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Original Paper

CXCL14 Inhibits Insulin Secretion Independently of CXCR4 or CXCR7 Receptor Activation or cAMP Inhibition

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Key Words
Insulin secretion • Type 2 diabetes • CXCL14 • MIN6 β-cells • Islets

Abstract
Background/Aims: CXCL14, a secreted chemokine peptide that promotes obesity-induced insulin resistance, is expressed by islets, but its effects on islet function are unknown. The aim of this study was to determine the role of CXCL14 in β-cells and investigate how it transduces these effects. ***Methods:*** *Cxcl14* and *Cxc-receptor* mRNA expression was quantified by qPCR and CXCL14 expression in the pancreas was determined by immunohistochemistry. The putative function of CXCL14 at CXCR4 and CXCR7 receptors was determined by β-arrestin recruitment assays. The effects of CXCL14 on glucose-stimulated insulin secretion, cAMP production, glucose-6-phosphate accumulation, ATP generation, apoptosis and proliferation were determined using standard techniques. ***Results:*** CXCL14 was present in mouse islets, where it was mainly localised to islet δ-cells. *Cxc-receptor* mRNA profiling indicated that *Cxcr4* and *Cxcr7* are the most abundant family members in islets, but CXCL14 did not promote β-arrestin recruitment at CXCR4 or CXCR7 or antagonise CXCL12 activation of these receptors. CXCL14 induced a concentration-dependent inhibition of glucose-stimulated insulin secretion, which was not coupled to $G_{\alpha i}$ signalling. However, CXCL14 inhibited glucose-6-phosphate generation and ATP production in mouse islets. ***Conclusion:*** CXCL14 is expressed by islet δ-cells where it may have paracrine effects to inhibit insulin secretion in a CXCR4/CXCR7-independent manner through reductions in β-cell ATP levels. These observations, together with the previously reported association of CXCL14 with obesity and impaired glucose homeostasis, suggest that inhibition of CXCL14 signalling could be explored to treat type 2 diabetes.

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Introduction

G protein-coupled receptors (GPCRs) are widely used as targets for clinically used drugs, but some GPCRs are orphans for which the endogenous ligands have not yet been identified [1, 2]. In addition, the receptors responsible for mediating biological effects of some ligands that are predicted to be GPCR agonists are unknown, which presents challenges in drug target discovery [3, 4]. CXCL14 (chemokine (C-X-C motif) ligand 14), is an orphan chemokine ligand belonging to the CXC-class chemokine family [5, 6]. Chemokines bind to receptors of their own class, of which there are four (C, CC, CXC, C3XC), and within each class some chemokines bind to several receptors [7]. However, despite this class-specific interaction between chemokine ligands and receptors, the receptor for CXCL14 is unknown. *Cxcl14* mRNA is abundantly expressed in a wide range of tissues, and it has been implicated in both tumour suppression and malignancy [8]. It has also been identified in human and mouse islets [9, 10], but there is no information on its role in islet function.

There is accumulating evidence that obesity results in a state of low-grade chronic inflammation that promotes release of pro-inflammatory cytokines, lipids and chemokines from adipose tissue, which consequently contribute to metabolic comorbidities such as type 2 diabetes (T2D) [11]. CXCL14 is secreted by adipocytes and it contributes to inflammatory processes by recruiting monocytes to the site of inflammation. A high-fat diet in mice markedly upregulates circulating CXCL14 and its expression by white adipose tissue [12], while CXCL14 knockout mice are protected from diet-induced obesity and show improved insulin sensitivity [13].

The association of CXCL14 with obesity, and improvement in glucose homeostasis following its deletion, suggest that inhibiting its activity may be a therapeutic option for treating obesity and T2D. However, as it is not yet clear whether CXCL14 directly affects insulin release the aim of the current study was to investigate its role in islet and β-cell function, and to identify the underlying mechanisms mediating its effects.

Materials and Methods

Reagents

DiscoverX Corporation, Ltd. (Birmingham, UK): mouse CXCR4 and CXCR7 β-arrestin assays; DAKO UK Ltd. (Ely, UK): insulin antibody; Abcam PLC (Cambridge, UK): glucagon and somatostatin antibodies, cytochalasin B and oligomycin A; Jackson ImmunoResearch (Suffolk, UK): anti- rabbit, guinea pig, rat and mouse secondary antibodies; PeproTech EC Ltd. (London, UK): CXCL14 antibody and murine TNFα, IFNγ and IL-1β; BioLegend (London, UK): Recombinant mouse CXCL14 and CXCL12; Sigma-Aldrich (Dorset, UK): BrdU cell proliferation kit; Promega UK (Southampton, UK): Caspase 3/7, CellTiter-Glo 3D and Glucose Uptake-Glo assay kits; Qiagen Ltd. (Manchester, UK): QuantiTect SYBR Green qPCR kits with QuantiTect qPCR assays; Cisbio Bioassays (Codolet, France): HTRF cAMP assays.

Islet and MIN6 β-cell culture

Islets were isolated from 10-12 week old male CD1 mice by collagenase digestion of the pancreas [14] and maintained in culture overnight in RPMI 1640 supplemented with 10% fetal calf serum (FCS), 100U/mL penicillin and 100μg/mL streptomycin. All animal procedures were approved by the King's College London Ethics Committee and carried out in accordance with the UK Home Office Animals (Scientific Procedures) Act 1986. MIN6 β-cells (passage 25-45) were maintained in DMEM supplemented with 10% FCS, 100U/mL penicillin, 100μg/mL streptomycin and 100μM 2-mercaptoethanol.

Cxcl14 and Cxc-receptor mRNA expression

RNA was extracted from MIN6 β-cells and mouse islets, brown adipose tissue (BAT) and gluteal white adipose tissue (GWAT) using a modified TRIzol protocol followed by RNA clean-up on RNEasy MinElute columns (Qiagen), then reverse transcribed into cDNAs. *Cxcl14* mRNA was quantified by qPCR on a LightCycler® 480 (Roche) using Qiagen QuantiTect primers and SYBR Green, and expressed relative to the

reference genes *Actb*, *Gapdh*, *Ppia*, *Tbp* and *Tfrc* amplified in the same samples [11]. Expression of *Cxc-receptors* (*Cxcr1-7*) by MIN6 β-cells and mouse islets was also quantified by qPCR. All qPCR amplification products were analysed by electrophoresis on 2% agarose gels to confirm product size. Efficiency values [15] of primers for *Cxcl14*, *Cxc-receptors* and reference genes were in the range of 1.85–2.15, and template cDNAs were diluted in such a way that all quantified genes returned Ct values <30.

