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Role of histamine H_3 receptor in glucagon-secreting $\alpha TC1.6$ cells



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ABSTRACT

Pancreatic α -cells secrete glucagon to maintain energy homeostasis. Although histamine has an important role in energy homeostasis, the expression and function of histamine receptors in pancreatic α -cells remains unknown. We found that the histamine H₃ receptor (H₃R) was expressed in mouse pancreatic α -cells and α TC1.6 cells, a mouse pancreatic α -cell line. H₃R inhibited glucagon secretion from α TC1.6 cells by inhibiting an increase in intracellular Ca²⁺ concentration. We also found that immepip, a selective H₃R agonist, decreased serum glucagon concentration in rats. These results suggest that H₃R modulates glucagon secretion from pancreatic α -cells.

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1. Introduction

Histamine has multiple functions; it is released during allergic reactions, affects gastric acid secretion, and impacts the sleep-wake cycle. These functions are mediated thorough interactions with four different subtypes (H_1 – H_4), which belong to the G protein-coupled receptor superfamily. Among these subtypes, histamine H_3 receptor (H_3R) is currently a hot topic because of its important role in energy homeostasis [1]. Neuronal histamine has an inhibitory effect on food intake through modulation of hypothalamic neuronal activities [2,3]. H_3R expressed in the central nervous system (CNS) regulates histamine release [4]. Therefore, H_3R is a prospective therapeutic target for obesity, which could potentially lead to reduced body weight [5].

The regulation of glucagon secretion from pancreatic α -cells is fundamental to the maintenance of glucose homeostasis; under normal conditions they secrete glucagon in response to starvation-induced hypoglycemia. Glucagon is a 29-amino acid peptide hormone that elevates blood glucose concentration by glycogenolysis and gluconeogenesis in the liver [6]. Glucagon secretion from pancreatic α -cells has recently become a hot topic as a new therapeutic target for diabetes mellitus [7], as dysfunctional pancreatic α -cells have been reported in diabetic patients [8]. In these patients, blood glucagon concentrations are elevated even in hyperglycemic states [9]. Conversely, glucagon secretion is insufficient in response to critical hypoglycemia [10]. This inappropriate glucagon secretion aggravates diabetes. Therefore, it is crucial to understand the mechanism of glucagon secretion.

The key secretagogue for glucagon is low serum glucose concentration [11]. Low glucose concentrations facilitate glucagon release from pancreatic α -cells through the activation of voltagedependent Ca²⁺ channels (VDCCs) [12]. In addition to glucose, many paracrine/endocrine products and neurotransmitters are involved in the modification of glucagon secretion [13]. For example, glucagon-like peptide (GLP)-1, an incretin secreted from the small intestine L-cells, decreases glucagon secretion from pancreatic α -cells [14].

There are some biological molecules that modulate pancreatic α -cells and CNS functions to regulate total energy homeostasis. GLP-1 has an important role in regulating eating behavior by modulating CNS activities. Turton et al. reported that administration of GLP-1 into the lateral ventricle resulted in inhibition of eating behavior [15]. This evidence indicates that pancreatic α -cells and CNS are targets of GLP-1. Additionally, leptin, a peptide adipokine secreted from peripheral adipose tissues [16], has inhibitory effects on pancreatic α -cells [17] and food intake [18]. Moreover, ghrelin, a 28-amino acid peptide synthesized in the gastrointestinal tract, directly stimulates glucagon secretion [19] and eating behavior

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Abbreviations: CNS, central nervous system; H_3R , histamine H_3 receptor; H_3KO , histamine H_3 receptor-gene knockout; KRB, Krebs–Ringer bicarbonate buffer; GLP, glucagon-like peptide; VDCCs, voltage-dependent Ca²⁺ channels

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[20]. As previously mentioned, hypothalamic H₃R has an important role in food intake; thus, it is interesting to examine whether H₃R is expressed in pancreatic α -cells and involved in the regulation of energy homeostasis.

Taking this evidence into account, we hypothesize that H_3R is expressed and involved in pancreatic α -cell function. We used a mouse pancreatic α -cell line, α TC1.6 cells, to evaluate the function of H_3R in pancreatic α -cells. These α TC1.6 cells have properties similar to those of pancreatic α -cells, and they are widely used in research on glucagon secretion [21]. We discovered inhibitory effects of H_3R on glucagon secretion *in vitro* and *in vivo* using pharmacological assays.

