 min). Then, two groups of animals received the injection of allantoin at 0.5 or 1 mg/kg into tail vein. Another group received a similar injection of vehicle at same volume to use as the control. At 30 min later, all rats received oral intake of glucose solution (1 g/kg body weight). Blood samples were obtained at desired time after the oral glucose challenge to determine the plasma glucose level. The insulin level in each sample was also estimated using an immunoassay kit (Mercodia, Uppsala, Sweden).

Cell cultures

The *Mus musculus insulinoma* cell line Min 6 (BCRC No. CRL-11506) and Chinese hamster ovary-K1 (CHO-K1) cells (BCRC No. CCL-61) were purchased from the Culture Collection and Research Center of the Food Industry Institute (Hsin-Chiu City, Taiwan). Following a previous method [24,25], Min 6 cells were maintained in RPMI 1640 medium, while CHO-K1 cells were maintained in F-12 K medium supplemented with 10% fetal bovine serum. The cells were sub-cultured once every three days by trypsinization (Gibco), and the culture medium was refreshed every 2–3 days.

Overexpression of NISCH in CHO-K1 cells

According to the previous report [26], CHO-K1 cells were transiently transfected with human nischarin (NISCH) gene, also known as human imidazoline receptor antiseraselective (IRAS) protein, and an expression vector (Origene, Rockville, MD, USA) using the TurboFect transfection reagent (Thermo Fisher Scientific, USA). At 24 h later, the transfected cells were used to treat with allantoin or agmatine at indicated concentrations.

Measurement of insulin secretion

To identify the direct effect of allantoin on insulin secretion, we used Min 6 cells to investigate the in vitro secretion as described previously [27]. The Min 6 cells were prepared at 1×10^5 cells per well in a 12-well plate containing 1 ml of DMEM before the experiment. Then, cells were incubated with KU14R (an I-3R antagonist) (Sigma-Aldrich, St. Louis, MO, USA) at effective concentrations or with same volume of vehicle, as the control, for 30 min. All cells were treated with allantoin at the indicated concentrations for 1 h. After the collection of media to store at −20°C, insulin levels in the media were estimated using an immunoassay kit (Mercodia).

Western blotting analysis

The cells were lysed with ice-cold lysis and the protein was extracted following a previous report [3]. Then, each sample at 30 micrograms was separated by 10% sodium dodecylsulfate polyacrylamide gel electrophoresis. The blots were then transferred to a polyvinyl difluoride membrane (Millipore, Billerica, MA, USA). After blocking with 10% skim milk for 1 h, immunoblots were developed with the primary antibody specific for NISCH and DDK (Origene, Rockville, MD, USA). The blots were subsequently hybridized using horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse IgG (Calbiochem, San Diego, CA, USA) and developed by a chemiluminescence kit (PerkinElmer). The optical densities of the bands (37 kD) were identified through Gel-Pro analyzer software 4.0 (Media Cybernetics, Silver Spring, MD, USA).

Measurement of intracellular calcium

The intracellular calcium concentrations were determined using the fluorescent probe fura-2 as described in our previous report [28]. In brief, the NISCH-CHO-K1 cells were placed in a buffered physiological saline solution (PSS) containing 5 mM fura-2. The cells were incubated for 1 h under 5% CO_2 in O_2 aeration at 37.8°C. After washing, the cells were incubated for another 30 min in PSS. The NISCH-CHO-K1 cells were inserted into a temperature-controlled (37°C) cuvette containing 2 ml of PSS and indicated doses of allantoin or agmatine for 1 h. The fluorescence was determined by a fluorescence spectrofluorometer (Hitachi F-2000). The intracellular calcium $[Ca^{2+}]i$ was calculated from the ratio R = F340/F380 by the following formula: $[Ca^{2+}]i = KdB (R - Rmin)/(Rmax - R)$, where Kd is 225 nM, F is fluorescence, and B is the ratio of the fluorescence of the free dye to that of the Ca^{2+}-bound dye measured at 380 nm. Rmax and Rmin were determined in separate experiments by using digoxin to equilibrate $[Ca^{2+}]i$ with ambient $[Ca^{2+}]$ (Rmax), and the addition of 0.1 mM $MnCl_2$ and 1 mmol/L EGTA (Rmin). Background autofluorescence was measured in unloaded cells and was subtracted from all measurements.