Immunohistochemical detection of CXCL14 in mouse pancreas

5μm sections of paraffin-embedded fixed mouse pancreas were boiled in 0.01M citric acid buffer (pH 6.0) for 2.5 min for antigen retrieval, blocked with 1% BSA, 10% normal goat serum, 0.1% Triton X-100 in PBS for 1 hour then incubated at 4°C overnight with rabbit anti-CXCL14 antibody (1:500), guinea pig anti-insulin (1:200), mouse anti-glucagon (1:50) and rat anti-somatostatin (1:25) antibodies. Sections were exposed for 1 hour at room temperature to Alexa Fluor® 488 anti-rabbit secondary antibody (1:150) and species-specific Alexa Fluor® 594 secondary antibodies (all at 1:200). Nuclei were detected using DAPI (1:500). Immunostained pancreas sections were analysed using Image J software and the proportion of islet cells expressing CXCL14 was calculated by dividing the mean number of CXCL14-positive cells by the number of β, α- or δ-cells per islet.

Insulin secretion

Groups of 20,000 MIN6 β-cells or 5 mouse islets were incubated in the absence or presence of increasing concentrations of CXCL14 for 1 hour at 37°C in a physiological buffer [16]. Insulin secreted into the supernatant was quantified by radioimmunoassay [17].

CXCR4- and CXCR7-dependent β-arrestin recruitment

CXCL14 interactions with CXCR4 and CXCR7 receptors were determined using mouse CXCR4 and CXCR7 PathHunter® eXpress β-arrestin assays. For these experiments groups of 10,000 CHO-K1 cells stably expressing CXCR4 or CXCR7 were incubated with a range of concentrations of CXCL12 or CXCL14 for 90 min at 37°C. Activity of β-arrestin driven by activated CXCR4 or CXCR7 was quantified using a Veritas luminometer plate reader.

Intracellular cAMP

Groups of 5,000 MIN6 β-cells were incubated with increasing concentrations of CXCL14 in the absence or presence of the adenylate cyclase activator forskolin (1μM) for 1 hour in Hanks' balanced salt solution (HBSS) supplemented with 10mM HEPES, 0.2% BSA and 2mM 3-isobutyl-1-methylxanthine (IBMX). MIN6 β-cell cAMP levels were quantified by measurement of the 665/620nm emission intensity ratio using a Pherastar FS microplate reader.

2-deoxyglucose-6-phosphate accumulation

Groups of 30,000 MIN6 β-cells or 5 mouse islets were incubated with increasing concentrations of CXCL14 for 1 hour at 37°C in the absence of glucose, then incubated for a further hour at 37°C with the glucose analogue 2-deoxy-D-glucose (2DG). Intracellular phosphorylated 2DG (2DG6P) was detected using the Glucose Uptake-Glo assay according to the manufacturer's protocol.

ATP generation

Groups of 3 mouse islets were incubated with increasing concentrations of CXCL14 for 1 hour at 37°C, lysed and ATP was quantified using the CellTiter-Glo 3D assay [18].

Apoptosis

Groups of 25,000 MIN6 β-cells were maintained in culture for 48 hours with increasing concentrations of CXCL14 in the absence or presence of 1U/μL TNFα, 1U/μL IFNγ and 0.25U/μL IL-1β for the last 20 hours of the 48 hour incubation period. Quantification of basal and cytokine-induced β-cell apoptosis was carried out using the Caspase-Glo 3/7 assay [19].

Proliferation

Groups of 20,000 MIN6 β-cells were maintained overnight in serum-free DMEM supplemented with 2mM glucose to ensure a quiescent state, then exposed to increasing concentrations of CXCL14 in the absence or presence of 10% FCS for 48 hours at 37°C. BrdU incorporation into proliferating cells was quantified using a plate reader measuring absorbance at 450nm [19].

Statistical analyses

Differences between selected pairs of data were analysed by unpaired Student's t-test and differences between several groups were analysed by one-way ANOVA followed by Dunnett's multiple comparison post-test, as appropriate, using GraphPad Prism 8.0. Values of $p<0.05$ were considered statistically significant.

Results

Quantification of Cxcl14 mRNA expression

Cxcl14 mRNA was detected at high levels in mouse brown (BAT) and white (GWAT) adipose tissue (Fig. 1A). There were lower levels of *Cxcl14* mRNA expression by mouse islets, but it was not detected when using MIN6 β-cell cDNA as a template. Agarose gel electrophoresis fractionation of the qPCR products indicated that an amplicon of the appropriate size (76bp) was generated (Fig. 1B).

Immunohistochemical localisation of CXCL14 in mouse pancreas

It can be seen from Fig. 2A that CXCL14 expression was confined to the endocrine pancreas, where it was localised to a minority of cells on the islet periphery. Co-staining with antibodies directed against glucagon, insulin and somatostatin indicated that there were high levels of co-localisation with somatostatin in δ-cells (Fig. 2A, merged panel). Quantification of co-expression of CXCL14 with islet hormones in multiple pancreas sections indicated that the majority of δ-cells expressed CXCL14 while it was absent in β-cells and only very few α-cells synthesised this peptide (Fig. 2B).