2. Methods

2.1. Animals

Wild-type (WT) and histamine H₃ receptor-gene KO (H₃KO) C57BL/6 [22] mice were bred in our laboratory. Wister rats were purchased from Japan SLC (Hamamatsu, Japan). All mice were maintained on a 12-h light/dark cycle in a humidity- and temper-ature-controlled room, and they were allowed *ad libitum* access to food and water. This study was approved by the Center for Laboratory Animal Research, Tohoku University, Sendai, Japan. This study had been performed in accordance with the EU Directive 2010/63/ EU for animal experiments.

2.2. Cell culture

 α TC1.6 cells were purchased from the American Type Culture Collection (Manassas, VA, USA). Cells were maintained in DMEM (Invitrogen, Carlsbad, CA, USA) containing 10% FCS (Thermo, Waltham, MA, USA), 15 mM HEPES (Gibco, Carlsbad, CA, USA), 0.1 mM non-essential amino acids (Gibco), 0.02% BSA (Sigma, St. Louis, MO, USA), 1.5 g/dL sodium bicarbonate (Sigma), 2 g/L p-glucose (Sigma), 75 mg/mL penicillin G potassium (Wako, Osaka, Japan), and 100 mg/L streptomycin sulfate (Wako) at 37 °C in a 5%-CO₂ humidified incubator.

2.3. RT-PCR

RT-PCR was performed using a previously described protocol [23]. Briefly, total RNA was isolated from α TC1.6 cells and reverse-transcribed. Samples (equivalent to 5 ng total RNA) were amplified by 35 cycles of PCR (10 s at 98 °C, 30 s at 60 °C, and 90 s at 72 °C).

2.4. Western blot

Western blot of H_3R was performed using a previously described protocol [23]. We used the hypothalamic lysate from WT and H_3KO mice as positive and negative controls, respectively. Beta-actin was detected by an antibody (Cell Signaling Technology, Danvers, MA, USA) as a loading control.

2.5. Immunohistochemical analysis

Pancreata were quickly removed from 12-week-old WT and H_3KO mice. Isolated pancreata were fixed in 10% paraformaldehyde and embedded in paraffin. The paraffin sections were deparaffinized and blocked with 10% goat serum at room temperature for 15 min. To detect glucagon, sections were incubated with mouse anti-glucagon antibody (Sigma; 1:2000 dilution) for 2 h at room temperature and then treated with the secondary antibody: Alexa Fluor (Invitrogen) 568 conjugated goat anti-mouse antibody (1:100 dilution). To detect H₃R, samples were incubated overnight at 4 °C with rabbit anti-mouse H₃R antibody (4 µg/mL) [23] and then treated with the secondary antibody: Alexa Fluor 488 conjugated goat anti-rabbit antibody (Invitrogen). For double staining of glucagon and H₃R, samples were incubated with anti-glucagon antibody for 2 h at room temperature, washed thrice with PBS, followed by overnight incubation with anti-H₃R antibody at 4 °C. Specimens from H₃KO mice were used as negative controls for H₃R. Finally, all samples were encapsulated with mounting medium containing DAPI.

2.6. Glucagon secretion assay

 α TC1.6 cells were seeded into a 24-well plate at a density of 2.0×10^5 cells per well and then preincubated in 500 µL of Krebs–Ringer bicarbonate buffer (KRB) containing 20 mM glucose for 30 min at 37 °C in 5% CO₂. Subsequently, α TC1.6 cells were incubated in 500 µL of KRB containing 20 or 2.8 mM glucose ± 1 µM histamine (Sigma), 1 and 100 µM of the selective H₃R agonist immepip (Sigma) [24,25], or the selective H₃R inverse agonist JNJ-5207852 (kindly gifted from Dr. Nicholas Carruthers, Johnson & Johnson Pharmaceutical Research and Development, USA) [26,27], or KRB containing 20 mM KCl ± 1 µM immepip, for 1 h at 37 °C in 5% CO₂. Immepip and JNJ-5207852 were dissolved in distilled water. We used a glucagon ELISA kit (Wako) to measure glucagon concentration in KRB.