Changes of blood glucose and insulin in rats

KU14R (an I-3R antagonist) (Sigma-Aldrich, St. Louis, MO, USA) at the effective dose (4 or 8 mg/kg) or

same volume of vehicle was used to treat the rats as described previously [29,30] for 30 minutes before the injection of allantoin (0.1, 1 or 2.5 mg/kg). Blood samples collected from the femoral vein at indicated times were centrifuged and the plasma glucose was measured in an automatic analyzer (Quik-Lab, Ames; Miles Inc., Elkhart, IN, USA). Plasma insulin was estimated from each sample using an immunoassay kit (Mercodia, Uppsala, Sweden).

Statistical analysis

Data are expressed as the mean ± SEM of each group. Means of the two groups were compared by *Student's t*-test using the software Microsoft EXCEL. The differences were analyzed by the unpaired *t-test* and considered significant at $P < 0.05$.

Results

Effect of allantoin on blood glucose in rats challenged with glucose

We injected allantoin (0.5 or 1 mg/kg) into fasted Wistar rats that received a glucose (1 g/kg) challenge to investigate the effectiveness of allantoin. Allantoin produced a marked reduction of hyperglycemia in these rats. This action of allantoin was more effective at 1 mg/kg than 0.5 mg/kg (Figure 1A).

Effects of allantoin on insulin secretion and basal blood glucose in rats

Normal Wistar rats were used to investigate the effects of allantoin on insulin secretion and basal blood glucose. The basal blood glucose was lowered by allantoin in a dose-dependent manner (Figure 1B). Plasma insulin was raised by allantoin in a same manner (Figure 1C). These results show that allantoin may lower blood glucose through an increase of plasma insulin.

Imidazoline receptor- mediated calcium influx in transfected CHO-K1 cells

Transfection efficiency was confirmed through Western blotting analysis. The over-expressed DDK and NISCH genes were identified in these transfected NISCH-CHO-K1 cells (Figure 2A and 2B). Additionally, calcium influx was dose- dependently increased in NISCH-CHO-K1 cells treated with agmatine (Figure 2C), an I-R agonist. The functional expression of NISCH in CHO-K1 cells was confirmed.

Changes in calcium influx caused by allantoin in NISCH-CHO-K1 cells

We tested the ability of allantoin to bind with imidazoline receptor. After incubation with allantoin, calcium influx was significantly raised in NISCH-CHO-K1 cells, similar to the increase stimulated by agmatine (Figure 2C).

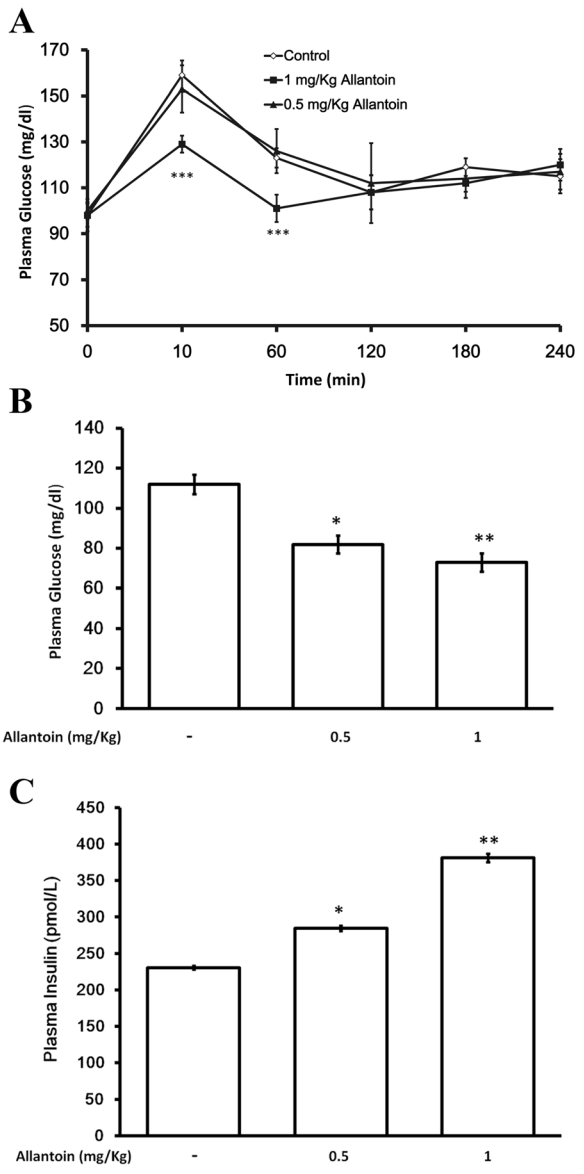

Figure 1 Effects of allantoin on insulin secretion and blood glucose in Wistar rats. Plasma glucose was reduced by intravenous (i.v.) injection of allantoin into fasted Wistar rats after glucose (1 g/kg) loading **(A)**. Basal plasma glucose **(B)** and basal plasma insulin **(C)** were also influenced by allantoin. Values are indicated as the mean and SEM from each group of eight rats. Vehicle only (0.9% NaCl in distilled water) was given at the same volume. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ versus data from animals treated with vehicle (control).

