BJP British Journal of Pharmacology

DOI:10.1111/bph.12429
www.brjpharmacol.org

RESEARCH PAPER

The expression and function of histamine H_3 receptors in pancreatic beta cells

T Nakamura[1], T Yoshikawa[1], N Noguchi[2], A Sugawara[3], A Kasajima[4], H Sasano[4] and K Yanai[1]

[1]*Department of Pharmacology, Tohoku University Graduate School of Medicine, Sendai, Japan,* [2]*Department of Otorhinolaryngology, Head and Neck Surgery, Tohoku University Graduate School of Medicine, Sendai, Japan,* [3]*Department of Molecular Endocrinology, Tohoku University Graduate School of Medicine, Sendai, Japan, and* [4]*Department of Pathology, Tohoku University Graduate School of Medicine, Sendai, Japan*

Correspondence
Takeo Yoshikawa, Department of Pharmacology, Tohoku University Graduate School of Medicine, 2-1 Seiryo-machi, Aoba, Sendai 980-8575, Japan. E-mail: tyoshikawa@med.tohoku.ac.jp

T N and T Y contributed equally to this work.

Keywords
histamine H_3 receptor; pancreatic beta cells; insulin secretion

Received
24 January 2013
Revised
6 September 2013
Accepted
16 September 2013

BACKGROUND AND PURPOSE

Histamine and its receptors in the CNS play important roles in energy homeostasis. Here, we have investigated the expression and role of histamine receptors in pancreatic beta cells, which secrete insulin.

EXPERIMENTAL APPROACH

The expression of histamine receptors in pancreatic beta cells was examined by RT-PCR, Western blotting and immunostaining. Insulin secretion assay, ATP measurement and calcium imaging studies were performed to determine the function and signalling pathway of histamine H_3 receptors in glucose-induced insulin secretion (GIIS) from MIN6 cells, a mouse pancreatic beta cell line. The function and signalling pathway of H_3 receptors in MIN6 cell proliferation were examined using pharmacological assay and Western blotting.

KEY RESULTS

Histamine H_3 receptors were expressed in pancreatic beta cells. A selective H_3 receptor agonist, imetit, and a selective inverse H_3 receptor agonist, JNJ-5207852, had inhibitory and facilitatory effects, respectively, on GIIS in MIN6 cells. Neither imetit nor JNJ-5207852 altered intracellular ATP concentration, or intracellular calcium concentration stimulated by glucose and KCl, indicating that GIIS signalling was affected by H_3 receptor signalling downstream of the increase in intracellular calcium concentration. Moreover, imetit attenuated bromodeoxyuridine incorporation in MIN6 cells. The phosphorylation of cAMP response element-binding protein (CREB), which facilitated beta cell proliferation, was inhibited, though not significantly, by imetit, indicating that activated H_3 receptors inhibited MIN6 cell proliferation, possibly by decreasing CREB phosphorylation.

CONCLUSIONS AND IMPLICATIONS

Histamine H_3 receptors were expressed in mouse beta cells and could play a role in insulin secretion and, possibly, beta cell proliferation.

Abbreviations

BrdU, bromodeoxyuridine; CaMK II, calcium/calmodulin-dependent PKs II; CREB, cAMP response element-binding protein; GIIS, glucose-induced insulin secretion; H_3KO, histamine H_3 receptor gene knockout;; Hdc, histidine decarboxylase; HdcKO, Hdc gene knockout; KRB, Krebs–Ringer bicarbonate buffer; PTX, *Pertussis* toxin; RT, reverse transcription; VMAT2, vesicular monoamine transporter 2

Introduction

Histamine is involved in a wide variety of physiological events including allergic reactions, gastric acid secretion, smooth muscle contraction and the sleep–wake cycle (Haas *et al.*, 2008). This amine exerts its diverse effects through interactions with four different histamine receptors: H_1 (Fukui *et al.*, 1994), H_2 (Gantz *et al.*, 1991), H_3 (Lovenberg *et al.*, 1999) and H_4 (Liu *et al.*, 2001), which belong to the GPCR superfamily (nomenclature of receptors follows Alexander *et al.*, 2013).

The human histamine H_3 receptor, first cloned by Lovenberg in 1999, is a $G_{i/o}$-coupled receptor composed of 445 amino acids. H_3 receptors are expressed in brain and neuroendocrine tissues such as enterochromaffin-like cells and adrenal cortex (Lovenberg *et al.*, 1999). When ligands bind to H_3 receptor-activated G_i proteins (Clark and Hill, 1996), they inhibit adenylyl cyclase and lead to decreased cAMP concentrations, followed by inactivation of PKA (Lovenberg *et al.*, 1999). In addition to the well-known cAMP–PKA pathway, H_3 receptors are involved in various types of intracellular signalling, such as the MAPK pathway (Drutel *et al.*, 2001), the PI3K pathway and changes in intracellular Ca^{2+} concentration (Leurs *et al.*, 2005). Interestingly, H_3 receptors display intrinsic, constitutive activity in the absence of ligands. The H_3 receptor agonists activate these receptors, whereas an H_3 receptor inverse agonist, which has the opposite effect of an agonist, reduces H_3 receptor activity to below the normal level (Morisset *et al.*, 2000). In fact, native brain H_3 receptors have a high constitutive activity, so the corresponding inverse agonists thioperamide and FUB465 increase histamine release with G_i inactivation, although the H_3 receptor agonist imetit reduces histamine release with G_i activation (Morisset *et al.*, 2000).

Since the inhibitory effect of neuronal histamine on food intake was first reported in 1973 (Clineschmidt and Lotti, 1973), many studies have shown that histamine acts as a neurotransmitter in energy homeostasis, modulating hypothalamic neuronal activities (Jorgensen *et al.*, 2007). A H_3 receptor inverse agonist increased the hypothalamic concentration of histamine, leading to reduced food intake and body weight (Malmlof *et al.*, 2005). Conversely, a H_3 receptor agonist suppressed hypothalamic histamine release and elicited feeding behaviour in rats (Chiba *et al.*, 2009). Betahistine, a partial inverse agonist at H_3 receptors, induced significant weight loss with minimal adverse events in women under 50 years of age (Barak *et al.*, 2008). Moreover, the centrally acting H_3 receptor protean agonist proxyfan reduced blood glucose level and exhibited anti-diabetic properties in mice (Henry *et al.*, 2011). Based on all these findings, neuronal H_3 receptors have attracted substantial attention as a potential therapeutic target in obesity (Leurs *et al.*, 2005).

Although peripheral metabolic organs, as well as the CNS, are involved in a rigorous control of energy homeostasis, the direct actions of histamine on peripheral metabolic organs, such as liver, muscles and pancreatic islets of Langerhans, remain to be elucidated. Previous reports showed that muscles (Lovenberg *et al.*, 2000) and liver (Heron *et al.*, 2001) did not express H_3 receptors, but it remained unclear whether pancreatic beta cells, which have similar properties to neurons, did express this receptor. Pancreatic beta cells in the islets of Langerhans crucially regulate energy homeostasis through appropriate insulin secretion in response to blood glucose level. These beta cells express a variety of GPCRs, modulating beta cell functions (Ahren, 2009). This is evident in the success of glucagon-like peptide 1 analogues, which increase insulin secretion and promote beta cell growth by activating stimulatory GPCRs (Drucker, 2006). Other GPCRs that regulate beta cell functions include neurotransmitter receptors such as muscarinic M_3 cholinoceptors (Gautam *et al.*, 2006) and α_{2A}-adrenoceptors (Devedjian *et al.*, 2000; Rosengren *et al.*, 2010).

Pharmacological analysis identified the H_3 receptor as an autoreceptor that inhibiting histamine release from neurons (Arrang *et al.*, 1983). Subsequently, extensive investigations have revealed that brain H_3 receptors regulate the release of various neurotransmitters, such as ACh, glutamate and GABA, and are involved in mediating the effect of histamine in numerous physiological processes, including the sleep–wake cycle and cognition (Haas *et al.*, 2008). In peripheral tissues, H_3 receptors are expressed in neuroendocrine organs and regulate their functions. For example, the activation of H_3 receptors inhibited the release of adrenocorticotropic hormone and prolactin from the pituitary gland (Knigge *et al.*, 1999) and of histamine from enterochromaffin-like cells (Kidd *et al.*, 1996).

These lines of evidence suggested that histamine receptors might be involved in the functions of pancreatic beta cells. Therefore, we investigated histamine receptor expression in pancreatic beta cells and the role of histamine receptors in insulin secretion and beta cell proliferation.

Methods

Animals

All animal care and experimental procedures used in this study were in accordance with the Principles for Care and Use of Research Animals of Tohoku University, Sendai, Japan. All experiments involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010). Male wild-type (WT) ICR mice were purchased from Japan SLC (Hamamatsu, Japan). Male H_3 receptor gene knockout (H_3KO) C57BL/6 mice (Okuda *et al.*, 2009) and male histidine decarboxylase gene knockout (HdcKO) C57BL/6 mice (Ohtsu and Watanabe, 2003) were maintained in our laboratory. All mice were maintained on a 12 h light/dark cycle (0800/2000 h) in a humidity- and temperature-controlled room, and were allowed *ad libitum* access to water and food (Labo MR Breeder; Nosan, Yokohama, Japan). Twenty-five WT ICR mice, five H_3KO mice and three HdcKO mice were used in this study.

Cell culture

MIN6 cells (Miyazaki *et al.*, 1990), a cell line derived from mouse pancreatic islet beta cells (kindly donated by Professor Jun-ichi Miyazaki, Faculty of Medicine, Osaka University; passage 20–35), were maintained in DMEM (Sigma, St. Louis, MO, USA) containing 4500 $mg \cdot L^{-1}$ D-glucose, 10% fetal calf serum (FCS; Thermo, Waltham, MA, USA), 100 $U \cdot mL^{-1}$ peni-

cillin G potassium (Wako, Osaka, Japan) and 50 mg·L^{-1} streptomycin sulphate (Wako) at 37°C in 5% CO_2 in a humidified incubator.

Isolation of mouse pancreatic islets

WT ICR mice (12 weeks old) were anaesthetized with isoflurane (initial dose 3.0%, subsequent dose 2.5%, air flow 2 L·min^{-1}) then laparotomized. The pancreata were perfused with Hank's solution (Nissui, Tokyo, Japan) through a 27 G needle placed into the common bile duct. The removed pancreata were then digested using collagenase (Wako) as described previously (Okamoto *et al.*, 1975), and pancreatic islets were isolated by handpicking under a stereomicroscope. We used ICR mice for the preparation of islets because bile ducts of C57BL/6 mice were smaller and difficult to cannulate.