2.7. Intracellular Ca²⁺concentration measurement

αTC1.6 cells were seeded into a 96-well plate (Greiner, Frickenhausen, Germany) at a density of 2.0×10^4 cells per well and were then preincubated in KRB containing 20 mM glucose, 5 µM fluo-4/ AM, 5 mM probenecid, and $1 \times$ Power load (all from Invitrogen) for 30 min at 37 °C. Preincubated KRB was replaced with KRB containing 20 mM glucose \pm 1 μ M immepip and the 96-well plate was placed into a microplate reader FlexStation 3 (Molecular Devices, Sunnvvale, CA. USA) to record changes in fluorescence (excitation: 495 nm/emission: 518 nm). Cells were excited at 495 nm and the light emitted through a 518-nm filter was detected. After a 1-min measurement of baseline fluorescence, glucose concentration in KRB in each well was diluted to 2.8 mM by adding KRB ± 1 µM immepip from injectors, or KRB containing high KCl ± 1 µM immepip was added from injectors to adjust KCl concentration to 20 mM. Fluorescent intensity before fluo-4/AM loading was measured as background.

2.8. Measurement of serum glucagon concentration in rats

We used rats instead of mice to evaluate the effect of immepip on serum glucagon concentration because a glucagon ELISA kit required 100 μ L of serum to measure glucagon correctly. 10-week-old Wister rats were fasted for 24 h prior to the experiment and were anesthetized with isoflurane. Immepip (30 mg/ kg) dissolved in normal saline or just normal saline was administered intraperitoneally. Blood samples were collected from the caudal vein before injection as the baseline and at 15 min after injection. Samples were centrifuged for 30 min at 5000 rpm at 4 °C and then the supernatant plasma was collected. Plasma glucagon concentration was measured using a glucagon ELISA kit (Wako).

2.9. Data analysis and presentation

All experiments were performed in triplicate. Statistical analysis was performed using Student's *t*-test or one-way ANOVA. Data are expressed as mean \pm SE. *P*-values of <0.05 were considered significant.

3. Results

3.1. H_3R were expressed in $\alpha TC1.6$ cells

First, we examined the expression of histamine receptors in α TC1.6 cells using RT-PCR and western blot. H₃R mRNA was pre-

dominantly expressed in α TC1.6 cells among the four histamine receptor subtypes (Fig. 1A). Histamine H₁ receptor mRNA was slightly detected, but histamine H₂ and H₄ receptor mRNAs were not detectable. H₃R protein expression in α TC1.6 cells was confirmed by western blotting (Fig. 1B). Immunohistochemical analysis of pancreatic islets showed that glucagon-positive (stained red) expressed H₃R protein (stained green), indicating that H₃R is expressed in glucagon-positive mouse pancreatic α -cells (Fig. 1C). H₁R protein expression was not detectable during western blot of α TC1.6 cells (data not shown).



Fig. 1. Histamine H₃ receptor expression in α TC1.6 cells and pancreatic islets. (A) RT-PCR detection of histamine receptors in α TC1.6 cells. RT + and – indicate RT-PCR in the presence or absence of reverse transcriptase, respectively. H₁R: histamine H₁ receptor, H₂R: histamine H₂ receptor, H₃R: histamine H₃ receptor, H₄R: histamine H₄ receptor. (B) Western blot of H₃R in α TC1.6 cells. "WT" is a wild-type mouse hypothalamus lysate used as a positive control. H₃KO is a negative control, a hypothalamus lysate from an H₃R-knockout mouse. Beta-actin (Actb) was used as a loading control (lower panel). (C) Immunohistochemical analysis of H₃R expression in mouse pancreatic islets of WT mice (upper panels) and H₃KO mice (lower panels) as negative controls. Glucagon and H₃R were visualized with Alexa 568 (red) and Alexa 488 (green), respectively. DAPI is depicted in merged images (right panels). Scale bar = 50 µM. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Activation of H₃R decreased glucagon secretion

Glucagon secretion from pancreatic α -cells is vital to enhance serum glucose concentration in hypoglycemia [6]. We investigated effects of histamine on glucagon secretion from α TC1.6 cells. Glucagon secretion increased 1.41-fold over basal concentration in response to low glucose concentrations (Fig. 2A). This secretion associated with low glucose concentrations was remarkably inhibited by 1 μ M histamine (0.67 ± 0.05 ng/well/h *versus* 0.96 ± 0.07 ng/well/h, *P* < 0.05), while basal secretion at 20 mM glucose with histamine was maintained at close to control concentrations (0.68 ± 0.06 ng/well/h *versus* 0.68 ± 0.06 ng/well/h, *P* > 0.05; Fig. 2A). These results indicate that histamine negatively regulates glucagon secretion from α TC1.6 cells in response to low glucose concentration.