After comparison, allantoin induced calcium influx was less effective than agmatine.

Comparison of the actions of allantoin and glibenclamide in Min 6 cells

Glibenclamide has widely been used to stimulate insulin secretion by increasing calcium influx and it was also

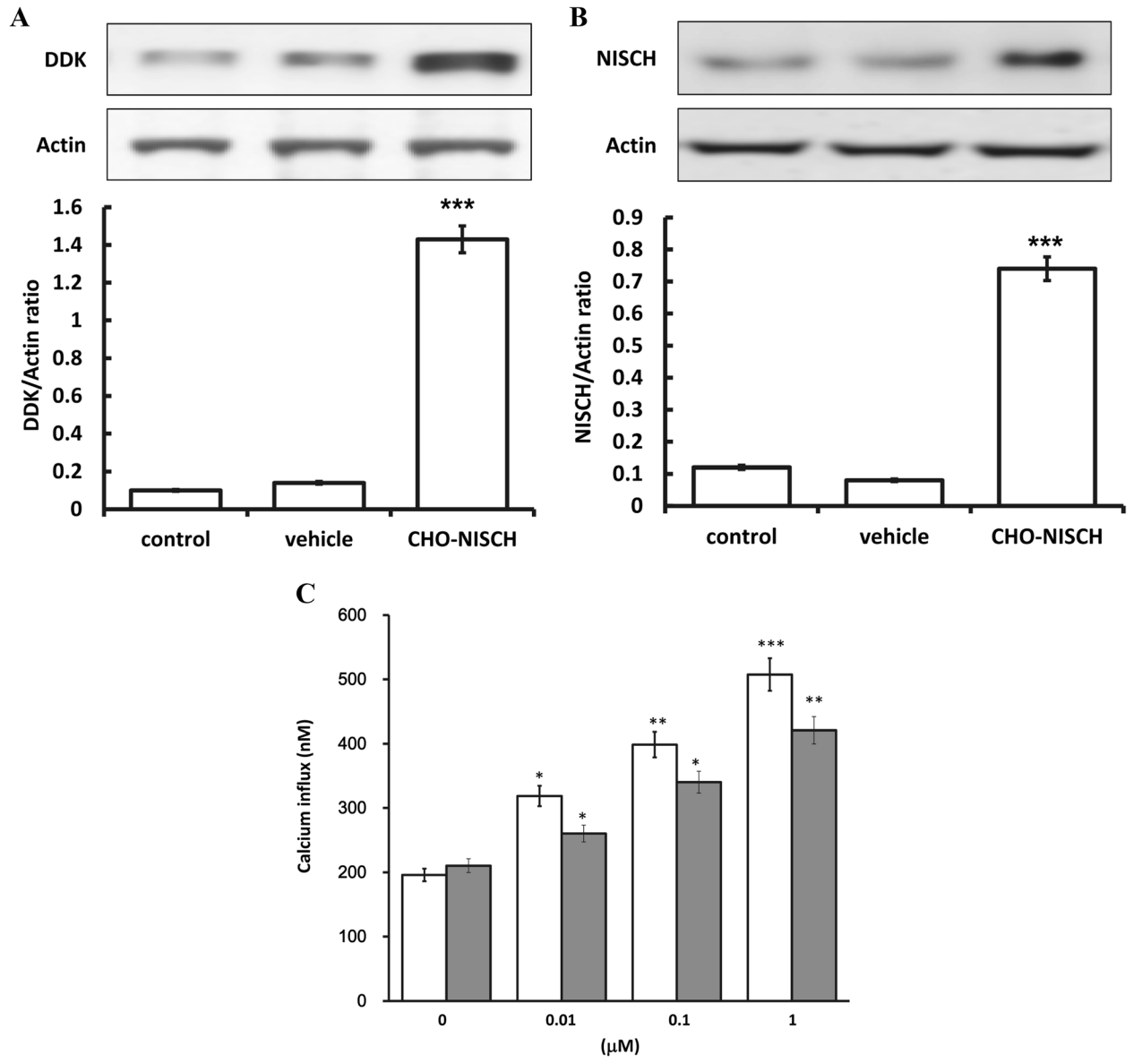

Figure 2 Allantoin induces calcium influx in CHO -cells transfected with imidazoline receptors. The expression of DDK **(A)** or NISCH **(B)** was identified using Western blotting analysis. Changes in intracellular calcium by agmatine (open bar) or allantoin (gray bar) were detected using the fura-2 probe by a fluorescence spectrofluorometer **(C)**. All of the values are expressed as the mean ± SEM (n = 6 per group). *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ compared with the control group.

applied as a positive control in this study. In Min 6 cells, glibenclamide significantly increased calcium influx at the concentration of 1 μM. Allantoin (1 μM) increased calcium influx to a level similar to that induced by 1 μM glibenclamide (Figure 3A).