Reverse transcription (RT)-PCR

Total RNA was isolated from mouse pancreatic islets and MIN6 cells using NucleoSpin RNAII mini spin kits (Macherey-Nagel, Duren, Germany), and 500 ng of this RNA was then reverse-transcribed using PrimeScriptII (Takara-Bio, Otsu, Japan). Diluted reverse-transcribed samples (equivalent to 5 ng total RNA) were amplified by 35 cycles of PCR (10 s at 98°C, 30 s at 58°C and 90 s at 72°C) with specific oligonucleotide primers using an ABI 2710 thermal cycler (Applied Biosystems, Foster City, CA, USA). The primers used to detect histamine receptors are shown in Table 1. RT-PCR in the absence of reverse transcriptase was used as negative control. For positive controls, we used cDNA from mouse hypothalamus for H_1 receptors and H_3 receptors, and cDNA from mouse cardiac muscle and mouse mast cells was used for H_2 and H_4 receptors respectively.

Mouse H_3 receptor antibody production

Rabbit anti-mouse H_3 receptor antibody was produced for us by Operon Biotechnology (Tokyo, Japan). Rabbits ($n = 2$) were immunized with keyhole limpet haemocyanin-conjugated mouse H_3 receptor peptides (amino acids: 142–155 and 428–442). An adequate increase in serum antibody titre was confirmed by ELISA. Collected serum was purified by antigen-specific affinity chromatography, and the purified antibody was used to detect mouse H_3 receptor protein.

H_3 receptor positive control for Western blot

The mouse H_3 receptor gene was cloned from WT ICR mouse islet cDNA and inserted into the pCI-neo vector (Promega, Fitchburg, WI, USA) using the In-Fusion PCR Cloning System (Clontech Bioinformatics, Mountain View, CA, USA). The plasmid construct was amplified in *Escherichia coli* and purified using a NucleoBond Xtra Maxi-prep kit (Macherey-Nagel). Purified plasmid was transfected into CHO cells using Lipofectamine LTX (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Transfected CHO cells were lysed with RIPA buffer (Thermo), and the cell lysate was used as the positive control in Western blots.

Western blotting

Isolated pancreatic islets from 12-week-old mice ($n = 5$) and MIN6 cells were lysed with RIPA buffer. We used the lysate of H_3 receptor-overexpressing CHO cells as positive control. The lysate of CHO cells transfected with plasmid DNA containing no insert was used as negative control. Samples were denatured at 75°C for 5 min. Aliquots containing 10 μg of protein were applied to 5–20% gradient polyacrylamide gels (Bio-Rad, Hercules, CA, USA) and separated by SDS-PAGE at constant voltage of 200 V for 30 min. After SDS-PAGE, the samples were transferred to a PVDF membrane by blotting at a constant current of 2.5 A for 3 min. The PVDF membrane was blocked by agitating in 5% non-fat dry milk solution for 1 h, then immunoblotted with rabbit anti-mouse H_3 receptor antibody (0.4 μg·mL^{-1} in 5% BSA solution) or rabbit anti-β-actin antibody (Cell Signaling Technology, Danvers, MA, USA; 1:2000 dilution), and incubated at room temperature for 1 h. At the end of the incubation period, the membrane was washed then incubated with a HRP-labelled donkey anti-rabbit antibody (Thermo) as the secondary antibody (1:25 000 dilution in non-fat dry milk solution) at room temperature for 1 h. Immunoreactive bands were detected using an ECL Western Blotting Analysis System (PerkinElmer, Waltham, MA, USA), and bands were recorded using the Ez-capture MG chemiluminescence imaging system (Atto, Tokyo, Japan).

Table 1

Primer sequences used for PCR

	Sense primers	Antisense primers	Product size (bp)
Hrh1	5′-GGGAAAGGGAAACAGTCACA-3′	5′- TGAAGTTCTCGTTGCACAGC-3′	555
Hrh2	5′-ATATGGACTGGTGGATGGGA-3′	5′-CAGAGATTCTTGACACGGCA-3′	709
Hrh3	5′-GCCATCCTGAGTTGGGAGTA-3′	5′-TCGTACCAGTAGTCGGGGAC-3′	677
Hrh4	5′-GGAATCTGCATGTTTTGGCT-3′	5′- GTGACCTGGCTAGCTTCCTG-3′	667
Hdc	5′- TGTGACGTCCCAGTTTCCA-3′	5′- GTGGATCACGAAGACCCTGT-3′	777
Actb	5′- TGTTACCAACTGGGACGACA-3′	5′-GTACTTGCGCTCAGGAGGAG -3′	687

The sequences of primers to detect mouse mRNA of histamine H_1 receptor (Hrh1), histamine H_2 receptor (Hrh2), histamine H_3 receptor (Hrh3), histamine H_4 receptor (Hrh4), Hdc and β-actin (Actb) are shown. The numbers in the left column indicate the size of the PCR product. bp, base pair.

Immunohistochemical analysis

Pancreata were quickly removed from 12-week-old WT (n = 5), H_3KO (n = 3) and HdcKO (n = 3) mice. Isolated pancreata were fixed in 10% paraformaldehyde and embedded in paraffin. The sections were then deparaffinized and blocked with 10% goat serum at room temperature for 15 min. To detect insulin, the sections were incubated with mouse anti-insulin 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 mouse H_3 receptors, Hdc and histamine in mouse specimens, samples were incubated with rabbit anti-mouse H_3 receptor antibody (4 μg·mL^{-1}), rabbit anti-Hdc antibody (Progen Biotechnik, Heidelberg, Germany; 1:200 dilution) and rabbit antihistamine antibody (Sigma; 1:50 dilution), respectively, overnight at 4°C, and then treated with the secondary antibody: Alexa Fluor 488-conjugated goat anti-rabbit antibody (Invitrogen). For double staining of insulin and H_3 receptors, Hdc or histamine, samples were incubated with anti-insulin antibody for 2 h at room temperature, then washed and labelled with antibodies to H_3 receptors, Hdc or histamine and incubated overnight at 4°C. Specimens from H_3KO and HdcKO mice were used as negative controls for H_3 receptors and Hdc respectively. As a negative control for histamine, the antihistamine antibody was incubated in 1 mM histamine buffer at room temperature for 30 min before it was added to the specimens.

Anonymized paraffinized human specimens (n = 2) were obtained from Tohoku University pathological library. The use of human pancreatic samples was approved by the ethical committee of Tohoku University Graduate School of Medicine. Because the human samples exhibited high autofluorescence, we were unable to use the same staining method as we used for the mouse samples and instead we used non-fluorescent methods to stain human sections. Deparaffinized sections were incubated with mouse anti-insulin antibody (Sigma; 1:2000 dilution) then treated with chromogenic Vector Red (Vector, Burlingame, CA, USA). After washing with PBS, rabbit anti-human H_3 receptor antibody (Acris, Herford, Germany; 1:100 dilution) was added to the incubation buffer, followed by treatment with chromogenic HistoGreen (Cosmobio, Tokyo, Japan). Parallel sections were incubated with non-immune rabbit Ig fraction (DAKO, Glostrup, Denmark) instead of human H_3 receptor antibody as a negative control. To detect Hdc and histamine in human specimens, deparaffinized sections were incubated with rabbit antihistamine antibody (Sigma) and rabbit anti-Hdc antibody (Progen Biotechnik), respectively, overnight at 4°C, and then treated with goat anti-rabbit antibody conjugated with HRP (Nissui, Tokyo, Japan). Colour was developed by diaminobenzidine, followed by haematoxylin contrast staining.

Histamine secretion

MIN6 cells, seeded into a 24 well plate at a density of 2.0×10^5 cells per well, were incubated in 1 mL of DMEM containing 5% horse serum (Gibco, Carlsbad, CA, USA) ±1 mM histidine (Wako) for 1, 2, 4, 8 and 24 h at 37°C in 5% CO_2. In this assay, we used horse serum as substitute for FCS because FCS contains much higher concentration of histaminase, a histamine-degrading enzyme (Dy *et al.*, 1982). After harvesting the medium from each well, the histamine concentration in the medium was measured by HPLC (Yamatodani *et al.*, 1985). Histamine contents in the medium without MIN6 cells were used as blank controls.

Insulin secretion

MIN6 cells, seeded into a 24 well plate at a density of 2.0×10^5 cells per well, were pre-incubated in 500 μL of Krebs–Ringer bicarbonate buffer (KRB) containing 2.8 mM glucose for 30 min at 37°C in 5% CO_2. Following pre-incubation, MIN6 cells were stimulated in 500 μL of KRB containing 2.8 or 16.7 mM glucose or 20 mM KCl ±1 μM imetit (Sigma), a selective H_3 receptor agonist (Garbarg *et al.*, 1992), or 1 μM JNJ-5207852 (kindly donated by Dr. Nicholas Carruthers, Johnson & Johnson Pharmaceutical Research and Development, USA), a potent and selective H_3 receptor inverse agonist, for 1 h at 37°C in 5% CO_2 (Barbier *et al.*, 2004; Jia *et al.*, 2006; Bonaventure *et al.*, 2007). Imetit and JNJ-5207852 were both dissolved in distilled water. The insulin concentration in KRB was determined using an insulin ELISA kit (Morinaga Institute of Biological Science, Yokohama, Japan). Another insulin secretion experiment was designed to examine the effects of imetit efflux after blockade of regulatory G proteins using *Pertussis* toxin (PTX; Sigma; Clark *et al.*, 1993). This was performed using the same insulin secretion experimental protocol described above, with the addition of KRB buffer containing PTX (10 ng·mL^{-1}).

Measurement of intracellular ATP concentration

We measured intracellular ATP concentration in static cells, which is a method commonly used in pancreatic beta cell research (Uchizono *et al.*, 2004). MIN6 cells, seeded into a 96 well plate at a density of 2.0×10^4 cells per well, were incubated under the same conditions used for insulin secretion. After the buffer was removed from the plate, intracellular ATP concentration was quantitated using Cell Titer-glo (Promega) using a luminometer (Berthold Technologies, Bad Wildbad, Germany; Lin and Anseth, 2011).