Subsequently, we examined the role of H₃R expressed in α TC1.6 cells in glucagon secretion using pharmacological assays with the H₃R agonist immepip or the inverse agonist JNJ-5207852. Glucagon secretion associated with low glucose concentrations was remarkably inhibited by 1 μ M immepip (0.73 ± 0.08 ng/well/h *versus* 1.01 ± 0.10 ng/well/h, *P* < 0.05; Fig. 2B). Conversely, 1 μ M JNJ-5207852 increased glucagon secretion (1.83 ± 0.08 ng/well/h)



Fig. 2. Effects of histamine, immepip, or JNJ-5207852 on glucagon secretion from α TC1.6 cells. (A) Glucagon secretion from α TC1.6 cells at low (2.8 mM) and high (20 mM) glucose concentrations ± 1 μ M histamine. *p < 0.05, n = 6. (B) Glucagon secretion from α TC1.6 cells at low (2.8 mM) and high (20 mM) glucose concentrations ± 1 or 100 μ M immepip. *p < 0.05, n = 6. (C) Glucagon secretion from α TC1.6 cells at low (2.8 mM) and high (20 mM) glucose concentrations ± 1 or 100 μ M immepip. *p < 0.05, n = 6. (C) Glucagon secretion from α TC1.6 cells at low (2.8 mM) and high (20 mM) glucose concentrations ± 1 or 100 μ M JNJ-5207852. *p < 0.05, n = 6.

versus 1.36 \pm 0.21 ng/well/h, P < 0.05; Fig. 2C). These results indicate that H₃R inhibits glucagon secretion in α TC1.6 cells.

3.3. Immepip inhibited the increase of intracellular Ca²⁺ concentration

 H_3R activation modulates cellular functions through several intracellular signaling pathways downstream of H_3R [28,29]. Silver et al. reported that H_3R activation attenuated norepinephrine exo-



Fig. 3. Analysis of intracellular signaling in α TC1.6 cells. (A) Changes in intracellular Ca²⁺ concentration in response to low (2.8 mM) glucose concentration ± 1 μ M immepip. The average of fluorescence intensity from fluo-4 before low glucose stimulation was arbitrarily set as 1. Intensity ratio on *y*-axis relates to the increase of intracellular Ca²⁺ concentration in direct proportion. 20 G: 20 mM glucose, 2.8 G: 2.8 mM glucose, n = 4. (B) Glucagon secretion from α TC1.6 cells at high (20 mM) glucose concentration and high (20 mM) KCl concentration ± 1 μ M immepip. **P* < 0.05, *n* = 6. (C) Changes in intracellular Ca²⁺ concentration in response to high (20 mM) KCl concentration ± 1 μ M immepip. The average of fluorescence intensity from fluo-4 before low glucose stimulation was arbitrarily set as 1. Intensity ratio on *y*-axis relates to the increase of intracellular Ca²⁺ concentration in direct proportion. 20 G: 20 mM glucose, 20 K: 20 mM KCl, *n* = 4.



Fig. 4. Effects of immepip on rat plasma glucagon concentration. Serum glucagon concentration of rats before and 15 min after intraperitoneal injection of 30 mg/kg immepip. *P < 0.05, n = 4.

cytosis mediated by a decrease in intracellular Ca²⁺ concentration, seemingly because of an impaired entrance of Ca²⁺ through VDCCs [30]. Glucagon exocytosis from pancreatic α -cells also requires an increase in intracellular Ca²⁺ concentration [31,32]. Thus, we examined the effect of activated H₃R on intracellular Ca²⁺ concentration. One micromole of immepip impaired the increase in intracellular Ca²⁺ concentration in response to 2.8 mM glucose (transient changes in Ca²⁺; 0.36 ± 0.03 *versus* 0.47 ± 0.04; Fig. 3A). This result indicates that H₃R is involved in the regulation of intracellular Ca²⁺ concentration in α TC1.6 cells.