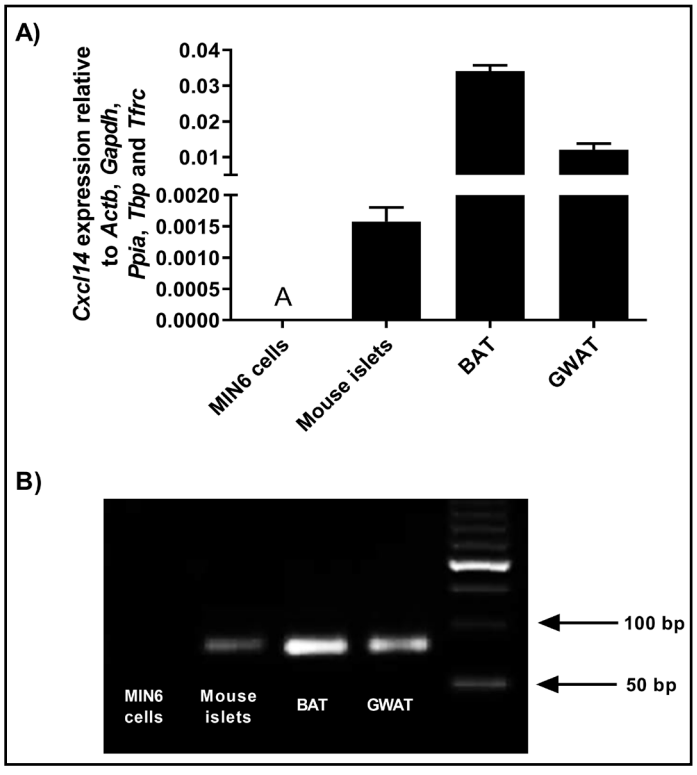

Fig. 1. *Cxcl14* mRNA expression. A) *Cxcl14* mRNA is expressed as mean+SEM, relative to *Actb, Gapdh, Ppia, Tbp* and *Tfrc* mRNA expression, n=4. A: absent expression. BAT: brown adipose tissue; GWAT: gluteal white adipose tissue. B) Agarose gel image of the *Cxcl14* qPCR products shown in panel A. The correct amplicon size (76bp) was detected for mouse islets, BAT and GWAT.

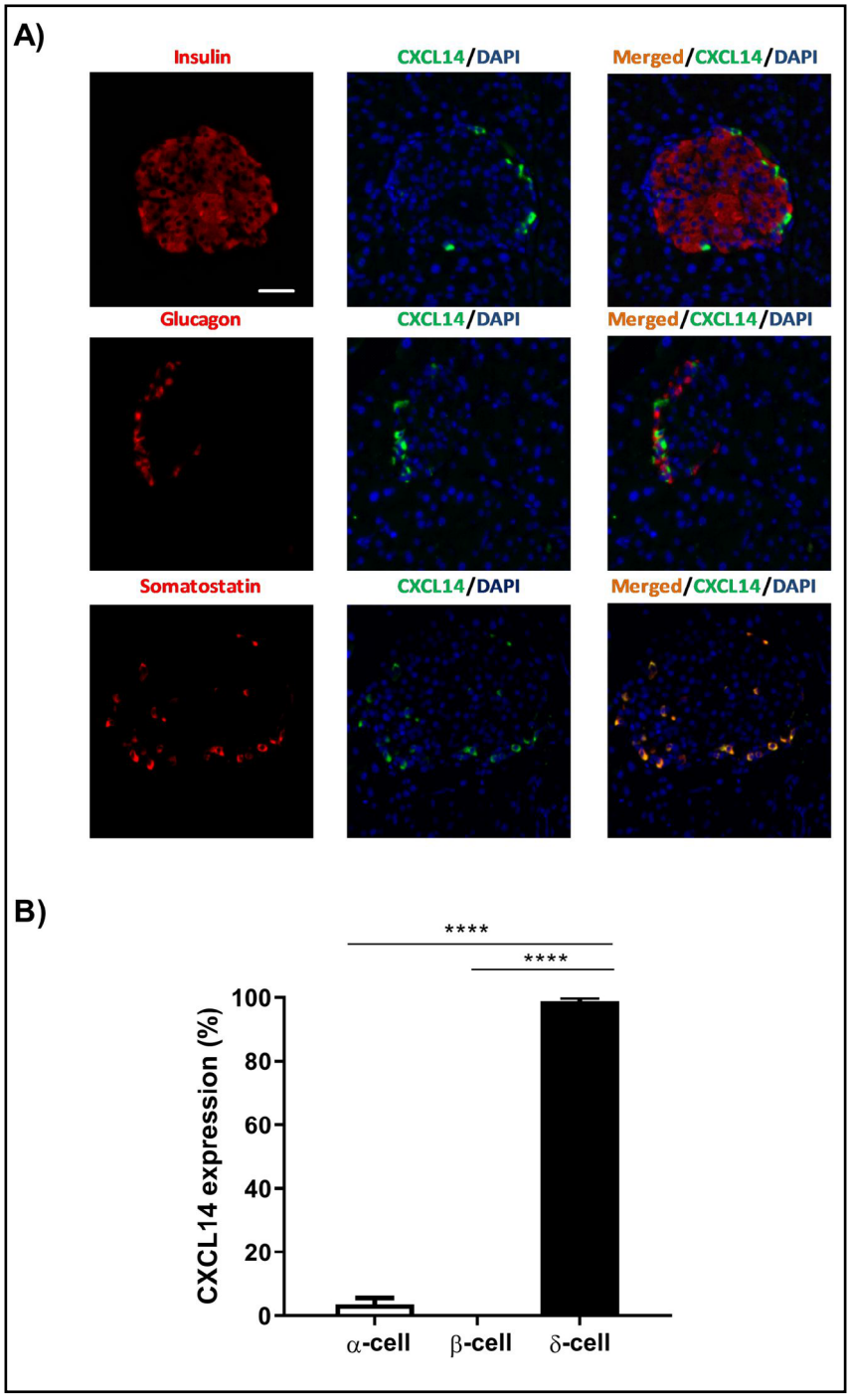

Fig. 2. CXCL14 expression by CD-1 mouse pancreas. A) Co-localisation of the islet hormones insulin, glucagon and somatostatin (red) with CXCL14 (green) by mouse islets. Nuclei have been counterstained with DAPI (blue). Scale bar indicates 50 µm. B) CXCL14 expression per islet cell population. Data are expressed as mean+SEM and represent multiple acquisitions of 20 to 35 islets per mouse (n=3), each with a minimum of 3 paraffin sections for analysis. (125±29 β-cells, 25±5 α-cells and 11±2 δ-cells); ****p<0.0001.