Measurement of intracellular Ca^{2+} concentration

Because intracellular Ca^{2+} concentration changes much faster than ATP, we could not use static measurement methods as for ATP but instead, it was necessary to measure changes dynamically by perfusion experiments. MIN6 cells, seeded into 35 mm diameter glass-bottomed dishes at a density of 6.0×10^5 cells per dish, were pre-incubated in KRB containing 2.8 mM glucose and 2 μM fura-2/AM (Invitrogen) for 30 min at 37°C (Nakazaki *et al.*, 1998). Following pre-incubation, MIN6 cells were continuously perfused with preheated buffer at 2 mL·min^{-1} on the stage of an inverted microscope (Olympus, Tokyo, Japan). Cells were excited alternately at 340 and 380 nm. Light emitted through a 510 nm filter was detected using a cooled CCD camera (Hamamatsu Photonics, Hamamatsu, Japan) and analysed with MetaMorph software (Molecular Devices, Sunnyvale, CA, USA).

Bromodeoxyuridine (BrdU) assay

MIN6 cells, seeded at equal numbers of 2.0×10^4 cells per well into a 96 well plate, were incubated in DMEM containing

2.8 mM glucose and 100 pM insulin (Sigma) at 37°C for 1, 2, 4, 8 or 24 h. Fifteen minutes after the start of the incubation, 1 μM BrdU was added for the remainder of the incubation period. BrdU incorporation was measured using a BrdU Labelling and Detection Kit3 (Roche, Basel, Switzerland), and absorbance at 405 nm was measured with a spectrophotometer (Molecular Devices).

Measurement of intracellular cAMP concentration

MIN6 cells seeded into a 24 well plate were stimulated using the same protocol as for insulin secretion. At the end of the incubation period, the cells were lysed in 0.1 M HCl. The intracellular cAMP concentration was measured using a cAMP EIA kit (Cayman, Ann Arbor, MI, USA). The protein concentration of the cell lysate was also measured using the Bradford protein assay.

cAMP response element-binding protein (CREB) and Akt phosphorylation assay

MIN6 cells, seeded into a 6 well plate at a density of 1.0×10^6 cells per well, were pre-incubated in KRB containing 2.8 mM glucose for 2 h then incubated in 16.7 mM glucose/KRB ±1 μM imetit for 15 min. After incubation, MIN6 cells were lysed with RIPA buffer (Thermo) containing protease inhibitor (Roche). SDS-PAGE was performed following the same protocol as for detection of H_3 receptor protein. Samples were then transferred to PVDF membranes and immunoblotted with rabbit anti-CREB antibody, anti-phosphorylated CREB antibody, anti-Akt antibody, anti-phosphorylated Akt antibody (all at 1:1000 dilution) and anti-β-actin antibody (1:2000 dilution; all from Cell Signaling Technology). Immunoreactive bands were detected using HRP-labelled anti-rabbit antibody (Thermo; 1:25 000 dilution) as a secondary antibody and visualized with an ECL Western Blotting Analysis System (PerkinElmer).

Data analysis

Data are expressed as the mean ± SE. Statistical analysis was performed using Student's *t*-test. Differences were considered significant at *P*-values less than 0.05. IC_{50}, I_{max}, EC_{50} and E_{max} parameters were calculated using Prism 5 software (GraphPad Software, La Jolla, CA, USA) with non-linear regression analysis. All experiments were performed more than once. No significant differences were found between repeated experiments.

Results

H_3 receptors are expressed in pancreatic beta cells

RT-PCR was used to identify the histamine receptor subtypes expressed in primary mouse islets. H_3 receptor mRNA was highly expressed in mouse islets (Figure 1A), whereas mRNA for none of the other histamine receptors were detectable. Expression of H_3 receptor protein in mouse pancreatic islets was confirmed by Western blotting (Figure 1B). Pancreatic islets consist mainly of α-cells and beta cells, and immunohistochemical analysis of pancreatic islets showed that insulin-positive beta cells (stained red) also expressed H_3 receptorprotein (stained green), indicating that H_3 receptors were expressed in insulin-positive mouse beta cells (Figure 1C). Moreover, immunohistochemical analysis showed that human pancreatic beta cells also express H_3 receptors (Figure 1D). These results together suggest that pancreatic beta cells, in general, express H_3 receptors.

Hdc and histamine are expressed in pancreatic beta cells

We next investigated the expression of histamine and of Hdc (Ohtsu, 2010), the enzyme essential for histamine synthesis, by RT-PCR and immunostaining pancreatic sections. MIN6 cells and mouse pancreatic islets both expressed Hdc mRNA (Figure 1A). The exocrine pancreas did not express histamine or Hdc; in contrast, mouse and human pancreatic islets expressed both (Figure 2A–C), indicating that histamine synthesized by Hdc in pancreatic islets could stimulate H_3 receptors in an autocrine or paracrine manner.

Histamine is secreted by MIN6 cells

To evaluate whether MIN6 cells generate and secrete histamine, we measured the amount of histamine in the medium after histidine loading. Histamine secretion from MIN6 cells increased in a time-dependent manner in the presence of histidine (Figure 2D), suggesting that mouse pancreatic beta cells synthesized and secreted histamine.

Activation of H_3 receptors inhibits glucose-induced insulin secretion (GIIS) in MIN6 cells

We used MIN6 cells to assay expression of functional H_3 receptors in pancreatic beta cells because pancreatic islets contain 10–30% of other endocrine cells, such as glucagon-releasing α-cells and somatostatin-releasing δ-cells. The MIN6 cell line, derived from mouse pancreatic beta cells, is widely used in research on pancreatic beta cell function because MIN6 cells are sensitive to the presence of glucose and secrete insulin in response to it. We confirmed that MIN6 cells expressed both H_3 receptor mRNA (Figure 1A) and protein (Figure 1B).

GIIS is one of the most important functions of pancreatic beta cells, enabling precise maintenance of glucose homeostasis. We investigated the role of H_3 receptors expressed in MIN6 cells by stimulating MIN6 cells with glucose-containing KRB buffer in the absence or presence of the H_3 receptor agonist imetit. Insulin secretion increased more than twofold over basal levels in response to high concentrations of glucose (16.7 mM). This insulin secretion associated with high glucose levels was inhibited by imetit ($P < 0.05$), whereas basal secretion at 2.8 mM glucose with imetit was maintained at close to control levels (Figure 3A). Subsequent experiments were therefore performed with high glucose (16.7mM). We examined the effects of various concentrations of imetit (10 nM to 100 μM) on insulin secretion. As shown in Figure 3B, imetit inhibited GIIS in a dose-dependent manner, with an IC_{50} of 1.95 μM and an I_{max} value of 68%. To confirm whether imetit reduced GIIS via the G_i activation of H_3 receptors, we treated MIN6 cells with PTX, an inhibitor of G_i protein activity (Clark *et al.*, 1993). The reduction in insulin secretion seen with 100 μM imetit was completely blocked by

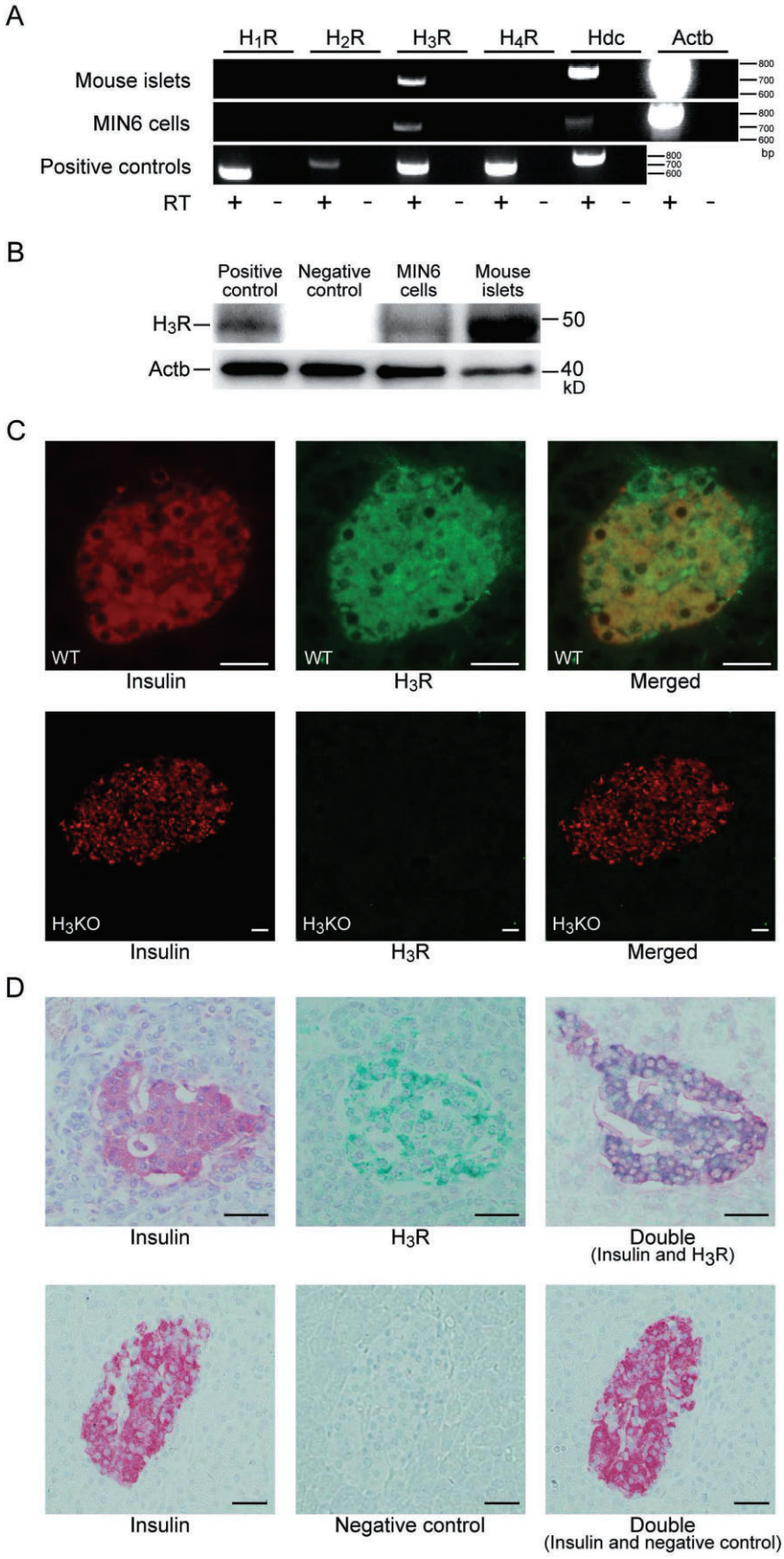
A
H1R
H2R
H3R
H4R
Hdc
Actb
Mouse islets
MIN6 cells
Positive controls
800
700
600
bp
RT + - + - + - + - + - + -
B
Positive control
Negative control
MIN6 cells
Mouse islets
H3R
Actb
50
40
kD
C
WT
Insulin
H3R
Merged
H3KO
D
Insulin
H3R
Double
(Insulin and H3R)
Negative control
Double
(Insulin and negative control)