To confirm whether intracellular Ca²⁺ concentration has a direct role in the inhibitory effect of H₃R on glucagon secretion, we stimulated aTC1.6 cells with 20 mM KCl solution, which directly depolarized plasma membranes, followed by Ca²⁺ influx through activated VDCCs [33]. We examined the effect of immepip on glucagon secretion stimulated by 20 mM KCl and found that glucagon secretion in response to 20 mM KCl was reduced by 1 µM immepip (1.82 ± 0.07 ng/well/h versus 2.29 ± 0.15 ng/well/h; Fig. 3B). This result suggests that intracellular signaling from H₃R affects the increase in intracellular Ca²⁺ concentration and/or downstream Ca²⁺ signaling, so we examined effects of immepip on the increase in intracellular Ca²⁺ concentration in response to 20 mM KCl. One micromole of immepip impaired the increase in intracellular Ca²⁺ concentration in response to 20 mM KCl (transient changes in Ca^{2+} ; 0.13 ± 0.07 versus 0.55 ± 0.08; Fig. 3C). These results indicate that H₃R signaling exerted an inhibitory effect on glucagon secretion through the blockage of Ca²⁺ influx.

3.4. H₃R agonist reduced serum glucagon concentration in rats

Finally, we investigated the role of H₃R in glucagon secretion *in vivo*. We examined the effect of immepip on serum glucagon concentration in rats. Serum glucagon concentration after a 24-h fast was remarkably inhibited by 1 μ M immepip (75.3 ± 14.5 pg/mL *versus* 130.0 ± 12.8 pg/mL, *P* < 0.05; Fig. 4), indicating that H₃R was involved in the regulation of serum glucagon concentration *in vivo*.

4. Discussion

We demonstrated for the first time that H_3R is expressed in α TC1.6 cells and mouse pancreatic α -cells. Using RT-PCR and western blot analysis, α TC1.6 cells were shown to express H_3R mRNA and protein (Fig. 1A, B). H_3R protein expression in pancreatic α -cells was confirmed by immunohistochemical analysis. Our data showed that activated H_3R inhibited glucagon secretion from α TC1.6 cells in response to low glucose concentrations (Fig. 2).

 H_3R is coupled to G_i protein [34]. Previous reports revealed that several G_i protein-coupled receptors expressed in pancreatic α -cells inhibited glucagon secretion. For example, group III metabotropic glutamate receptors, coupled with inhibitory G_i protein, are expressed in rat pancreatic α -cells and inhibit glucagon secretion from rat islets under low glucose conditions [35]. Moreover, somatostatin from pancreatic δ -cells also inhibits glucagon secretion through somatostatin receptor 2 coupled to G_i proteins [36]. This evidence corroborates the function of H₃R in pancreatic α -cells.

To elucidate the regulatory mechanism of H₃R on glucagon secretion, we investigated effects of immepip on intracellular Ca^{2+} concentration, which is essential to glucagon secretion [13]. Immepip inhibited increases in intracellular Ca²⁺ concentrations in response to 20 mM KCl stimulation (Fig. 3B). KCl stimulation directly elicits plasma membrane depolarization, leading to the activation of VDCCs. It is possible that H₃R signaling might inhibit the activity of VDCCs in pancreatic α-cells because H₃R in neurons reduced Ca²⁺ influx through VDCCs [37]. However, the inhibitory effect of immepip on Ca²⁺ concentration was more moderate during low glucose conditions than during KCl stimulation (Fig. 3B, C). although immepip inhibited glucagon secretion during low glucose conditions more strongly than during KCl stimulation (Figs. 2B and 3A). This suggests that the intracellular Ca²⁺-independent signaling pathway might be associated with the inhibitory effect of H₃R on glucagon secretion. Recently, De Marinis showed that the cAMP-PKA pathway increased glucagon secretion by the recruitment of glucagon granules toward the cell membrane [38]. Activated H₃R inhibits the cAMP-PKA pathway [24,39]; thus, any involvement of this pathway might be confirmed by using gene silencing technologies or a pharmacological approach. Further studies are necessary to elucidate the signaling induced by H₃R stimulation in pancreatic α -cells.