Effect of KU14R on the action of allantoin in Min 6 cells

The antagonist of I-3R, KU14R, influenced the action of allantoin markedly (Figure 3B). Insulin secretion increased by allantoin was inhibited by KU14R in a concentration-dependent manner. At the highest concentration, KU14R abolished the action of allantoin. This result indicates the activation of I-3R by allantoin.

Effect of I-3R blockade by KU14R on the action of allantoin in rats

The allantoin action in rats was also influenced by KU14R. The increase in insulin secretion and the decrease in blood glucose caused by allantoin were both markedly inhibited by KU14R (Figure 4) at the dose sufficient to block I-3R [29,30]. Thus, the mediation of I-3R in actions of allantoin was identified *in vivo*.

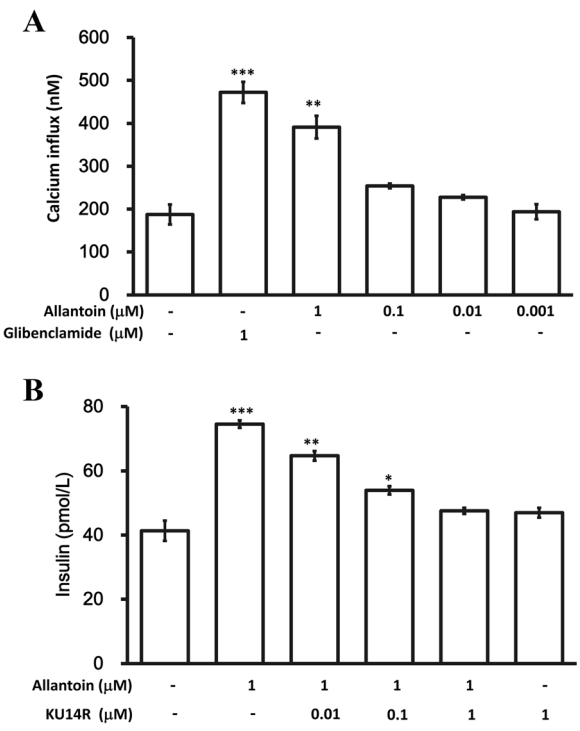

Figure 3 Characterization of allantoin induced actions in Min 6 cells. Changes in calcium influx by glibenclamide or allantoin were determined in Min 6 cells **(A)**. The effect of KU14R on allantoin induced insulin secretion **(B)**. All of the values are expressed as the mean ± SEM (n = 6 per group). $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with the control group.

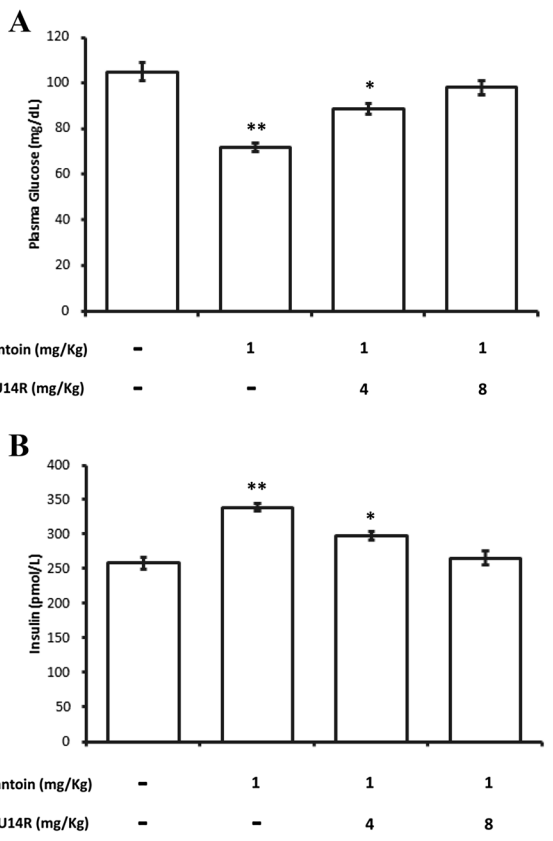

Figure 4 Effects of I-3R blockade on allantoin-induced insulin secretion and blood glucose reduction in Wistar rats. Data show the effects of an I-3R antagonist (KU14R) on allantoin-induced blood glucose reduction **(A)** and insulin secretion **(B)** in Wistar rats. Values are expressed as the mean and SEM from each group of eight rats. Vehicle (0.9% NaCl in distilled water) was given at the same volume. $^{*}P < 0.05$ and $^{**}P < 0.01$ versus data from the vehicle-treated control.