Figure 1

Histamine receptor expression in mouse islets, MIN6 cells and human islets. (A) RT-PCR detection of histamine receptors in mouse islets and MIN6 cells. RT + and – indicate RT-PCR in the presence or absence of reverse transcriptase respectively. The results shown are representative of three experiments performed. (B) Western blot of H_3 receptor protein in mouse islets and MIN6 cells. The bands corresponding to H_3 receptor protein and β-actin (Actb) bands are indicated. Lysates of CHO cells overexpressing H_3 receptors and of CHO cells transfected with plasmid DNA containing no insert were used as the positive and negative control respectively. The results shown are representative of three experiments performed. (C) Immunohistochemical analysis of H_3 receptor expression in mouse pancreatic islets of WT mice (upper panels) and of H_3KO mice (lower panels) used as negative controls. Insulin and H_3 receptors were visualized with Alexa 568 (red) and Alexa 488 (green) respectively. Merged images of insulin and H_3 receptor staining are shown in the right panel. Scale bar, 20 μm. The results shown are representative of three experiments performed, each of which involved staining of pancreata from five mice. At least 10 islets were observed in each pancreas. (D) Immunohistochemical analysis of H_3 receptor expression in human pancreatic islets. Insulin and H_3 receptors were visualized with Vector Red (red) and HistoGreen (green) respectively. Double staining of insulin and H_3 receptors is shown in the right panels. The panels in the lower row show the negative control staining of anti- H_3 receptor antibody. Scale bar, 50 μm. The results shown are representative of two experiments performed.

exposure to PTX (Figure 3E), confirming that imetit inhibited GIIS by activating the G_i protein associated with the H_3 receptors. These results indicate that H_3 receptors negatively regulate insulin secretion in pancreatic beta cells, via G_i proteins.

The H_3 receptor inverse agonist, JNJ-5207852, increases GIIS in a dose-dependent manner

Next, we investigated the effects of an H_3 receptor inverse agonist, JNJ-5207852, on insulin secretion. We assumed that an H_3 receptor inverse agonist would increase insulin secretion from MIN6 cells because of the high constitutive activity of H_3 receptors in neurons (Morisset *et al.*, 2000). As expected, the level of insulin secretion stimulated by 16.7 mM glucose was increased by 25% in the presence of 1 μM JNJ-5207852 ($P < 0.05$; Figure 3C), whereas basal insulin secretion was not affected by the inverse agonist. JNJ-5207852 increased GIIS in a dose-dependent manner, with an EC_{50} of 13.8 μM and an E_{max} of 103% (Figure 3D). These results indicate that H_3 receptors expressed in pancreatic beta cells are constitutively active and that H_3 receptor inverse agonists could be used as insulin secretagogues.

Inhibition of GIIS mediated by H_3 receptors is not accompanied by changes in ATP or intracellular Ca^{2+} concentration

We next investigated which part of the GIIS pathway was affected by intracellular H_3 receptor signalling. The GIIS signalling pathway can be divided into four steps, as follows (Leibiger *et al.*, 2008): (i) glucose imported into beta cells is metabolized to ATP; (ii) increased ATP induces closure of ATP-sensitive potassium channels followed by plasma membrane depolarization; (iii) depolarization opens voltage-dependent calcium channels, leading to Ca^{2+} influx; and (iv) increased intracellular Ca^{2+} evokes insulin granule exocytosis.

Because an increase in ATP concentration due to glucose uptake and metabolism is necessary for insulin secretion, we first examined the effects of imetit on ATP concentrations. The intracellular ATP concentration was increased by stimulation with 16.7 mM glucose, but imetit did not reduce the intracellular ATP concentration at either a low or a high glucose concentration (Figure 4A), suggesting that intracellular signalling from the H_3 receptor affected the process of GIIS signalling after the increase in intracellular ATP concentration. Next, we investigated the effect of imetit on insulin secretion induced by 20 mM KCl, which directly depolarizes the plasma membrane and leads to a raised intracellular Ca^{2+} concentration, followed by insulin secretion. Insulin secretion in response to 20 mM KCl was reduced by imetit (Figure 4C). The extent of inhibition of KCl-induced insulin secretion by imetit was quite similar to that of GIIS (22 vs. 24% reduction), suggesting that intracellular signalling from the H_3 receptor affects the steps downstream of membrane depolarization, increasing intracellular Ca^{2+} concentration and/or insulin granule exocytosis.

We next examined the effects of imetit on the increase in intracellular Ca^{2+} concentration in response to 20 mM KCl or 16.7 mM glucose. Imetit did not affect the increase in intracellular Ca^{2+} concentration in response to 20 mM KCl (transient changes in Ca^{2+}; 1.74 ± 0.21 vs. 1.75 ± 0.09; n = 4; Figure 4E) and 16.7 mM glucose (transient changes in Ca^{2+}; 0.87 ± 0.08 vs. 0.81 ± 0.10; n = 4; Figure 4G). These results suggest that intracellular signalling from the H_3 receptor inhibits GIIS downstream of Ca^{2+} influx.

We also investigated the effect of JNJ-5207852 and found that it did not change the ATP concentration at either low or high glucose concentrations (Figure 4B). The extent of the stimulation of KCl-induced insulin secretion by JNJ-5207852 was similar to that of GIIS (38 vs. 39%; Figures 3C and 4D). Moreover, the intracellular Ca^{2+} concentration induced by 20 mM KCl (transient changes in Ca^{2+}; 1.88 ± 0.20 vs. 1.83 ± 0.20; n = 4; Figure 4F) or 16.7 mM glucose (transient changes in Ca^{2+}; 1.35 ± 0.22 vs. 1.32 ± 0.25; n = 4; Figure 4H) was not affected by JNJ-5207852.

To summarize all the results of the GIIS signalling experiments, H_3 receptor signalling did not alter either the intracellular ATP or Ca^{2+} concentration.

Imetit inhibits the proliferation of MIN6 cells

We investigated the effect of H_3 receptors on the proliferation of MIN6 cells because a variety of GPCRs expressed in pancreatic beta cells are known to regulate beta cell proliferation (Ahren, 2009). BrdU incorporation increased more than threefold in response to insulin and glucose, compared with basal BrdU incorporation (Figure 5A). This BrdU incorporation in the presence of insulin and glucose was significantly inhibited by imetit after 24 h incubation (Figure 5A). Imetit inhibited BrdU incorporation in a time-dependent manner (Figure 5A). Insulin stimulates beta cell proliferation through the phosphorylation of Akt (Saltiel and Kahn, 2001), but Akt phosphorylation was not affected by imetit (Figure 5B).

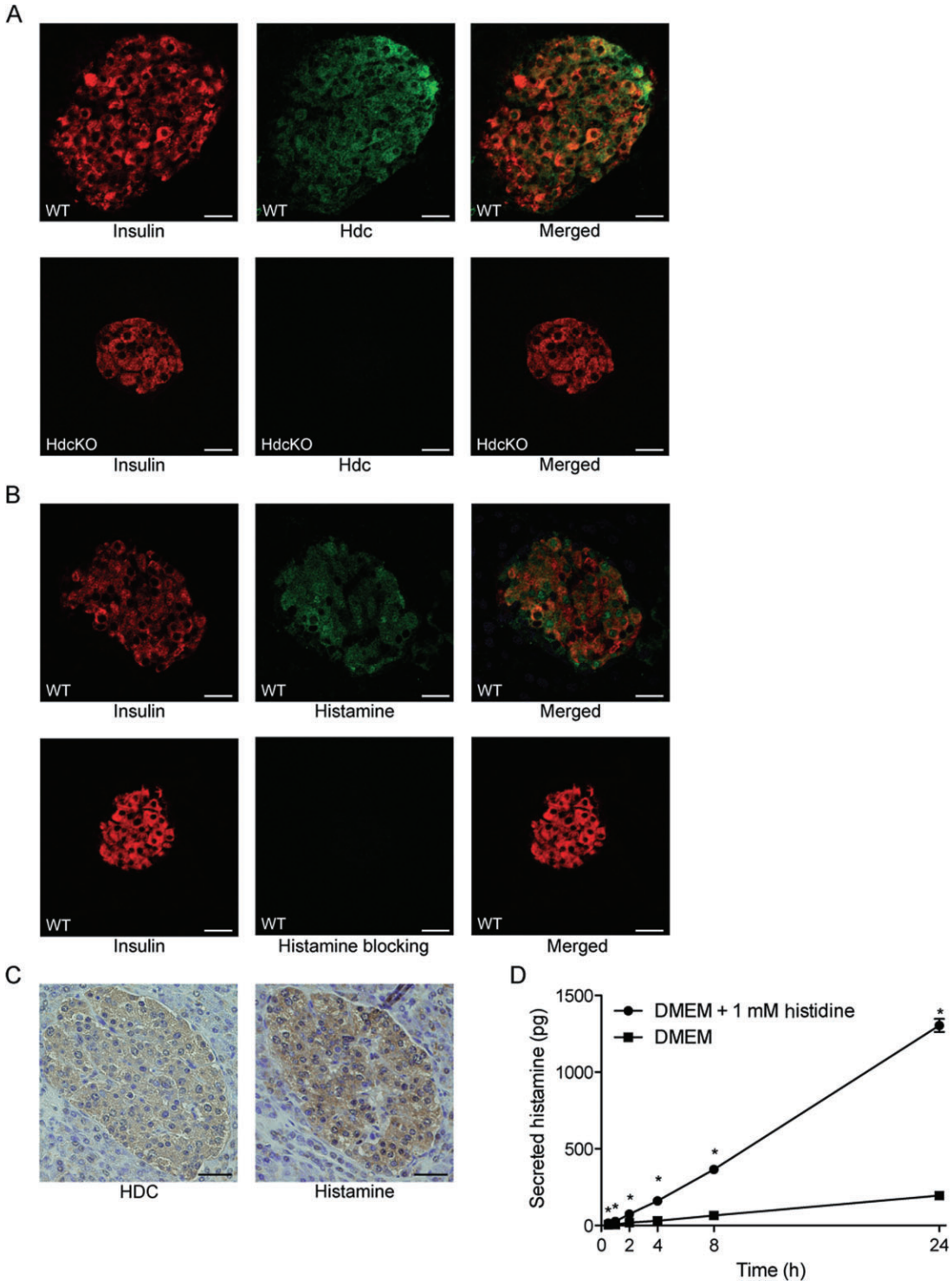

Figure 2

Hdc and histamine expression in mouse and human islets. (A) Immunohistochemical analysis of Hdc expression in mouse pancreatic islets of WT mice (upper panels) and HdcKO mice (lower panels) as negative controls. Insulin and Hdc were visualized with Alexa 568 (red) and Alexa 488 (green) respectively. Merged images of insulin and Hdc staining are shown in the right panel. Scale bar, 20 μm. The experiment shown is representative of two experiments performed. (B) Immunohistochemical analysis of histamine expression in mouse pancreatic islets. Insulin and histamine were visualized with Alexa 568 (red) and Alexa 488 (green) respectively. A merged image of insulin and histamine staining is shown in the right panel. The antihistamine antibody that was absorbed by histamine was used as a negative control (lower panel). Scale bar, 20 μm. The experiment shown is representative of two experiments performed. (C) Immunohistochemical analysis of Hdc (left) and histamine (right) expression in human pancreatic islets. Scale bar, 50 μm. The experiment shown is representative of two experiments performed. (D) Histamine secretion from MIN6 cells stimulated by 1 mM histidine. *$P < 0.05$, $n = 4$. The experiment shown is representative of two experiments performed.