Immepip remarkably inhibited plasma glucagon concentration 15 min after intraperitoneal injection, indicating that H₃R has an important role in glucagon concentration *in vivo*. Our data suggests that immepip acts on pancreatic α -cells leading to direct inhibition of glucagon secretion. Additionally, immepip might indirectly modulate glucagon secretion through sympathetic nerves. Sympathetic nerve endings innervate pancreatic α -cells and modulate glucagon secretion [32,40]. Schlicker et al. reported that postgan-glionic sympathetic nerves expressed H₃R and that this H₃R regulates neurotransmitter release [41]. This evidence indicates that immepip might act on H₃R in sympathetic nerves and indirectly regulate glucagon secretion. However, further studies are needed to elucidate the role of H₃R in pancreatic α -cells or sympathetic nerves in the regulation of serum glucagon secretion.

As previously mentioned, abnormal glucagon secretion from pancreatic α -cells has been observed in diabetic patients [8]. H₃R agonists that decrease glucagon secretion could improve excessive glucagon secretion in hyperglycemic conditions; however, our data only indicates the inhibitory effect of H₃R on glucagon secretion in hypoglycemic conditions. Therefore, further studies are needed to clarify therapeutic effects of H₃R agonists in diabetic animal models, which have abnormal glucagon secretion during hyperglycemia [42].

We found a weak band of H_1R mRNA in α TC1.6 cells (Fig. 1A). Based on our previous study that clearly indicated the absence of H_1R mRNA in pancreatic islets [23], its expression might be undetectably low or absent in pancreatic islets and it may be specific to the cell-line. Additionally, we confirmed that glucagon secretion was not affected by H_1R -specific agonists (data not shown). Accordingly, it is suggested that H_1R is not involved in the regulation of glucagon secretion in pancreatic α -cells.

In this study, we found that H_3R in pancreatic α -cells had an inhibitory effect on glucagon secretion possibly by decreasing intracellular Ca²⁺ concentration. We also found that H_3R was involved in glucagon secretion *in vivo*. It will be interesting to investigate roles of H_3R in glucagon secretion in a diabetic animal model.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgments

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References

- [1] Passani, M.B. and Blandina, P. (2011) Histamine receptors in the CNS as targets for therapeutic intervention. Trends Pharmacol. Sci. 32, 242–249.
- [2] Clineschmidt, B.V. and Lotti, V.J. (1973) Histamine: intraventricular injection suppresses ingestive behavior of the cat. Arch. Int. Pharmacodyn. Ther. 206, 288–298.
- [3] Jorgensen, E.A., Knigge, U., Warberg, J. and Kjaer, A. (2007) Histamine and the regulation of body weight. Neuroendocrinology 86, 210–214.
- [4] Arrang, J.M., Garbarg, M. and Schwartz, J.C. (1983) Auto-inhibition of brain histamine release mediated by a novel class (H3) of histamine receptor. Nature 302, 832–837.
- [5] Barak, N., Greenway, F.L., Fujioka, K., Aronne, L.J. and Kushner, R.F. (2008) Effect of histaminergic manipulation on weight in obese adults: a randomized placebo controlled trial. Int. J. Obes. 32, 1559–1565.
- [6] Jiang, G. and Zhang, B.B. (2003) Glucagon and regulation of glucose metabolism. Am J. Physiol. Endocrinol. Metab. 284, E671–E678.
- [7] D'Alessio, D. (2011) The role of dysregulated glucagon secretion in type 2 diabetes. Diabetes Obes. Metab. 13, 126–132.
- [8] Muller, W.A., Faloona, G.R., Aguilar-Parada, E. and Unger, R.H. (1970) Abnormal alpha-cell function in diabetes. Response to carbohydrate and protein ingestion,. New Engl. J. Med. 283, 109–115.
- [9] Greenbaum, C.J., Prigeon, R.L. and D'Alessio, D.A. (2002) Impaired beta-cell function, incretin effect, and glucagon suppression in patients with type 1 diabetes who have normal fasting glucose. Diabetes 51, 951–957.
- [10] Cryer, P.E. (2002) Hypoglycaemia: the limiting factor in the glycaemic management of Type I and Type II diabetes. Diabetologia 45, 937–948.
- [11] Rorsman, P., Salehi, S.A., Abdulkader, F., Braun, M. and MacDonald, P.E. (2008) K(ATP)-channels and glucose-regulated glucagon secretion. Trends Endocrinol. Metab. 19, 277–284.
- [12] Huang, Y.C., Rupnik, M. and Gaisano, H.Y. (2011) Unperturbed islet alpha-cell function examined in mouse pancreas tissue slices. J. Physiol. 589, 395–408.
- [13] Walker, J.N., Ramracheya, R., Zhang, Q., Johnson, P.R., Braun, M. and Rorsman, P. (2011) Regulation of glucagon secretion by glucose: paracrine, intrinsic or both? Diabetes Obes. Metab. 13, 95–105.
- [14] Dunning, B.E., Foley, J.E. and Ahren, B. (2005) Alpha cell function in health and disease: influence of glucagon-like peptide-1. Diabetologia 48, 1700–1713.
- [15] Turton, M.D., O'Shea, D., Gunn, I., Beak, S.A., Edwards, C.M., Meeran, K., Choi, S.J., Taylor, G.M., Heath, M.M., Lambert, P.D., Wilding, J.P., Smith, D.M., Ghatei, M.A., Herbert, J. and Bloom, S.R. (1996) A role for glucagon-like peptide-1 in the central regulation of feeding. Nature 379, 69–72.
- [16] Friedman, J.M. and Halaas, J.L. (1998) Leptin and the regulation of body weight in mammals. Nature 395, 763–770.
- [17] Tuduri, E., Marroqui, L., Soriano, S., Ropero, A.B., Batista, T.M., Piquer, S., Lopez-Boado, M.A., Carneiro, E.M., Gomis, R., Nadal, A. and Quesada, I. (2009) Inhibitory effects of leptin on pancreatic alpha-cell function. Diabetes 58, 1616–1624.
- [18] Campfield, L.A., Smith, F.J., Guisez, Y., Devos, R. and Burn, P. (1995) Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science 269, 546–549.
- [19] Chuang, J.C., Sakata, I., Kohno, D., Perello, M., Osborne-Lawrence, S., Repa, J.J. and Zigman, J.M. (2011) Ghrelin directly stimulates glucagon secretion from pancreatic alpha-cells. Mol. Endocrinol. 25, 1600–1611.