CXCL14 inhibits glucose-stimulated insulin secretion

Quantification of insulin secretion from MIN6 β-cells (Fig. 3A) and mouse islets (Fig. 3B) indicated that exogenous CXCL14 (1-40ng/mL) induced a concentration-dependent inhibition of glucose-stimulated insulin secretion. Insulin secretion in the presence of 40ng/mL CXCL14 was inhibited to levels not significantly different from those obtained in the presence of a sub-stimulatory concentration of glucose (2mM), in both MIN6 β-cells and mouse islets.

Assessment of CXCL14 interactions with CXCR4 and CXCR7 receptors

CXCL14 is an orphan ligand, but its inhibitory effects on insulin secretion suggest that it may act as a $G_{\alpha i}$-coupled GPCR agonist in β-cells. Other CXC family peptides are ligands for CXC-receptors that inhibit adenylate cyclase activity via $G_{\alpha i}$ signalling [20], so mRNA expression profiles of all *Cxc-receptors* in MIN6 β-cells and mouse islets were determined (Fig. 4A). This expression analysis revealed that mRNAs encoding *Cxcr1*, *Cxcr2*, *Cxcr3*, *Cxcr5* and *Cxcr6* were absent in mouse islets, or only expressed at trace levels. However, transcripts for *Cxcr4* and *Cxcr7* were readily detectable in both mouse islets and MIN6 β-cells suggesting that either (or both) of these receptors could be activated by CXCL14. This was investigated using β-arrestin recruitment assays specific for CXCR4 and CXCR7. As expected, the natural ligand, CXCL12, induced a concentration-dependent increase in β-arrestin recruitment at CXCR4 (Fig. 4B) and CXCR7 (Fig. 4C), but when CXCL14 was used over the same concentration range it failed to promote β-arrestin recruitment to either receptor (Fig. 4B and 4C). Although CXCR4 and CXCR7 classically couple to $G_{\alpha i}$, signalling via stimulatory cascades have also been identified [20], so the potential antagonistic properties of CXCL14 for CXCR4 and CXCR7 were assessed by performing concentration-response curves to the natural agonist, CXCL12, in the presence of increasing concentrations of CXCL14. These assays indicated that CXCL14 did not alter the EC_{50} of CXCL12 for CXCR4 (Fig. 4D) or CXCR7 (Fig. 4E) at any of the concentrations tested. These observations rule out the involvement of CXCR4 and CXCR7 in mediating CXCL14 signalling.

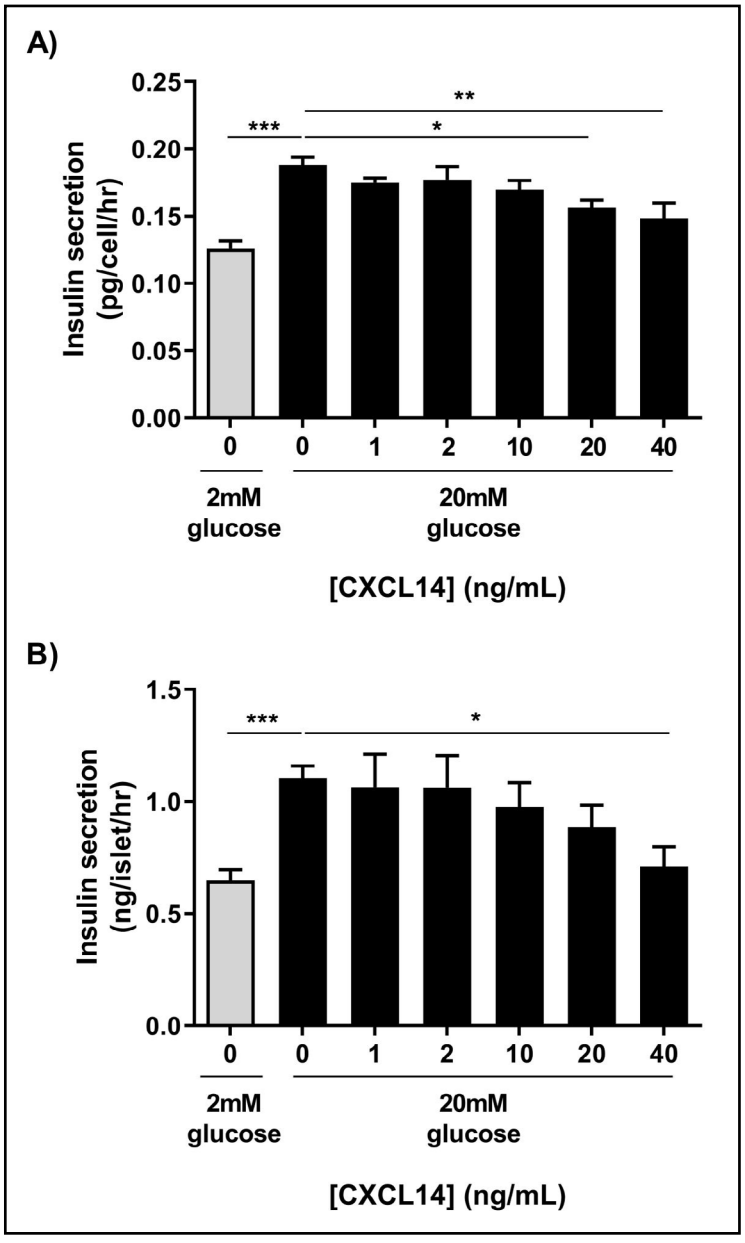

Fig. 3. Effect of CXCL14 on insulin secretion. A,B) CXCL14 induced a concentration-dependent reduction in glucose-stimulated insulin secretion from MIN6 β-cells (A) and mouse islets (B). Data are expressed as mean+SEM, n=8; *p<0.05, **p<0.01, ***p<0.001.