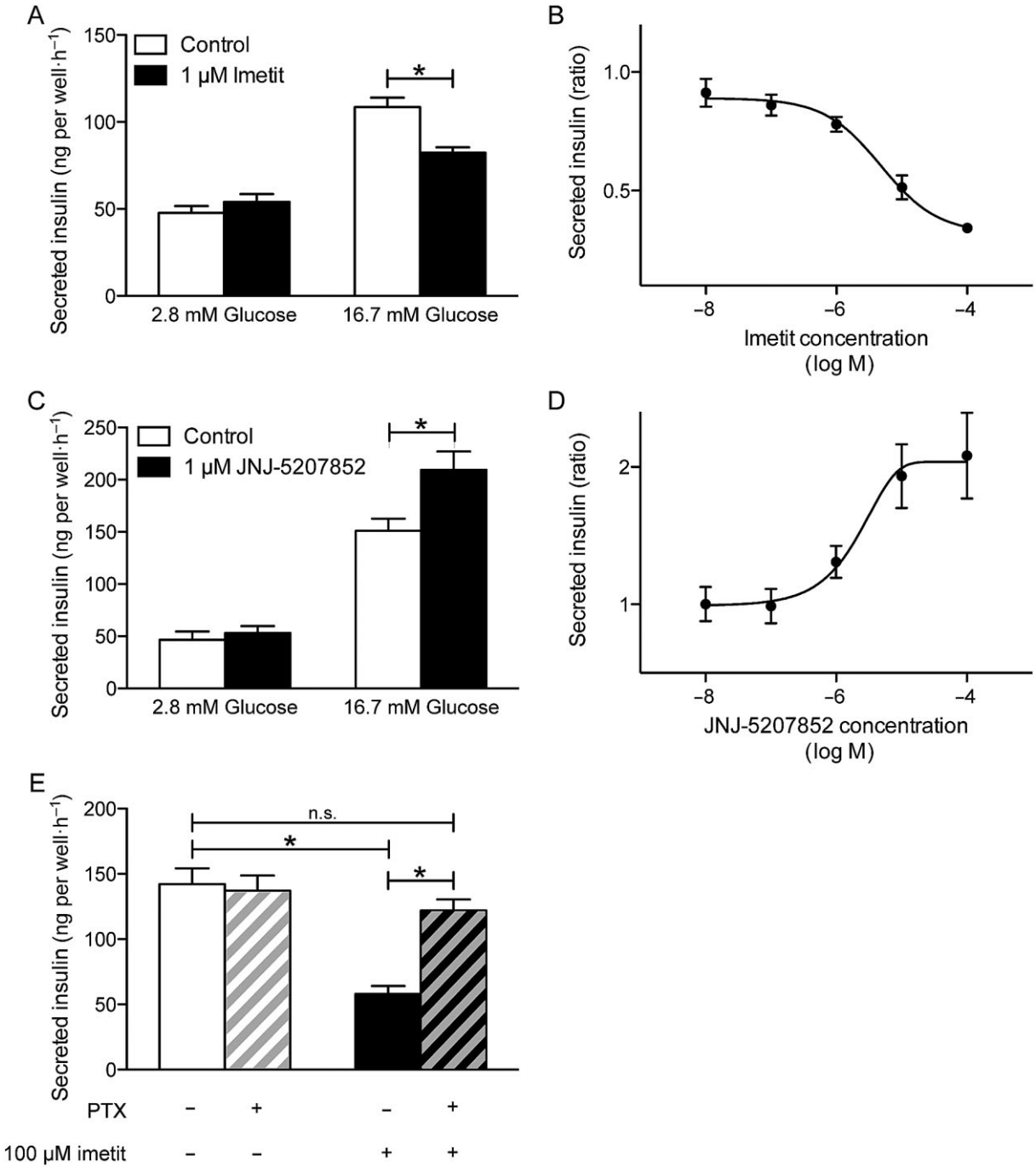

Figure 3

Effects of imetit and JNJ-5207852 on GIIS from MIN6 cells. (A) Insulin secretion from MIN6 cells at low (2.8 mM) and high (16.7 mM) glucose concentrations ±1 μM imetit. *$P < 0.05$, $n = 4$. The experiment shown is representative of three experiments performed. (B) Insulin secretion from MIN6 cells induced by 16.7 mM glucose at different concentrations of imetit. Data are expressed as insulin secretion relative to control secretion with 16.7 mM glucose without agonist/antagonist (which was arbitrarily set as 1), $n = 4$. The experiment shown is representative of two experiments performed. (C) Insulin secretion from MIN6 cells at low (2.8 mM) and high (16.7 mM) glucose concentrations with or without 1 μM JNJ-5207852. *$P < 0.05$, $n = 4$. The experiment shown is representative of three experiments performed. (D) Insulin secretion from MIN6 cells induced by 16.7 mM glucose at different concentrations of JNJ-5207852. Data are expressed as insulin secretion relative to control secretion with 16.7 mM glucose without agonist/inverse agonist (which was arbitrarily set as 1), $n = 8$. The experiment shown is representative of two experiments performed. (E) Effects of 10 ng·μL^{-1} PTX, a G_i inhibitor, on insulin secretion in response to 16.7 mM glucose with or without 100 μM imetit. *$P < 0.05$, $n = 4$. The experiment shown is representative of three experiments performed.

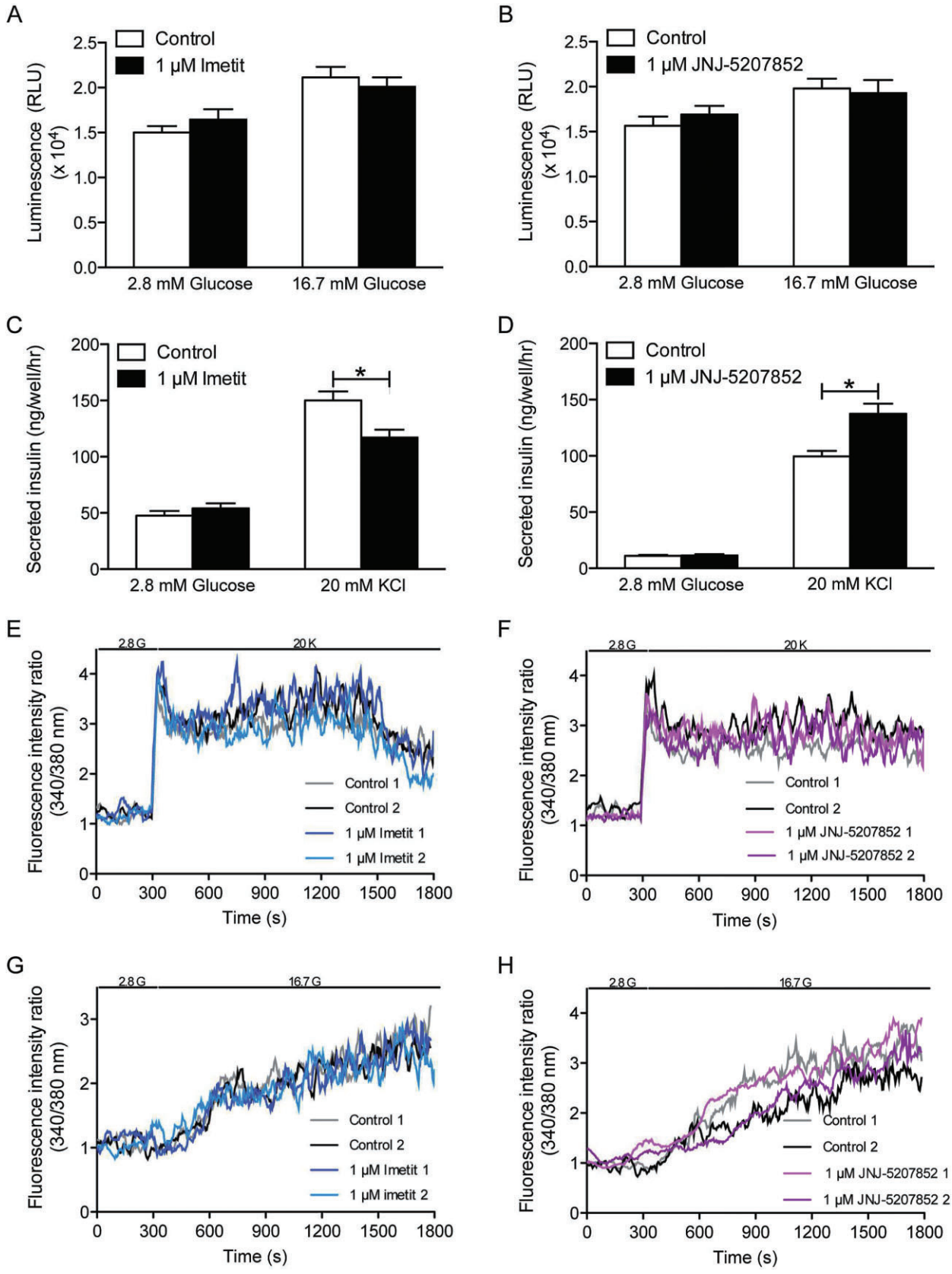
A
Control
1 μM Imetit
Luminescence (RLU) (x 10⁴)
2.8 mM Glucose
16.7 mM Glucose
B
Control
1 μM JNJ-5207852
Luminescence (RLU) (x 10⁴)
2.8 mM Glucose
16.7 mM Glucose
C
Control
1 μM Imetit
Secreted insulin (ng/well/hr)
2.8 mM Glucose
20 mM KCl
D
Control
1 μM JNJ-5207852
Secreted insulin (ng/well/hr)
2.8 mM Glucose
20 mM KCl
E
2.8 G
20 K
Fluorescence intensity ratio (340/380 nm)
Control 1
Control 2
1 μM Imetit 1
1 μM Imetit 2
Time (s)
F
2.8 G
20 K
Fluorescence intensity ratio (340/380 nm)
Control 1
Control 2
1 μM JNJ-5207852 1
1 μM JNJ-5207852 2
Time (s)
G
2.8 G
16.7 G
Fluorescence intensity ratio (340/380 nm)
Control 1
Control 2
1 μM Imetit 1
1 μM imetit 2
Time (s)
H
2.8 G
16.7 G
Fluorescence intensity ratio (340/380 nm)
Control 1
Control 2
1 μM JNJ-5207852 1
1 μM JNJ-5207852 2
Time (s)