Discussion

Yam containing allantoin is a widely used nutrient [2]. Allantoin ameliorates hyperglycemia in diabetic rats by activating I-2R [7]. In the present study, we found that allantoin also can activate I-3R, linking its increase of insulin secretion to reduce blood glucose in Wistar rats. Additionally, this is the first report demonstrated that allantoin can activate I-R directly using the response in CHO -cells transfected with imidazoline receptor gene (NISCH-CHO-K1 cells). Moreover, we applied a pharmacological antagonist named KU14R at a dose sufficient to block I-3R, as described previously [31,32], to block the actions of allantoin in both pancreatic β-cells (Min 6 cells) and Wistar rats, indicating the mediation of I-3R in insulin secretion induced by allantoin.

Allantoin is easily degraded in the intestinal tract [33] and loses its activity after oral administration [34,35]. Thus, we treated rats with allantoin using intravenous injection (iv). Allantoin (1 mg/kg, iv) attenuated the hyperglycemia in fasting rats challenged with glucose. Moreover, allantoin decreased blood sugar and increased blood insulin in normal rats in a dose-dependent manner. Thus, bolus injection of allantoin induces lowering of blood glucose in a way associated with the increased insulin secretion in rats.

Imidazoline receptors have been established [36-38], but research tools to study them are still not well developed. There is no ideal radioligand to perform ligand-receptor binding assay. Also, the antagonist specific for each subtype of imidazoline receptor is not sufficient. In the present study, we transfected the imidazoline receptor gene (NISCH) into CHO cells. Success of the transfection was confirmed using Western blotting analysis. Agmatine, a well-known ligand of I-Rs, induced an increase in calcium influx in these cells, indicating that the transfected CHO-cells were functional. Additionally, allantoin enhanced calcium influx into NISCH- expressing CHO-K1 cells in a manner similar to agmatine. The activation of imidazoline receptor by allantoin was thus confirmed. These results show that CHO -cells transfected with imidazoline receptor gene can be used to identify the direct effect of allantoin.

Allantoin can activate I-2R to lower blood sugar and to improve insulin sensitivity [39-42]. However, these results were observed in the absence of endogenous insulin. We applied Min 6 cells to investigate the effect of allantoin on I-3R in pancreatic cells and used glibenclamide as positive control. Glibenclamide has been applied to enhance insulin secretion through an induction of calcium influx in pancreatic β-cells [43,44]. We observed that 1 μM allantoin increases calcium influx in Min 6 cells to a level similar to that produced by 1 μM glibenclamide. Glibenclamide is known to inhibit ATP-regulated potassium (K_{ATP}) channels in pancreatic β-cells, thereby causing calcium influx to result in the increase of intracellular calcium [45]. In the present study, we identified that allantoin increases calcium influx in pancreatic cells in a manner similar to glibenclamide.

The binding site(s) of imidazolines in pancreatic β-cell has been distinguished with I-1 and I-2 receptors [46]. I-1 sites were not expressed in β-cells and I-2 sites were mentioned as not reliable for the activity of imidazoline ligands [10]. Thus, imidazolines induced insulin secretion after binding to a single site named I-3 site. In the present study, we found that an antagonist specific for I-3R (KU14R) inhibited allantoin-induced insulin secretion in Min 6 cells in a dose-dependent manner. Similar results were observed in rats showing the plasma insulin increasing action of allantoin blocked by KU14R. Thus, activation of I-3R is involved in the insulin secretion induced by allantoin.

Allantoin can activate I-1R in brain to produce antihypertension [47] and decrease feeding behaviors [48]. Allantoin can stimulate I-2R to lower blood sugar and improve insulin sensitivity in type 1-like diabetic rats [39-42]. In this study, allantoin activates I-3R to increase insulin secretion. Taken together, these findings indicate that allantoin could be useful for treating metabolic syndrome. However, more data are needed to confirm this hypothesis, especially from the clinical trials, in the future.

Conclusion

Allantoin can enhance insulin secretion by activating I-3R to lower blood glucose in rats. Thus, allantoin or the related compound(s) that activates I-3R could be developed for the supplementary treatment of diabetic disorders.