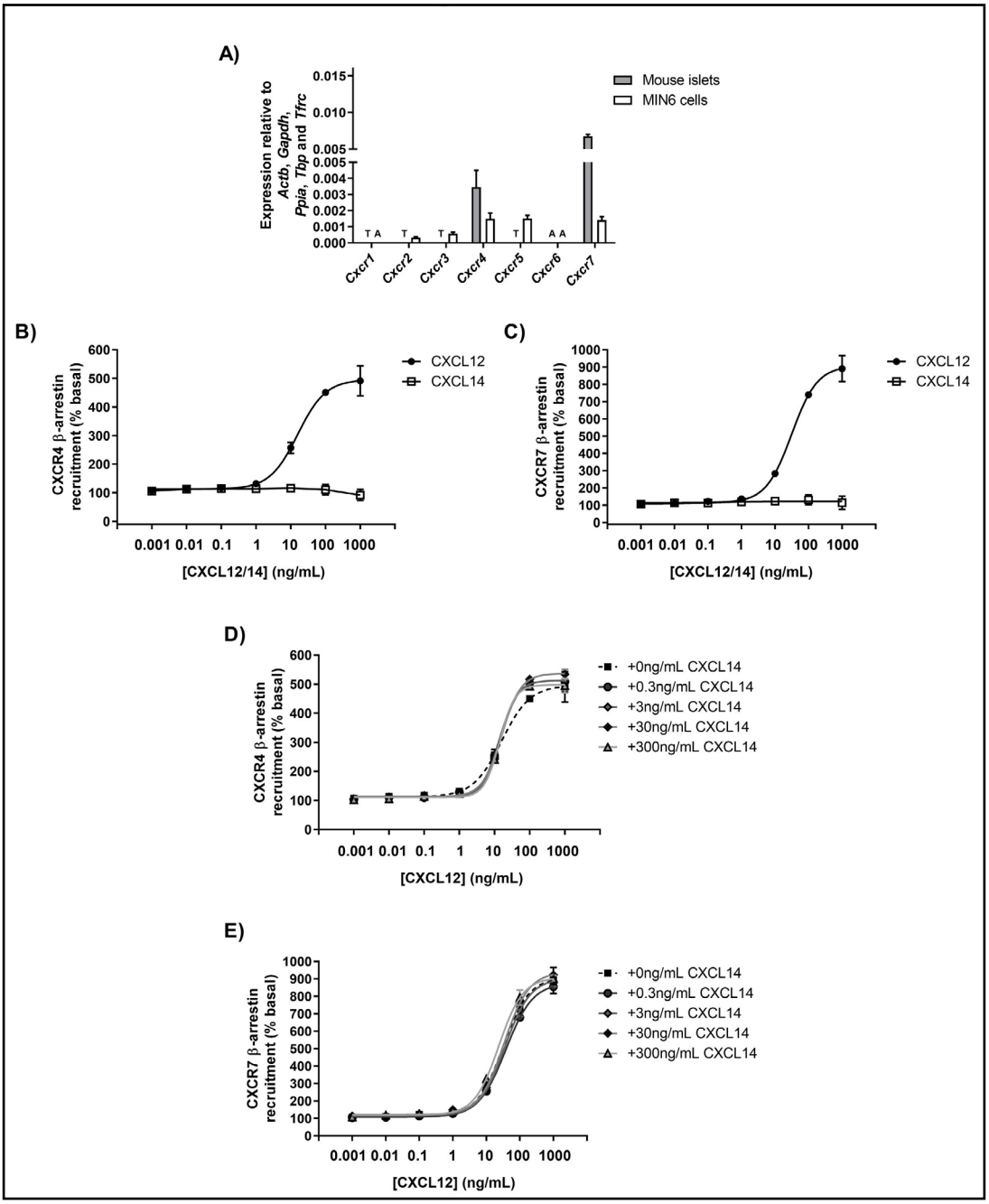

Fig. 4. Assessment of CXCL14 interactions with CXCR4 and CXCR7 receptors. A) mRNA profiling of *Cxc*-family receptors by mouse islets and MIN6 β-cells. Data are expressed as mean+SEM expression relative to *Actb, Gapdh, Ppia, Tbp* and *Tfrc*, n=4. T: trace expression; A: absent expression. B,C) CXCL12 induced β-arrestin recruitment at the mouse CXCR4 (B) and CXCR7 (C) receptors, with EC_{50} values of 15.86ng/mL and 31.29ng/mL and Hill slope values of 1.079 and 1.134, respectively. CXCL14 did not promote CXCR4 or CXCR7 β-arrestin recruitment. Data are expressed as mean±range, n=2. D,E) Increasing concentrations of CXCL14 did not significantly affect CXCL12 concentration-response profiles at CXCR4 (D) or CXCR7 (E) receptors. Data are expressed as mean±range, n=2.

CXCL14 does not reduce β-cell cAMP generation

CXCL14 (0.0625-512ng/mL) had no effect on cAMP accumulation in MIN6 β-cells stimulated by the direct adenylate cyclase activator forskolin, while 1μM clonidine, an α2-adrenergic agonist, completely inhibited forskolin-induced increases in intracellular cAMP, as expected (Fig. 5A). The ability of CXCL14 to function as an inverse agonist of a $G_{\alpha s}$-coupled GPCR was also assessed, but it did not reduce basal cAMP levels in MIN6 β-cells, thus ruling out this possibility. In the same experiments the $G_{\alpha s}$-coupled GPCR agonist exendin-4 produced the expected increase in cAMP accumulation, as did forskolin (Fig. 5B).

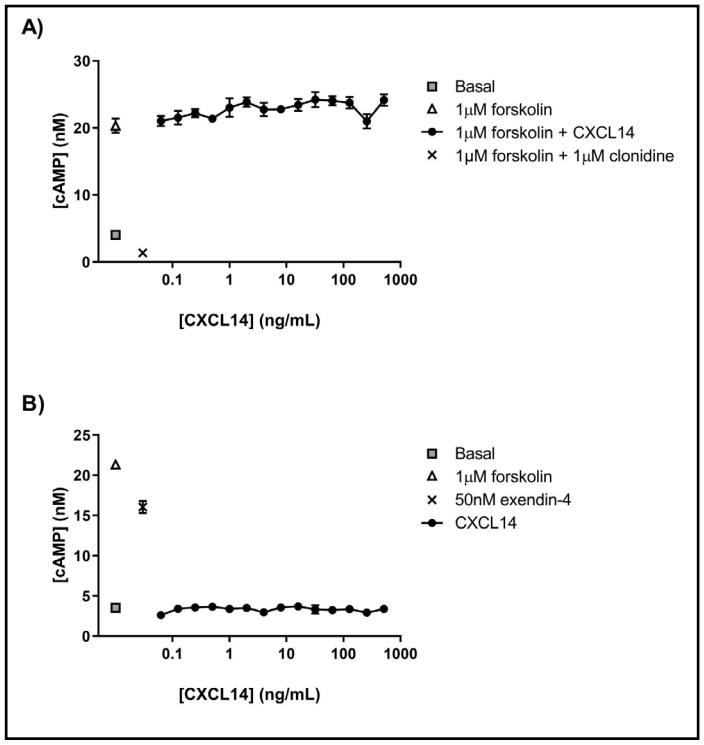

Fig. 5. Effect of CXCL14 intracellular cAMP levels. A,B) CXCL14 did not inhibit forskolin-induced increase in cAMP (A), nor did it affect basal cAMP levels (B) in MIN6 β-cells. 1μM clonidine and 50nM exendin-4 were included as positive $G_{\alpha i}$ and $G_{\alpha s}$ controls, respectively. Data are expressed as mean+SEM, n=3.