Figure 4

Analysis of H_3 receptor intracellular signalling in MIN6 cells. (A) Changes in intracellular ATP concentration in response to low (2.8 mM) and high (16.7 mM) glucose concentrations with or without 1 μM imetit. *$P < 0.05$, $n = 4$. The experiment shown is representative of two experiments performed. (B) The increase of intracellular ATP concentration in response to low (2.8 mM) and high (16.7 mM) glucose concentrations in the presence or absence of 1 μM JNJ-5207852. *$P < 0.05$, $n = 4$. ATP concentration with 2.8 mM glucose was set as 1. The experiment shown is representative of two experiments performed. (C) Insulin secretion from MIN6 cells at low (2.8 mM) glucose concentration and high (20 mM) KCl concentrations, with or without 1 μM imetit. *$P < 0.05$, $n = 4$. The experiment shown is representative of three experiments performed. (D) Insulin secretion from MIN6 cells at low (2.8 mM) glucose concentration and high (20 mM) KCl concentration with or without 1 μM JNJ-5207852. *$P < 0.05$, $n = 4$. The experiment shown is representative of three experiments performed. (E) Changes in intracellular Ca^{2+} concentration in response to high (20 mM) KCl concentrations, with or without 1 μM imetit. Representative results of each group are shown. MIN6 cells seeded into a glass-bottomed dish formed multicellular island-shaped clusters. The number of clusters per visual field was 8–10, and 80–90% of clusters responded to the excitation. Ca^{2+} transient was measured in all MIN6 cell clusters that responded to the excitation. The results shown are representative of four experiments performed. (F) Changes in intracellular Ca^{2+} concentration in response to high (20 mM) KCl concentration with or without 1 μM JNJ-5207852. Representative results of each group are shown. The experiment shown is representative of four experiments performed. (G) Changes in intracellular Ca^{2+} concentration in response to high (16.7 mM) glucose concentration with or without 1 μM imetit. Representative results of each group are shown. The experiment shown is representative of four experiments performed. (H) Changes in intracellular Ca^{2+} concentration in response to high (16.7 mM) glucose concentrations, with or without 1 μM JNJ-5207852. Representative results of each group are shown. The experiment shown is representative of four experiments performed.

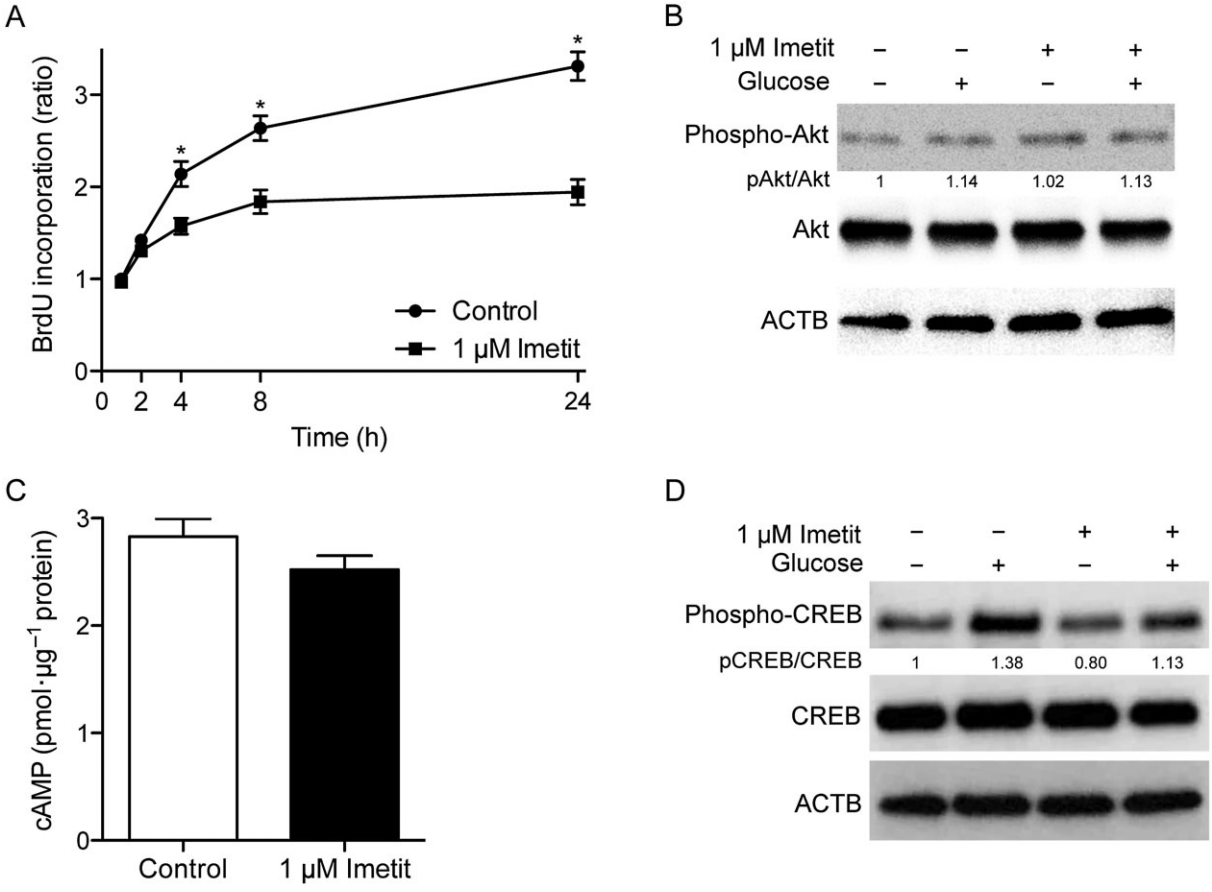

Figure 5

Effects of imetit on MIN6 cell proliferation. (A) Time course of imetit effect on BrdU incorporation in MIN6 cells stimulated by glucose and insulin. *$P < 0.05$, $n = 5$. BrdU incorporation in the absence of imetit after 1 h incubation was set as 1. The experiment shown is representative of two experiments performed. (B) Western blot of Akt in MIN6 cells, stimulated with or without glucose. Phosphorylated Akt (pAkt) was correlated with total Akt, as calculated using ImageJ software. The experiment shown is representative of two experiments performed. (C) Effect of imetit on the concentration of intracellular cAMP in MIN6 cells, $n = 3$. The experiment shown is representative of two experiments performed. (D) Western blot of CREB in MIN6 cells stimulated with or without glucose. Phosphorylated CREB (pCREB) correlated with total CREB, as calculated using ImageJ software. The experiment shown is representative of two experiments performed.

However, glucose stimulates beta cell proliferation through the cAMP–CREB pathway (Jhala *et al.*, 2003; Hussain *et al.*, 2006). Imetit tended to reduce the intracellular concentration of cAMP stimulated by 16.7 mM glucose, although this reduction was not significant (Figure 5C), and also led to a small reduction in the level of phosphorylated CREB (Figure 5D). These results indicate that agonists at the H_3 receptor inhibit MIN6 cell proliferation, possibly through the cAMP–CREB pathway.

Discussion and conclusions

We demonstrate for the first time that H_3 receptors were expressed in mouse and human pancreatic beta cells. Some H_3 receptor-positive cells at the margin of mouse pancreatic islets did not correspond to insulin-positive cells, as shown in Figure 1C. WT mouse pancreatic islets typically exhibit a core and mantle structure, comprising a core of beta cells surrounded by a mantle of α-, δ- and PP-cells (Boyer *et al.*, 2006). H_3 receptors could be expressed not only in beta cells but also in these other cell types. H_3 receptor protein was detected in the cytoplasm in addition to the plasma membrane (Figure 1C,D), suggesting that some of the H_3 receptors are internalized by ligand binding in pancreatic beta cells (Shi *et al.*, 2012).

We also show for the first time that the H_3 receptor plays an important role in insulin secretion and in beta cell proliferation. Interestingly, an H_3 receptor inverse agonist, JNJ-5207852, increased GIIS, indicating that H_3 receptor inverse agonists could be novel insulin secretagogues. Although conventional insulin secretagogues, such as the sulfonylureas, have strong effects on insulin secretion from pancreatic beta cells, they induce weight gain because of the elevated glucose utilization of metabolic organs (UK Prospective Diabetes Study Group, 1998). As a result, obese patients with diabetes taking sulfonylureas need to diet to lose weight, but it is sometimes difficult to alter their dietary habits (Halford *et al.*, 2010). In contrast, H_3 receptor inverse agonists have been shown to suppress food intake and reduce body weight by modulating histaminergic neurons (Malmlof *et al.*, 2005; Barak *et al.*, 2008; Chiba *et al.*, 2009).

Pancreatic islets and/or autonomic nerves adjacent to the islets contain neurotransmitters such as ACh and noradrenaline and also express the enzymes that synthesize them (Gautam *et al.*, 2006; Rosengren *et al.*, 2010). These neurotransmitters bind to their GPCRs in beta cells and modulate beta cell functions. The physiological serum concentration of histamine is about 5 nM (Maintz and Novak, 2007), which is insufficient to stimulate H_3 receptors. In fact, pancreatic beta cells have been shown to express vesicular monoamine transporter 2 (VMAT2), which transports histamine to secretory vesicles (Saisho *et al.*, 2008). A VMAT2-specific antagonist, tetrabenazine, reportedly plays an important role in histamine transport into secretory vesicles (Erickson *et al.*, 1995). Therefore, it is possible that histamine synthesized by Hdc is transported to secretory vesicles via VMAT2 and that histamine released from beta cells regulates beta cell functions in an autocrine and/or paracrine manner.