Abbreviations

I-3R: Imidazoline I_3 receptor; I-R: Imidazoline receptors; CHO-K1: Chinese Overy Hamster-K1; I-2R: Imidazoline I_2 receptor; I-1R: Imidazoline I_1 receptor; NISCH: Human nischarin; IRAS: Mouse homologue of human imidazoline receptor antisera-selective; K_{ATP}: ATP-regulated potassium channels.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

CCT and KCL conceived the study and designed the experimental plan. LJC and HSN performed all of the experiments and contributed to data collection. KMC and LJC contributed to data analysis, interpretation and manuscript writing. JTC and KCL contributed to data interpretation and manuscript submission. All authors read and approved the final manuscript.

Acknowledgements

We thank Miss Mei-Jen Wang, Yang-Lian Yan and Pei-Ru Liao for their skillful assistance in conducting the experiments. The present study was supported in part by a grant (CMFHR10302) from Chi-Mei Medical Center, Yong Kang, Tainan City, Taiwan.

Author details

[1]Department of Neurosurgery, Mackay Hospital, and Graduate Institute of Injury Prevention and Control, College of Medicine, Taipei Medical University, Taipei City 10361, Taiwan. [2]Institute of Basic Medical Sciences, College of Medicine, National Cheng Kung University, Tainan City 70101, Taiwan. [3]Department of Nursing, Tzu Chi College of Technology, Hualien City 97005, Taiwan. [4]Department of Internal Medicine, Chi-Mei Medical Center, Yong Kang City, Tainan County 73101, Taiwan. [5]Department of Medical Research, Chi-Mei Medical Center, Yong Kang City, Tainan County 73101, Taiwan. [6]Institute of Biotechnology, Southern Taiwan University, Tainan City, Yong Kang 71004, Taiwan.

Received: 18 May 2014 Accepted: 28 August 2014
Published: 31 August 2014

References

1. Sagara K, Ojima M, Suto K, Yoshida T: **Quantitative determination of allantoin in Dioscorea rhizome and an Oriental pharmaceutical preparation, hachimi-gan, by high-performance liquid chromatography.** *Planta Med* 1989, **55:**93.
2. Shestopalov AV, Shkurat TP, Mikashinovich ZI, Kryzhanovskaia IO, Bogacheva MA, Lomteva SV, Prokof'ev VP, Gus'kov EP: **Biological functions of allantoin.** *Izv Akad Nauk Ser Biol* 2006, 541–545.
3. Sato K, Iemitsu M, Aizawa K, Ajisaka R: **DHEA improves impaired activation of Akt and PKC zeta/lambda-GLUT4 pathway in skeletal muscle and improves hyperglycaemia in streptozotocin-induced diabetes rats.** *Acta Physiol (Oxf)* 2009, **197:**217–225.
4. Gao X, Li B, Jiang H, Liu F, Xu D, Liu Z: **Dioscorea opposita reverses dexamethasone induced insulin resistance.** *Fitoterapia* 2007, **78:**12–15.
5. Hsu JH, Wu YC, Liu IM, Cheng JT: **Dioscorea as the principal herb of Die-Huang-Wan, a widely used herbal mixture in China, for improvement of insulin resistance in fructose-rich chow-fed rats.** *J Ethnopharmacol* 2007, **112:**577–584.
6. Wang SJ, Liu HY, Gao WY, Chen HX, Yu JG, Xiao PG: **Characterization of new starches separated from different Chinese yam (Dioscorea opposita Thunb.) cultivars.** *Food Chem* 2006, **99:**30–37.
7. Cheng JT, Niu CS, Chen W, Wu HT, Cheng KC, Wen YJ, Lin KC: **Decrease of plasma glucose by Allantoin, an active principle of Yam (Dioscorea spp.), in streptozotocin-induced diabetic rats.** *J Agric Food Chem* 2010, **58:**12031–12035.
8. Dardonville C, Rozas I: **Imidazoline binding sites and their ligands: an overview of the different chemical structures.** *Med Res Rev* 2004, **24:**639–661.
9. Ernsberger P, Graves ME, Graff LM, Zakieh N, Nguyen P, Collins LA, Westbrooks KL, Johnson GG: **I1-imidazoline receptors. Definition, characterization, distribution, and transmembrane signaling.** *Ann N Y Acad Sci* 1995, **763:**22–42.
10. Morgan NG, Chan SL, Mourtada M, Monks LK, Ramsden CA: **Imidazolines and pancreatic hormone secretion.** *Ann N Y Acad Sci* 1999, **881:**217–228.
11. Lui TN, Tsao CW, Huang SY, Chang CH, Cheng JT: **Activation of imidazoline I2B receptors is linked with AMP kinase pathway to increase glucose uptake in cultured C2C12 cells.** *Neurosci Lett* 2010, **474:**144–147.
12. Cheng JT, Chang CH, Wu HT, Cheng KC, Lin HJ: **Increase of beta-endorphin secretion by agmatine is induced by activation of imidazoline I(2A) receptors in adrenal gland of rats.** *Neurosci Lett* 2010, **468:**297–299.
13. Eglen RM, Hudson AL, Kendall DA, Nutt DJ, Morgan NG, Wilson VG, Dillon MP: **'Seeing through a glass darkly': casting light on imidazoline 'I' sites.** *Trends Pharmacol Sci* 1998, **19:**381–390.