CXCL14 inhibits 2DG6P accumulation in β -cells

CXCL14 caused a concentration-dependent inhibition of accumulation of the glucose-6-phosphate analogue 2DG6P, in MIN6 β-cells (Fig. 6A), and mouse islets (Fig. 6B). Maximal inhibition of 2DG6P generation was observed when the MIN6 β-cells or islets were exposed to 50μM cytochalasin B, a non-competitive inhibitor of all GLUT isoforms (Fig. 6A and 6B).

CXCL14 inhibits islet ATP generation

20mM glucose stimulated a significant elevation in ATP production in mouse islets and this was inhibited in a concentration-dependent manner by CXCL14 (Fig. 7), consistent with its inhibitory effects on 2DG6P accumulation. 5μM oligomycin A, an inhibitor of mitochondrial ATP synthase, caused a substantial inhibition of islet ATP production, as expected.

CXCL14 does not affect β -cell apoptosis or proliferation

Potential effects of CXCL14 on MIN6 β-cell apoptosis were investigated in the absence and presence of pro-apoptotic cytokines (TNFα, IFNγ and IL-1β), which promoted a 3-fold elevation in β-cell caspase 3/7 activities. CXCL14 had no effect on basal or cytokine-induced apoptosis at any of the concentrations used (Fig. 8A). In parallel experiments, it was observed that 10% FCS induced a 5-fold increase in BrdU incorporation into proliferating MIN6 β-cells, but CXCL14 neither stimulated basal proliferation nor inhibited the stimulation induced by 10% FCS (Fig. 8B).

Discussion

It has been known for some time that CXCL14 is up-regulated in obesity [12, 21], and that its deletion in mice leads to reduced food intake and body weight [13], and protects against obesity-induced insulin resistance and hyperglycaemia [21]. The deleterious effects of CXCL14 on glucose homeostasis are evident from the observations that its overexpression in CXCL14$^{-/-}$ mice restored insulin resistance [13] and that it inhibited glucose uptake into myocytes *in vitro* [21, 22]. These earlier studies focused on the beneficial effects of reducing CXCL14 levels to improve insulin sensitivity. In contrast, a recent report has implicated CXCL14 in inducing browning of white adipose tissue and improving glucose homeostasis in obese mice [23]. Thus, *in vivo* studies support either a deleterious [13] or beneficial [23] effect of CXCL14 on glucose homeostasis, but nothing is known about its direct effects on islet function. The identification of *Cxcl14* expression by mouse and human islets [9, 10] suggests that this chemokine may also play a role in regulating islet function through autocrine or paracrine signalling, so in the current study we therefore aimed to quantify the effects of CXCL14 on insulin secretion, β-cell proliferation and apoptosis, and investigate the receptors and intracellular pathways responsible for transducing its effects.

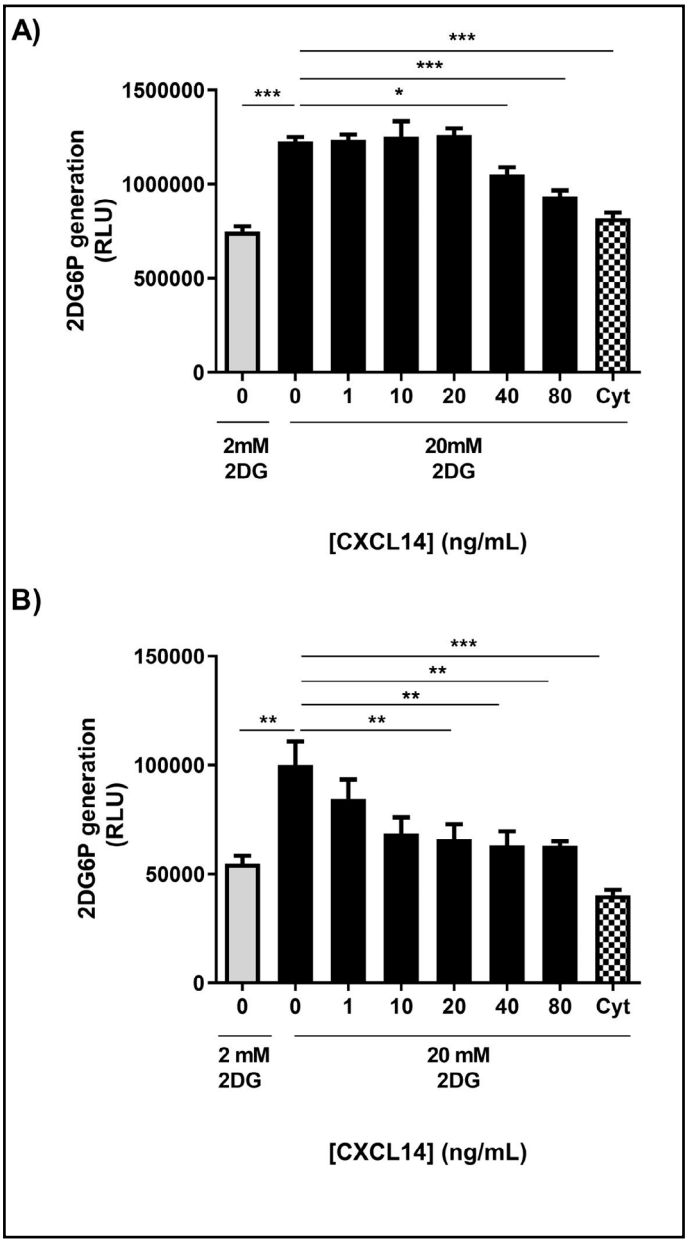

Fig. 6. Effect of CXCL14 on 2DG6P accumulation. A,B) CXCL14 induced a concentration-dependent reduction in 2DG6P generation in MIN6 β-cells (A) and mouse islets (B). The glucose transporter inhibitor cytochalasin-B (Cyt, 50μM) also significantly reduced 2DG6P accumulation. Data are expressed as mean+SEM, n=6; *p<0.05, **p<0.01, ***p<0.001.