The potencies of the H_3 receptor ligands in regulating GIIS by MIN6 cells were somewhat lower (Fig. 3B,D) than expected from their high potencies in other systems. The IC_{50} of imetit at the H_3 receptors affecting insulin secretion by MIN6 cells was 100 times higher than its IC_{50} for histamine release by neurons (Garbarg *et al.*, 1992). Because the level of receptor expression influences drug potency (Wilson *et al.*, 1996), the high potency of H_3 receptor ligands in neurons could be attributed to the high neuronal expression of H_3 receptors. In fact, neurons express the highest levels of H_3 receptors and thus agonists have a much lower IC_{50} value, compared with those in other cells and tissues. The IC_{50} value of imetit at the H_3 receptors in MIN6 cells (about 2 μM) is not extremely high, compared with those in other non-neuronal tissues. For example, in rat mesenteric resistance arteries, another potent H_3 receptor agonist, α-methyl histamine, had a IC_{50} value of about 10 μM (Sun *et al.*, 2010). Moreover, the expression of H_3 receptors in pancreatic beta cells was higher than that in MIN6 cells (Figure 1B). In addition, histamine and Hdc are both expressed in both mouse and human pancreatic islets (Figure 2), so the *local* histamine concentration might be high enough to stimulate the H_3 receptors in pancreatic beta cells.

To elucidate the regulatory mechanisms underlying the effects of activated H_3 receptors, we also investigated the effects of imetit and JNJ-5207852 on the insulin secretion signalling pathway. Neither imetit nor JNJ-5207852 increased intracellular ATP or Ca^{2+} concentrations, even though both ligands significantly affected insulin secretion. These results suggest that H_3 receptor-mediated signalling is involved in insulin granular exocytosis, secondary to the increases in ATP and Ca^{2+}. Renstrom *et al.* (1996) reported that adrenaline reduced insulin secretion by interfering with exocytosis downstream of the elevation in intracellular Ca^{2+} concentration through the activation of calcineurin. Recently, Rosengren *et al.* (2010) confirmed that activation of α_{2A}-adrenoceptors, $G_{i/o}$-type GPCRs, inhibited insulin granule exocytosis through calcineurin activation, indicating that H_3 receptors might inhibit insulin granule exocytosis through the same activation of calcineurin. GIIS is also amplified by calcium/calmodulin-dependent PK II (CaMK II). CaMK II phosphorylates synapsin I which, in turn, increases insulin exocytosis (Yamamoto *et al.*, 2003). Activation of H_3 receptors down-regulated CaMK II activity (Torrent *et al.*, 2005), suggesting that H_3 receptors might inhibit insulin granule exocytosis by reducing CaMK II activity coupled to the reduction of synapsin I phosphorylation. However, the precise mechanism(s) that are utilised following H_3 receptor activation need to be confirmed in further studies.

Although proliferation of human beta cells *in vivo* is still controversial (Cnop *et al.*, 2009), pancreatic beta cells need to proliferate to maintain their numbers and the number of pancreatic beta cells is dramatically reduced in patients with diabetes (Butler *et al.*, 2003), so the molecular mechanism underlying impaired beta cell proliferation is interesting in terms of therapeutic potential. Various studies have shown that H_3 receptors are involved in cell proliferation, but their role was different among different tissues. Activation of H_3 receptors decreased the proliferation of biliary cells (Francis *et al.*, 2007) and hepatoma cells (Davenas *et al.*, 2008) through the inhibition of a cAMP-dependent pathway. In contrast, H_3 receptor activation protected against neurotoxicity by stimulating the PI3K/Akt pathway (Bongers *et al.*, 2007). The effects of activating H_3 receptors on proliferation

probably depend on cell-specific, intracellular signalling. We showed here that H_3 receptor activation by imetit inhibited MIN6 cell proliferation (Figure 5A). Imetit also showed a trend towards decreased cAMP concentration and inhibition of the phosphorylation of CREB, a downstream target of cAMP. This pathway plays an important role in mediating intracellular signalling from the factors that promote beta cell proliferation, such as incretin (Jansson *et al.*, 2008) and calcineurin (Soleimanpour *et al.*, 2010). Therefore, H_3 receptor activation in pancreatic beta cells might transduce signals to the cAMP–CREB pathway, but not the PI3K/Akt pathway, resulting in inhibition of beta cell proliferation. H_3 receptor antagonists/inverse agonists may have proliferative effects on pancreatic beta cells by enhancing the phosphorylation of CREB, and this is supported by the fact that JNJ-5207852 was shown to increase intracellular cAMP concentration in a human neuroblastoma cell line (Barbier *et al.*, 2004). However, further details of the effects of H_3 receptor activation on beta cell proliferation need to be elucidated.

One of the limitations of this study is that all the functional analyses of H_3 receptors were performed in MIN6 cells, a cell line derived from a mouse pancreatic tumour (Miyazaki *et al.*, 1990). Although MIN6 cells secrete amounts of insulin equivalent to cultured normal islet cells, the MIN6 cell line may not retain all the physiological properties of pancreatic beta cells. Moreover, the local histamine concentration in pancreatic islets *in vivo* has not been determined, so further studies are needed to elucidate the *in vivo* function of H_3 receptors expressed in pancreatic beta cells.

In the present study, we investigated the role of H_3 receptors in pancreatic beta cells using imetit and JNJ-5207852. The insulin secretion study clearly demonstrates that activation of H_3 receptors inhibits insulin secretion, possibly by regulating exocytosis. In addition, H_3 receptors could be involved in the proliferation of pancreatic beta cells, possibly through the phosphorylation of CREB. These lines of evidence might indicate that the H_3 receptor could play an important role in beta cell functions.

Acknowledgements

This study was supported by a grant from the HIROMI Medical Research Foundation. We thank Professors T. Watanabe and H. Okamoto at Tohoku University for encouraging us to perform this study. We also express our gratitude to Professor Jun-ichi Miyazaki at Osaka University, Dr. Nicholas Carruthers at Johnson & Johnson Pharmaceutical Research and Development and Dr. Jun Sukegawa at Tohoku University for providing experimental materials. Finally, we appreciate the technical assistance provided by F. Naganuma, R. Harada, A. Mohsen, K. Shibuya and Tohoku University Graduate School of Medicine Pathological Platform in the experiments.

Conflicts of interest

The authors declare no conflict of interest.

References

Ahren B (2009). Islet G protein-coupled receptors as potential targets for treatment of type 2 diabetes. Nat Rev Drug Discov 8: 369–385.

Alexander SPH *et al.* (2013). The Concise Guide to PHARMACOLOGY 2013/14: Overview. Br J Pharmacol 170: 1449–1867.

Arrang JM, Garbarg M, Schwartz JC (1983). Auto-inhibition of brain histamine release mediated by a novel class (H3) of histamine receptor. Nature 302: 832–837.

Barak N, Greenway FL, Fujioka K, Aronne LJ, Kushner RF (2008). Effect of histaminergic manipulation on weight in obese adults: a randomized placebo controlled trial. Int J Obes (Lond) 32: 1559–1565.

Barbier AJ, Berridge C, Dugovic C, Laposky AD, Wilson SJ, Boggs J *et al.* (2004). Acute wake-promoting actions of JNJ-5207852, a novel, diamine-based H3 antagonist. Br J Pharmacol 143: 649–661.

Bonaventure P, Letavic M, Dugovic C, Wilson S, Aluisio L, Pudiak C *et al.* (2007). Histamine H3 receptor antagonists: from target identification to drug leads. Biochem Pharmacol 73: 1084–1096.

Bongers G, Sallmen T, Passani MB, Mariottini C, Wendelin D, Lozada A *et al.* (2007). The Akt/GSK-3beta axis as a new signaling pathway of the histamine H(3) receptor. J Neurochem 103: 248–258.

Boyer DF, Fujitani Y, Gannon M, Powers AC, Stein RW, Wright CV (2006). Complementation rescue of Pdx1 null phenotype demonstrates distinct roles of proximal and distal cis-regulatory sequences in pancreatic and duodenal expression. Dev Biol 298: 616–631.

Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC (2003). Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes 52: 102–110.

Chiba S, Itateyama E, Sakata T, Yoshimatsu H (2009). Acute central administration of immepip, a histamine H3 receptor agonist, suppresses hypothalamic histamine release and elicits feeding behavior in rats. Brain Res Bull 79: 37–40.

Clark EA, Hill SJ (1996). Sensitivity of histamine H3 receptor agonist-stimulated [35S]GTP gamma[S] binding to pertussis toxin. Eur J Pharmacol 296: 223–225.

Clark MA, Korte A, Egan RW (1993). Guanine nucleotides and pertussis toxin reduce the affinity of histamine H3 receptors on AtT-20 cells. Agents Actions 40: 129–134.

Clineschmidt BV, Lotti VJ (1973). Histamine: intraventricular injection suppresses ingestive behavior of the cat. Arch Int Pharmacodyn Ther 206: 288–298.

Cnop M, Hughes SJ, Igoillo-Esteve M, Hoppa MB, Sayyed F, Laar L *et al.* (2009). The long lifespan and low turnover of human islet beta cells estimated by mathematical modelling of lipofuscin accumulation. Diabetologia 53: 321–330.

Davenas E, Rouleau A, Morisset S, Arrang JM (2008). Autoregulation of McA-RH7777 hepatoma cell proliferation by histamine H3 receptors. J Pharmacol Exp Ther 326: 406–413.

Devedjian JC, Pujol A, Cayla C, George M, Casellas A, Paris H *et al.* (2000). Transgenic mice overexpressing alpha2A-adrenoceptors in pancreatic beta-cells show altered regulation of glucose homeostasis. Diabetologia 43: 899–906.

Drucker DJ (2006). The biology of incretin hormones. Cell Metab 3: 153–165.

Drutel G, Peitsaro N, Karlstedt K, Wieland K, Smit MJ, Timmerman H *et al*. (2001). Identification of rat H3 receptor isoforms with different brain expression and signaling properties. Mol Pharmacol 59: 1–8.

Dy M, Lebel B, Schneider E (1982). Presence of histaminase in fetal calf sera. J Immunol Methods 48: 261–265.

Erickson JD, Eiden LE, Schafer MK, Weihe E (1995). Reserpine- and tetrabenazine-sensitive transport of ^{3}H-histamine by the neuronal isoform of the vesicular monoamine transporter. J Mol Neurosci 6: 277–287.