- [20] Malik, S., McGlone, F., Bedrossian, D. and Dagher, A. (2008) Ghrelin modulates brain activity in areas that control appetitive behavior. Cell Metab. 7, 400–409.
- [21] Rouille, Y., Westermark, G., Martin, S.K. and Steiner, D.F. (1994) Proglucagon is processed to glucagon by prohormone convertase PC2 in alpha TC1-6 cells. Proc. Natl. Acad. Sci. U.S.A. 91, 3242–3246.
- [22] Okuda, T., Zhang, D., Shao, H., Okamura, N., Takino, N., Iwamura, T., Sakurai, E., Yoshikawa, T. and Yanai, K. (2009) Methamphetamine- and 3,4methylenedioxymethamphetamine-induced behavioral changes in histamine H3-receptor knockout mice. J. Pharmacol. Sci. 111, 167–174.
- [23] Nakamura, T., Yoshikawa, T., Noguchi, N., Sugawara, A., Kasajima, A., Sasano, H. and Yanai, K. (2014) The expression and function of histamine H3 receptor in pancreatic β-cells. Br. J. Pharmacol. 171, 171–185.
- [24] Lovenberg, T.W., Pyati, J., Chang, H., Wilson, S.J. and Erlander, M.G. (2000) Cloning of rat histamine H3 receptor reveals distinct species pharmacological profiles. J. Pharmacol. Exp. Ther. 293, 771–778.
- [25] Berlin, M., Boyce, C.W. and Ruiz Mde, L. (2011) Histamine H3 receptor as a drug discovery target. J. Med. Chem. 54, 26–53.
- [26] Barbier, A.J., Berridge, C., Dugovic, C., Laposky, A.D., Wilson, S.J., Boggs, J., Aluisio, L., Lord, B., Mazur, C., Pudiak, C.M., Langlois, X., Xiao, W., Apodaca, R., Carruthers, N.I. and Lovenberg, T.W. (2004) Acute wake-promoting actions of JNJ-5207852, a novel, diamine-based H3 antagonist. Br. J. Pharmacol. 143, 649–661.
- [27] Bonaventure, P., Letavic, M., Dugovic, C., Wilson, S., Aluisio, L., Pudiak, C., Lord, B., Mazur, C., Kamme, F., Nishino, S., Carruthers, N. and Lovenberg, T. (2007) Histamine H3 receptor antagonists: from target identification to drug leads. Biochem. Pharmacol. 73, 1084–1096.
- [28] Leurs, R., Bakker, R.A., Timmerman, H. and de Esch, I.J. (2005) The histamine H3 receptor: from gene cloning to H3 receptor drugs. Nat. Rev. Drug Discov. 4, 107–120.
- [29] Bongers, G., Bakker, R.A. and Leurs, R. (2007) Molecular aspects of the histamine H3 receptor. Biochem. Pharmacol. 73, 1195–1204.
- [30] Silver, R.B., Poonwasi, K.S., Seyedi, N., Wilson, S.J., Lovenberg, T.W. and Levi, R. (2002) Decreased intracellular calcium mediates the histamine H3-receptorinduced attenuation of norepinephrine exocytosis from cardiac sympathetic nerve endings. Proc. Natl. Acad. Sci. U.S.A. 99, 501–506.
- [31] Ashby, J.P. and Speake, R.N. (1975) Insulin and glucagon secretion from isolated islets of Langerhans. The effects of calcium ionophores. Biochem. J. 150, 89–96.