14. Chan SL, Dunne MJ, Stillings MR, Morgan NG: **The alpha 2-adrenoceptor antagonist efaroxan modulates K + ATP channels in insulin-secreting cells.** *Eur J Pharmacol* 1991, **204:**41–48.
15. Brown CA, Loweth AC, Smith SA, Morgan NG: **Stimulation of insulin secretion by imidazoline compounds is not due to interaction with non-adrenoceptor idazoxan binding sites.** *Br J Pharmacol* 1993, **108:**312–317.
16. Chan SL, Pallett AL, Clews J, Ramsden CA, Chapman JC, Kane C, Dunne MJ, Morgan NG: **Characterisation of new efaroxan derivatives for use in purification of imidazoline-binding sites.** *Eur J Pharmacol* 1998, **355:**67–76.
17. Cheng JT, Lee JP, Chen W, Wu HT, Lin KC: **Metformin can activate imidazoline I-2 receptors to lower plasma glucose in type 1-like diabetic rats.** *Horm Metab Res* 2011, **43:**26–30.
18. Chung HH, Yang TT, Chen MF, Chou MT, Cheng JT: **Improvement of hyperphagia by activation of cerebral I(1)-imidazoline receptors in streptozotocin-induced diabetic mice.** *Horm Metab Res* 2012, **44:**645–649.
19. Hwang SL, Liu IM, Tzeng TF, Cheng JT: **Activation of imidazoline receptors in adrenal gland to lower plasma glucose in streptozotocin-induced diabetic rats.** *Diabetologia* 2005, **48:**767–775.
20. Jou SB, Liu IM, Cheng JT: **Activation of imidazoline receptor by agmatine to lower plasma glucose in streptozotocin-induced diabetic rats.** *Neurosci Lett* 2004, **358:**111–114.
21. Gongadze NV, Antelava NA, Kezeli TD: **Imidazoline receptors.** *Georgian Med News* 2008, **160–161:**44–47.
22. Morgan NG, Chan SL: **Imidazoline binding sites in the endocrine pancreas: can they fulfil their potential as targets for the development of new insulin secretagogues?** *Curr Pharm Des* 2001, **7:**1413–1431.
23. Baron AD: **Postprandial hyperglycaemia and alpha-glucosidase inhibitors.** *Diabetes Res Clin Pract* 1998, **40**(Suppl):S51–S55.
24. Cheng JY, Whitelock J, Poole-Warren L: **Syndecan-4 is associated with beta-cells in the pancreas and the MIN6 beta-cell line.** *Histochem Cell Biol* 2012, **138:**933–944.
25. Osmond RI, Martin-Harris MH, Crouch MF, Park J, Morreale E, Dupriez VJ: **G-protein-coupled receptor-mediated MAPK and PI3-kinase signaling is maintained in Chinese hamster ovary cells after gamma-irradiation.** *J Biomol Screen* 2012, **17:**361–369.
26. Zambad SP, Tuli D, Mathur A, Ghalsasi SA, Chaudhary AR, Deshpande S, Gupta RC, Chauthaiwale V, Dutt C: **TRC210258, a novel TGR5 agonist, reduces glycemic and dyslipidemic cardiovascular risk in animal models of diabesity.** *Diabetes Metab Syndr Obes* 2013, **7:**1–14.
27. Cheng K, Delghingaro-Augusto V, Nolan CJ, Turner N, Hallahan N, Andrikopoulos S, Gunton JE: **High passage MIN6 cells have impaired insulin secretion with impaired glucose and lipid oxidation.** *PLoS One* 2012, **7:**e40868.
28. Lee PY, Chen W, Liu IM, Cheng JT: **Vasodilatation induced by sinomenine lowers blood pressure in spontaneously hypertensive rats.** *Clin Exp Pharmacol Physiol* 2007, **34:**979–984.
29. Chan SL, Mourtada M, Morgan NG: **Characterization of a KATP channel-independent pathway involved in potentiation of insulin secretion by efaroxan.** *Diabetes* 2001, **50:**340–347.
30. Mourtada M, Chan SL, Smith SA, Morgan NG: **Multiple effector pathways regulate the insulin secretory response to the imidazoline RX871024 in isolated rat pancreatic islets.** *Br J Pharmacol* 1999, **127:**1279–1287.