Francis H, Franchitto A, Ueno Y, Glaser S, DeMorrow S, Venter J *et al*. (2007). H3 histamine receptor agonist inhibits biliary growth of BDL rats by downregulation of the cAMP-dependent PKA/ERK1/2/ELK-1 pathway. Lab Invest 87: 473–487.

Fukui H, Fujimoto K, Mizuguchi H, Sakamoto K, Horio Y, Takai S *et al*. (1994). Molecular cloning of the human histamine H1 receptor gene. Biochem Biophys Res Commun 201: 894–901.

Gantz I, Munzert G, Tashiro T, Schaffer M, Wang L, DelValle J *et al*. (1991). Molecular cloning of the human histamine H2 receptor. Biochem Biophys Res Commun 178: 1386–1392.

Garbarg M, Arrang JM, Rouleau A, Ligneau X, Tuong MD, Schwartz JC *et al*. (1992). S-[2-(4-imidazolyl)ethyl]isothiourea, a highly specific and potent histamine H3 receptor agonist. J Pharmacol Exp Ther 263: 304–310.

Gautam D, Han SJ, Hamdan FF, Jeon J, Li B, Li JH *et al*. (2006). A critical role for beta cell M3 muscarinic acetylcholine receptors in regulating insulin release and blood glucose homeostasis *in vivo*. Cell Metab 3: 449–461.

UK Prospective Diabetes Study Group (1998). Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. Lancet 352: 837–853.

Haas HL, Sergeeva OA, Selbach O (2008). Histamine in the nervous system. Physiol Rev 88: 1183–1241.

Halford JC, Boyland EJ, Blundell JE, Kirkham TC, Harrold JA (2010). Pharmacological management of appetite expression in obesity. Nat Rev Endocrinol 6: 255–269.

Henry MB, Zheng S, Duan C, Patel B, Vassileva G, Sondey C *et al*. (2011). Antidiabetic properties of the histamine H3 receptor protean agonist proxyfan. Endocrinology 152: 828–835.

Heron A, Rouleau A, Cochois V, Pillot C, Schwartz JC, Arrang JM (2001). Expression analysis of the histamine H(3) receptor in developing rat tissues. Mech Dev 105: 167–173.

Hussain MA, Porras DL, Rowe MH, West JR, Song WJ, Schreiber WE *et al*. (2006). Increased pancreatic beta-cell proliferation mediated by CREB binding protein gene activation. Mol Cell Biol 26: 7747–7759.

Jansson D, Ng AC, Fu A, Depatie C, Al Azzabi M, Screaton RA (2008). Glucose controls CREB activity in islet cells via regulated phosphorylation of TORC2. Proc Natl Acad Sci U S A 105: 10161–10166.

Jhala US, Canettieri G, Screaton RA, Kulkarni RN, Krajewski S, Reed J *et al*. (2003). cAMP promotes pancreatic beta-cell survival via CREB-mediated induction of IRS2. Genes Dev 17: 1575–1580.

Jia F, Kato M, Dai H, Xu A, Okuda T, Sakurai E *et al*. (2006). Effects of histamine H_3 antagonists and donepezil on learning and mnemonic deficits induced by pentylenetetrazol kindling in weanling mice. Neuropharmacology 50: 404–411.

Jorgensen EA, Knigge U, Warberg J, Kjaer A (2007). Histamine and the regulation of body weight. Neuroendocrinology 86: 210–214.

Kidd M, Tang LH, Miu K, Lawton GP, Sandor A, Modlin IM (1996). Autoregulation of enterochromaffin-like cell histamine secretion via the histamine 3 receptor subtype. Yale J Biol Med 69: 9–19.

Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG (2010). Animal research: reporting *in vivo* experiments: the ARRIVE guidelines. Br J Pharmacol 160: 1577–1579.

Knigge U, Soe-Jensen P, Jorgensen H, Kjaer A, Moller M, Warberg J (1999). Stress-induced release of anterior pituitary hormones: effect of H3 receptor-mediated inhibition of histaminergic activity or posterior hypothalamic lesion. Neuroendocrinology 69: 44–53.

Leibiger IB, Leibiger B, Berggren PO (2008). Insulin signaling in the pancreatic beta-cell. Annu Rev Nutr 28: 233–251.

Leurs R, Bakker RA, Timmerman H, de Esch IJ (2005). The histamine H3 receptor: from gene cloning to H3 receptor drugs. Nat Rev Drug Discov 4: 107–120.

Lin CC, Anseth KS (2011). Cell-cell communication mimicry with poly(ethylene glycol) hydrogels for enhancing beta-cell function. Proc Natl Acad Sci U S A 108: 6380–6385.

Liu C, Ma X, Jiang X, Wilson SJ, Hofstra CL, Blevitt J *et al*. (2001). Cloning and pharmacological characterization of a fourth histamine receptor (H_4) expressed in bone marrow. Mol Pharmacol 59: 420–426.

Lovenberg TW, Roland BL, Wilson SJ, Jiang X, Pyati J, Huvar A *et al*. (1999). Cloning and functional expression of the human histamine H3 receptor. Mol Pharmacol 55: 1101–1107.

Lovenberg TW, Pyati J, Chang H, Wilson SJ, Erlander MG (2000). Cloning of rat histamine H_3 receptor reveals distinct species pharmacological profiles. J Pharmacol Exp Ther 293: 771–778.

Maintz L, Novak N (2007). Histamine and histamine intolerance. Am J Clin Nutr 85: 1185–1196.

McGrath JC, Drummond GB, McLachlan EM, Kilkenny C, Wainwright CL (2010). Guidelines for reporting experiments involving animals: the ARRIVE guidelines. Br J Pharmacol 160: 1573–1576.

Malmlof K, Zaragoza F, Golozoubova V, Refsgaard HH, Cremers T, Raun K *et al*. (2005). Influence of a selective histamine H3 receptor antagonist on hypothalamic neural activity, food intake and body weight. Int J Obes (Lond) 29: 1402–1412.

Miyazaki J, Araki K, Yamato E, Ikegami H, Asano T, Shibasaki Y *et al*. (1990). Establishment of a pancreatic beta cell line that retains glucose-inducible insulin secretion: special reference to expression of glucose transporter isoforms. Endocrinology 127: 126–132.

Morisset S, Rouleau A, Ligneau X, Gbahou F, Tardivel-Lacombe J, Stark H *et al*. (2000). High constitutive activity of native H3 receptors regulates histamine neurons in brain. Nature 408: 860–864.

Nakazaki M, Ishihara H, Kakei M, Inukai K, Asano T, Miyazaki JI *et al*. (1998). Repetitive mitochondrial Ca^{2+} signals synchronize with cytosolic Ca^{2+} oscillations in the pancreatic beta-cell line, MIN6. Diabetologia 41: 279–286.

Ohtsu H (2010). Histamine synthesis and lessons learned from histidine decarboxylase deficient mice. Adv Exp Med Biol 709: 21–31.

Ohtsu H, Watanabe T (2003). New functions of histamine found in histidine decarboxylase gene knockout mice. Biochem Biophys Res Commun 305: 443–447.

Okamoto H, Noto Y, Miyamoto S, Mabuchi H, Takeda R (1975). Inhibition by somatostatin of insulin release from isolated pancreatic islets. FEBS Lett 54: 103–105.

Okuda T, Zhang D, Shao H, Okamura N, Takino N, Iwamura T *et al*. (2009). Methamphetamine- and 3,4-methylenedioxymethamphetamine-induced behavioral changes in histamine H3-receptor knockout mice. J Pharmacol Sci 111: 167–174.

Renstrom E, Ding WG, Bokvist K, Rorsman P (1996). Neurotransmitter-induced inhibition of exocytosis in insulin-secreting beta cells by activation of calcineurin. Neuron 17: 513–522.

Rosengren AH, Jokubka R, Tojjar D, Granhall C, Hansson O, Li DQ *et al*. (2010). Overexpression of alpha2A-adrenergic receptors contributes to type 2 diabetes. Science 327: 217–220.

Saisho Y, Harris PE, Butler AE, Galasso R, Gurlo T, Rizza RA *et al*. (2008). Relationship between pancreatic vesicular monoamine transporter 2 (VMAT2) and insulin expression in human pancreas. J Mol Histol 39: 543–551.

Saltiel AR, Kahn CR (2001). Insulin signalling and the regulation of glucose and lipid metabolism. Nature 414: 799–806.

Shi Y, Sheng R, Zhong T, Xu Y, Chen X, Yang D *et al*. (2012). Identification and characterization of ZEL-H16 as a novel agonist of the histamine H_3 receptor. PLoS ONE 7: e42185.

Soleimanpour SA, Crutchlow MF, Ferrari AM, Raum JC, Groff DN, Rankin MM *et al*. (2010). Calcineurin signaling regulates human islet β-cell survival. J Biol Chem 285: 40050–40059.

Sun P, Jin X, Koyama T, Li S, Kitamura Y, Kawasaki H (2010). R-(-)-alpha-methylhistamine, a histamine H3 receptor agonist, induces endothelium-dependent vasodilation in rat mesenteric resistance arteries. Biol Pharm Bull 33: 58–63.

Torrent A, Moreno-Delgado D, Gomez-Ramirez J, Rodriguez-Agudo D, Rodriguez-Caso C, Sanchez-Jimenez F *et al*. (2005). H3 autoreceptors modulate histamine synthesis through calcium/calmodulin- and cAMP-dependent protein kinase pathways. Mol Pharmacol 67: 195–203.

Uchizono Y, Iwase M, Nakamura U, Sasaki N, Goto D, Iida M (2004). Tacrolimus impairment of insulin secretion in isolated rat islets occurs at multiple distal sites in stimulus–secretion coupling. Endocrinology 145: 2264–2272.

Wilson S, Chambers JK, Park JE, Ladurner A, Cronk DW, Chapman CG *et al*. (1996). Agonist potency at the cloned human beta-3 adrenoceptor depends on receptor expression level and nature of assay. J Pharmacol Exp Ther 279: 214–221.

Yamamoto H, Matsumoto K, Araki E, Miyamoto E (2003). New aspects of neurotransmitter release and exocytosis: involvement of Ca2+/calmodulin-dependent phosphorylation of synapsin I in insulin exocytosis. J Pharmacol Sci 93: 30–34.

Yamatodani A, Fukuda H, Wada H, Iwaeda T, Watanabe T (1985). High-performance liquid chromatographic determination of plasma and brain histamine without previous purification of biological samples: cation-exchange chromatography coupled with post-column derivatization fluorometry. J Chromatogr 344: 115–123.