- [32] Gromada, J., Bokvist, K., Ding, W.G., Barg, S., Buschard, K., Renstrom, E. and Rorsman, P. (1997) Adrenaline stimulates glucagon secretion in pancreatic Acells by increasing the Ca²⁺ current and the number of granules close to the Ltype Ca²⁺ channels. J. Gen. Physiol. 110, 217–228.
- [33] Nelson, P.L., Zolochevska, O., Figueiredo, M.L., Soliman, A., Hsu, W.H., Feng, J.M., Zhang, H. and Cheng, H. (2011) Regulation of Ca²⁺-entry in pancreatic alpha-cell line by transient receptor potential melastatin 4 plays a vital role in glucagon release. Mol. Cell. Endocrinol. 335, 126–134.
- [34] Chen, J., Liu, C. and Lovenberg, T.W. (2003) Molecular and pharmacological characterization of the mouse histamine H3 receptor. Eur. J. Pharmacol. 467, 57–65.
- [35] Tong, Q., Ouedraogo, R. and Kirchgessner, A.L. (2002) Localization and function of group III metabotropic glutamate receptors in rat pancreatic islets. Am. J. Physiol. Endocrinol. Metab. 282, E1324–E1333.
- [36] Fehmann, H.C., Strowski, M. and Goke, B. (1995) Functional characterization of somatostatin receptors expressed on hamster glucagonoma cells. Am. J. Physiol. 268, E40–E47.
- [37] Brown, R.E. and Haas, H.L. (1999) On the mechanism of histaminergic inhibition of glutamate release in the rat dentate gyrus. J. Physiol. 515, 777– 786.
- [38] De Marinis, Y.Z., Salehi, A., Ward, C.E., Zhang, Q., Abdulkader, F., Bengtsson, M., Braha, O., Braun, M., Ramracheya, R., Amisten, S., Habib, A.M., Moritoh, Y., Zhang, E., Reimann, F., Rosengren, A.H., Shibasaki, T., Gribble, F., Renstrom, E., Seino, S., Eliasson, L. and Rorsman, P. (2010) GLP-1 inhibits and adrenaline stimulates glucagon release by differential modulation of N- and L-type Ca²⁺ channel-dependent exocytosis. Cell Metab. 11, 543–553.
- [39] Lovenberg, T.W., Roland, B.L., Wilson, S.J., Jiang, X., Pyati, J., Huvar, A., Jackson, M.R. and Erlander, M.G. (1999) Cloning and functional expression of the human histamine H3 receptor. Mol. Pharmacol. 55, 1101–1107.
- [40] Gromada, J., Franklin, I. and Wollheim, C.B. (2007) Alpha-cells of the endocrine pancreas: 35 years of research but the enigma remains. Endocr. Rev. 28, 84– 116.
- [41] Schlicker, E., Malinowska, B., Kathmann, M. and Gothert, M. (1994) Modulation of neurotransmitter release via histamine H3 heteroreceptors. Fundam. Clin. Pharmacol. 8, 128–137.
- [42] Flatt, P.R., Bailey, C.J. and Buchanan, K.D. (1982) Regulation of plasma immunoreactive glucagon in obese hyperglycaemic (ob/ob) mice. J. Endocrinol. 95, 215–227.