We have previously reported that *Cxcl14* mRNA is expressed by islets isolated from both inbred and outbred mouse strains [10], and the data presented here confirm islet expression of this chemokine and also indicate that it is almost exclusively expressed by δ-cells in the pancreases of outbred CD1 mice. These observations are in agreement with a previous report of CXCL14 co-localisation with somatostatin in islet δ-cells of BALB/c and C57BL/6N

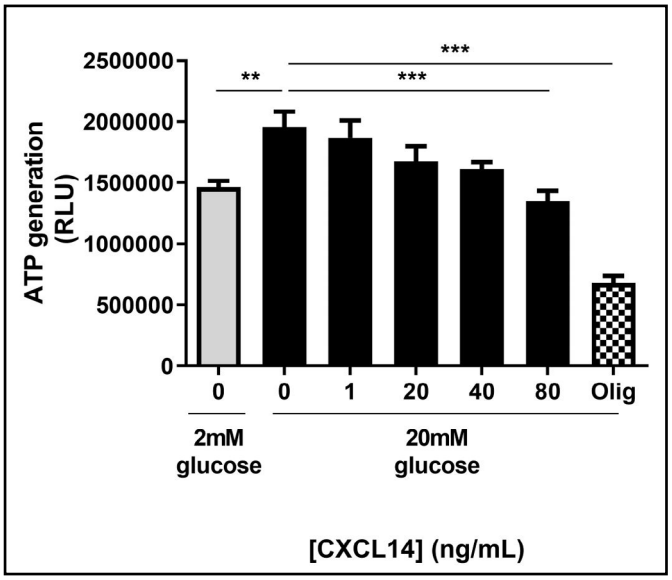

Fig. 7. Effect of CXCL14 on ATP generation. CXCL14 caused a concentration-dependent reduction in ATP generation in mouse islets at 20mM glucose, as did the positive control oligomycin A (Olig, 5μM). Data are expressed as mean+SEM, n=8; **p<0.01, ***p<0.001.

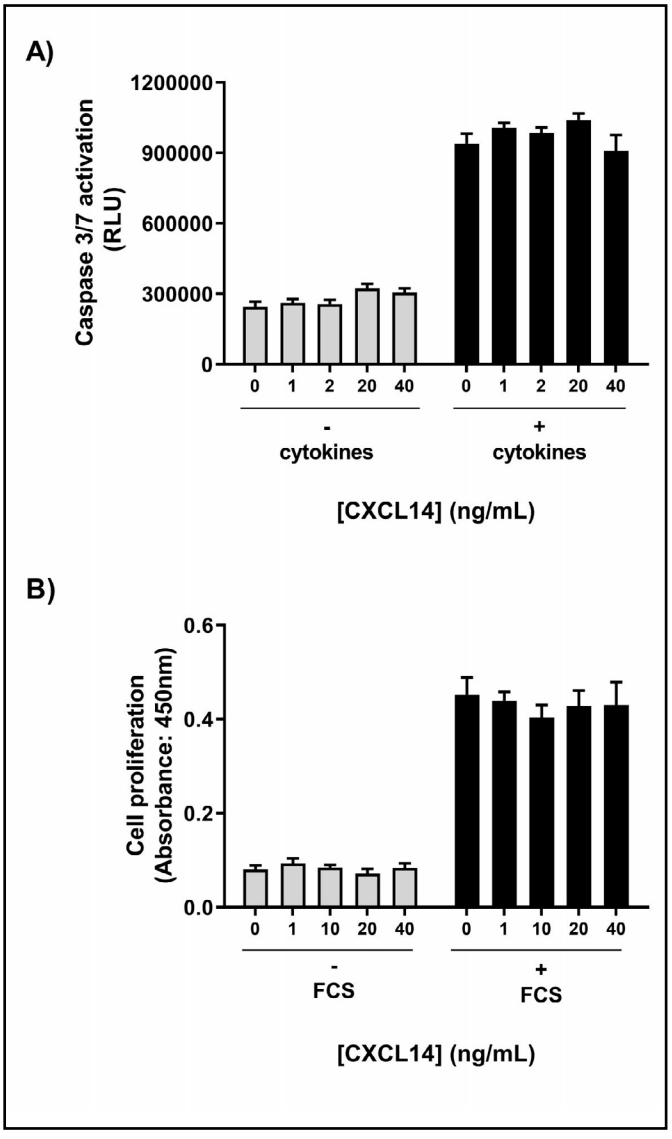

Fig. 8. Effect of CXCL14 on β-cell apoptosis and proliferation. A,B) Increasing concentrations of CXCL14 had no effect on basal (grey bars) or stimulated (black bars) MIN6 β-cell apoptosis (A) or proliferation (B). Data are expressed as mean+SEM, n=6.

inbred strains of mice, in which it was suggested that CXCL14 could be co-released with somatostatin to regulate insulin secretion in a paracrine fashion [9]. Our functional studies support this, as exposure of isolated islets to exogenous CXCL14 resulted in a concentration-dependent inhibition of glucose-dependent insulin secretion. These data are consistent with the improved glucose homeostasis observed in CXCL14$^{-/-}$ mice [12, 21], and the direct effects of CXCL14 on islet function demonstrated here indicate that it acts at multiple sites to impair glucose tolerance. We observed maximal inhibitory effects of CXCL14 on insulin release at 40ng/mL, which is higher than the reported plasma level of approximately 1ng/mL [24], but locally released intra-islet CXCL14 levels are likely to be much higher than circulating levels. The expression of CXCL14 by islet cells and its inhibitory effects on insulin secretion therefore support paracrine signalling, but we cannot rule out a contribution of CXCL14 released from macrophages, fibroblasts and endothelial cells, and it is likely that there are both paracrine and systemic interactions.