31. Bleck C, Wienbergen A, Rustenbeck I: **Essential role of the imidazoline moiety in the insulinotropic effect but not the KATP channel-blocking effect of imidazolines; a comparison of the effects of efaroxan and its imidazole analogue, KU14R.** *Diabetologia* 2005, **48:**2567–2575.
32. Mayer G, Taberner PV: **Effects of the imidazoline ligands efaroxan and KU14R on blood glucose homeostasis in the mouse.** *Eur J Pharmacol* 2002, **454:**95–102.
33. Kahn LP, Nolan JV: **Kinetics of allantoin metabolism in sheep.** *Br J Nutr* 2000, **84:**629–634.
34. Pak N, Donoso G, Tagle MA: **Allantoin excretion in the rat.** *Br J Nutr* 1973, **30:**107–112.
35. Koguchi T, Nakajima H, Wada M, Yamamoto Y, Innami S, Maekawa A, Tadokor T: **Dietary fiber suppresses elevations of uric acid and allantoin in serum and urine induced by dietary RNA and increases its excretion to feces in rats.** *J Nutr Sci Vitaminol (Tokyo)* 2002, **48:**184–193.
36. Nebieridze DV, Kamyshova TV: **Imidazoline receptor agonists: are they actual today?** *Kardiologiia* 2012, **52:**90–93.
37. Tanabe M: **Imidazoline receptor.** *Nihon Yakurigaku Zasshi* 2008, **131:**478–480.
38. Zhang J, Abdel-Rahman AA: **Nischarin as a functional imidazoline (I1) receptor.** *FEBS Lett* 2006, **580:**3070–3074.
39. Chung HH, Lee KS, Cheng JT: **Decrease of obesity by allantoin via imidazoline I 1 -receptor activation in high fat diet-fed mice.** *Evid Based Complement Alternat Med* 2013, **2013:**589309.
40. Yang TT, Chiu NH, Chung HH, Hsu CT, Lee WJ, Cheng JT: **Stimulatory effect of allantoin on imidazoline I(1) receptors in animal and cell line.** *Horm Metab Res* 2012, **44:**879–884.
41. Chen MF, Yang TT, Yeh LR, Chung HH, Wen YJ, Lee WJ, Cheng JT: **Activation of imidazoline I-2B receptors by allantoin to increase glucose uptake into C(2)C(1)(2) cells.** *Horm Metab Res* 2012, **44:**268–272.
42. Lin KC, Yeh LR, Chen LJ, Wen YJ, Cheng KC, Cheng JT: **Plasma glucose-lowering action of allantoin is induced by activation of imidazoline I-2 receptors in streptozotocin-induced diabetic rats.** *Horm Metab Res* 2012, **44:**41–46.
43. Marigo V, Courville K, Hsu WH, Feng JM, Cheng H: **TRPM4 impacts on Ca2+ signals during agonist-induced insulin secretion in pancreatic beta-cells.** *Mol Cell Endocrinol* 2009, **299:**194–203.
44. Ali MY, Whiteman M, Low CM, Moore PK: **Hydrogen sulphide reduces insulin secretion from HIT-T15 cells by a KATP channel-dependent pathway.** *J Endocrinol* 2007, **195:**105–112.
45. Tominaga M, Horie M, Sasayama S, Okada Y: **Glibenclamide, an ATP-sensitive K + channel blocker, inhibits cardiac cAMP-activated Cl- conductance.** *Circ Res* 1995, **77:**417–423.
46. Chan SL, Brown CA, Scarpello KE, Morgan NG: **The imidazoline site involved in control of insulin secretion: characteristics that distinguish it from I1- and I2-sites.** *Br J Pharmacol* 1994, **112:**1065–1070.
47. Chen MF, Tsai JT, Chen LJ, Wu TP, Yang JJ, Yin LT, Yang YL, Chiang TA, Lu HL, Wu MC: **Antihypertensive action of allantoin in animals.** *Biomed Res Int* 2014, **2014:**690135.
48. Chung HH, Cheng J: **Improvement of obesity by activation of I1-imidazoline receptors in high fat diet-fed mice.** *Horm Metab Res* 2013, **45:**581–585.

doi:10.1186/1743-7075-11-41
Cite this article as: Tsai *et al.*: Allantoin activates imidazoline I-3 receptors to enhance insulin secretion in pancreatic β-cells. *Nutrition & Metabolism* 2014 **11**:41.