Despite observations that CXCL14 has a variety of functional effects, including promoting chemotaxis [25, 26] and inhibiting insulin signalling [22], that its expression is up-regulated in obesity [12, 21] and cancer [24], and that its deletion improves glucose tolerance [13, 21], the receptor through which it signals has not been identified. All chemokines whose receptors have been identified activate members of the rhodopsin-like family of GPCRs [27], but CXCL14 is an orphan for which no receptor has yet been confirmed. It is an alpha chemokine, in which the first two cysteine (C) residues are separated by another amino acid (X). There are 16 members of this family, and CXCL1-CXCL13 are known to signal via a family of CXC receptors. Our mRNA expression analysis of the *Cxc* family receptors in mouse islets and MIN6 β-cells revealed abundant expression of *Cxcr4* and *Cxcr7*, which are the cognate receptors for CXCL12. CXCL14 has been reported to antagonise CXCL12-mediated chemotaxis of CD34+ hematopoietic progenitor cells and bind with high affinity to CXCR4 in THP-1 monocytic cells over-expressing this receptor [26]. However, data from another study indicated that CXCL14 was without effect on CXCL12-induced CXCR4 phosphorylation, calcium mobilisation, ERK1/2 phosphorylation or CXCR4 internalisation in CXCR4 transfected HEK293 and Jurkat T cells [28], suggesting that CXCL14 does not bind to CXCR4. Further complexity is introduced by the recent proposal that CXCL14 may be a positive allosteric modulator of CXCR4, enhancing the potency of CXCR4 ligands [29].

Given the lack of consensus on whether CXCL14 can regulate CXCR4 and the lack of information on its capacity to interact with CXCR7, we therefore used β-arrestin technology to investigate the ability of CXCL14 to function as an agonist, antagonist or allosteric modulator at CXCR4 and CXCR7. These experiments demonstrated that CXCL14 did not induce β-arrestin recruitment at either CXCR4 or CXCR7, nor did it antagonise or modulate CXCL12 affinity or efficacy at these receptors. It is possible that CXCL14 signals via an as yet undefined GPCR, and systematic screening with the PRESTO-Tango interrogation system [30] may lead to deorphanisation of this chemokine. However, it should be borne in mind that the assumption that CXCL14 is a GPCR-activating ligand is largely based on the GPCR-activity relationships of fellow CXC-family ligands and it is possible that CXCL14 has a separate mode of action. In this context, non-GPCR-mediated effects of CXCL14 are supported by vertebrate CXCL14 homologues differing from all other chemokines by possessing an uncharacteristically short amino-terminus of only two amino acids located before the first disulphide bridge, a region which is typically required for triggering GPCR activation [31]. This structural difference suggests that unlike other CXC-family ligands, CXCL14 effects may not be GPCR-mediated and thus efforts to elucidate the target responsible for mediating CXCL14 function should also consider alternative target classes.

An obvious mechanism responsible for CXCL14 inhibition of insulin secretion is by reducing intracellular cAMP, a process that is utilised by well-established inhibitors such as noradrenaline and somatostatin [32]. However, CXCL14 did not inhibit the stimulatory effects of forskolin or affect basal cAMP levels in MIN6 β-cells, indicating that its effects on insulin secretion are transduced via cAMP-independent mechanisms. Glucose uptake into β-cells and its glycolytic and oxidative metabolism are essential pre-requisites for glucose-

stimulated insulin secretion. We found that CXCL14 inhibited accumulation of 2DG6P, the product of glucokinase-mediated phosphorylation of the glucose analogue 2DG, in MIN6 β-cells and mouse islets. Although CXCL14 is reported to reduce glucose uptake into myocytes via insulin-dependent GLUT4 [21], it is unlikely that the decrease in 2DG6P production in β-cells is secondary to inhibition of GLUT2, the predominant transporter in mouse β-cells, as GLUT2-dependent glucose uptake is not rate-limiting for glucose-induced insulin secretion [33]. It is therefore more likely that CXCL14 inhibits 2DG6P accumulation through inhibition of glucokinase, the rate-limiting enzyme in glucose metabolism. It is not clear how CXCL14 inhibits glucokinase, but it could be through inhibition of Ca^{2+} accumulation since β-cell glucokinase is known to be activated by elevations in cytoplasmic Ca^{2+} concentration [34]. Inhibition of glucokinase by CXCL14 would be expected to impair glucose metabolism and the elevation in intracellular ATP that is required for glucose-induced insulin release [35]. Direct measurement of ATP production in islets confirmed that CXCL14 induced a concentration-dependent inhibition, consistent with its inhibitory effects on 2DG6P generation and insulin secretion.

In addition to a reduction in insulin secretory capacity, T2D is characterised by reduced β-cell mass [36, 37], and agents that enhance β-cell apoptosis or reduce their proliferative capacity will exacerbate insulin secretory deficiency. CXCL14 is known to promote apoptosis of renal cancer cells [38] and its over-expression stimulates apoptosis of HepG2 liver cells [39]. However, our quantification of β-cell 3/7 caspase activities indicated that CXCL14, when used over the same concentration range that significantly inhibited insulin secretion, did not increase either basal or cytokine-induced apoptosis. Furthermore, it had no effect on β-cell proliferation, suggesting that this chemokine is not directly linked to changes in β-cell mass.

Conclusion

In summary, our data reveal that CXCL14 exhibits direct effects on islet β-cells that are distinct from its well-recognised roles as an immune and inflammatory modulator. We have established that CXCL14 does not signal via CXCR4 or CXCR7 receptors, that its inhibition of insulin secretion is independent of reductions in cAMP production, but is most likely a consequence of impaired glucokinase activity, and a subsequent decrease in intracellular ATP generation. These observations imply that the deleterious effects of CXCL14 up-regulation in obesity are not only secondary to its induction of insulin resistance and compromised insulin signalling, but also to impaired insulin secretion, and highlight the utility of CXCL14 inhibition as a possible therapeutic approach for T2D. However, it is not feasible to block CXCL14 generation, so therapeutic tractability is dependent on identification of its cognate receptor.

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Disclosure Statement

The authors declare that no conflicts of interest exist.

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