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Exenatide blocks JAK1-STAT1 in pancreatic beta cells

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Abstract

Exenatide (Ex-4) is an antidiabetic drug that acts through the glucagon-like peptide 1 receptor and has recently been approved for the treatment of type 2 diabetes mellitus. Ex-4 also has been shown to affect beta cell gene expression and increase beta cell mass in rodent models of type 1 diabetes mellitus, but the mechanisms are not fully understood. We therefore analyzed the pathways affected by Ex-4 in human islets by using oligonucleotide microarrays and the PathwayStudio software (Ariadne Genomics, Rockville, MD). We identified the JAK1-STAT1 pathway as a novel target of Ex-4 and confirmed the Ex-4–mediated down-regulation of JAK1 and STAT1 by quantitative reverse transcription-polymerase chain reaction in human islets and INS-1 cells. JAK1-STAT1 is the major signaling pathway mediating the interferon γ effects on beta cell apoptosis in type 1 diabetes mellitus. Thus, these findings suggest that Ex-4 treatment may also be beneficial in type 1 diabetes mellitus, where it may help protect beta cells from cytokine-induced cell death by inhibiting JAK1-STAT1.

1. Introduction

Exenatide/exendin-4 (Ex-4) is an antidiabetic drug recently approved for the treatment of type 2 diabetes mellitus. It was originally isolated from the venom of the lizard Gila monster and discovered to be a potent stimulator of the glucagon-like peptide 1 (GLP-1) receptor [1]. In contrast to GLP-1, Ex-4 is not a substrate for cleavage by dipeptidyl peptidase IV and, therefore, has a longer half-life, which makes it suitable as a therapeutic agent.

Glucagon-like peptide 1 and Ex-4 have also been shown to increase beta cell mass in models of type 1 diabetes mellitus, such as partially pancreatectomized and streptozotocin-treated rats, by enhancing proliferation and neogenesis and decreasing beta cell apoptosis [2]. However, the exact mechanisms are not fully understood. Although activation of the GLP-1 receptor affects transcription of several genes including insulin and *PDX-1* [3-7], its effects on overall islet gene expression remain unknown. The goal of the present study was therefore to investigate changes in the gene expression profile of human islets treated with Ex-4 and to identify novel target pathways.

2. Materials and methods

2.1. Human islet and cell culture

Islets were isolated from brain-dead organ donors [8] by using continuous Ficoll purification under a protocol approved by the University of Wisconsin Hospitals and Clinics Institutional Review Board. Islets were incubated for 24 hours with or without Ex-4 (100 nmol/L) (Sigma-Aldrich, St Louis, MO) (2500 islets per condition) in RPMI 1640 (Invitrogen, Carlsbad, CA) containing 11.1 mmol/L glucose and supplemented with 10, fetal bovine serum, 1% penicillin-streptomycin, 1 mmol/L sodium pyruvate, 2 mmol/L L-glutamine, and 10 mmol/L HEPES. We chose 11.1 mmol/L glucose because GLP-1/Ex-4 is only effective at elevated glucose levels [5,9] and the 24-hour incubation time was based on our previous experiments using Ex-4 [8].

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Table 1
Oligonucleotides used in this study

Sequence 5′ -3′	Use
TTCCCTGGCCTTCTTCGAG	Human JAK1 5′ taqman primer
TTCCACAAACTCTTCCACCATG	Human JAK1 3′ taqman primer
CGGGACATTTCACTGGCCT	Rat JAK1 5′ taqman primer
AACCTGTCRCATCATGCTGGC	Rat JAK1 3′ taqman primer
CCCAGAATGCCCTGATTAATG	Human STAT1 5′ taqman primer
CTGCAGCTGATCCAAGCAAG	Human STAT1 3′ taqman primer
AACGGTCCCAAAATGGAGGT	Rat STAT1 5′ taqman primer
TGTAGGGCTCAACAGCATGG	Rat STAT1 3′ taqman primer

Rat insulinoma INS-1 beta cells were maintained as described previously [8] and incubated for 24 hours with or without Ex-4 (100 nmol/L) in serum-free RPMI 1640 supplemented with 0.1% bovine serum albumin.

2.2. Oligonucleotide microarray

Total RNA was extracted from Ex-4–treated and untreated human islets isolated from the same donor by using Trizol reagent (Invitrogen), and RNA samples were hybridized to Human Genome U133Plus2.0 chips (Affymetrix, Stanford, CA) representing approximately 39 500 human genes (54 675 probe sets).

2.3. Data analysis

Robust multiarray average method was used to get the log scale (base 2) measure of expression. The measure of expression was then analyzed by using the empirical Bayes methodology EBarrays and a gamma gamma model as described previously [10]. Genes were considered differentially expressed if their posterior probability exceeded the threshold of .95, which controls the conditional false discovery rate at 5%. Differentially expressed genes were imported into PathwayStudio 4.0 (Ariadne Genomics, Rockville, MD). The "build pathway" function and the option "find only direct interactions between selected entities" was used in conjunction with the ResNet 3.0 database released in February 2005, which contains more than 1 million biological interactions.

2.4. Quantitative real-time reverse transcription-polymerase chain reaction

RNA was extracted from human islets and INS-1 cells by using Trizol and was converted to complementary DNA with the First Strand cDNA synthesis kit (Roche, Nutley, NJ). Quantitative reverse transcription-polymerase chain reaction (RT-PCR) was performed on a Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA)

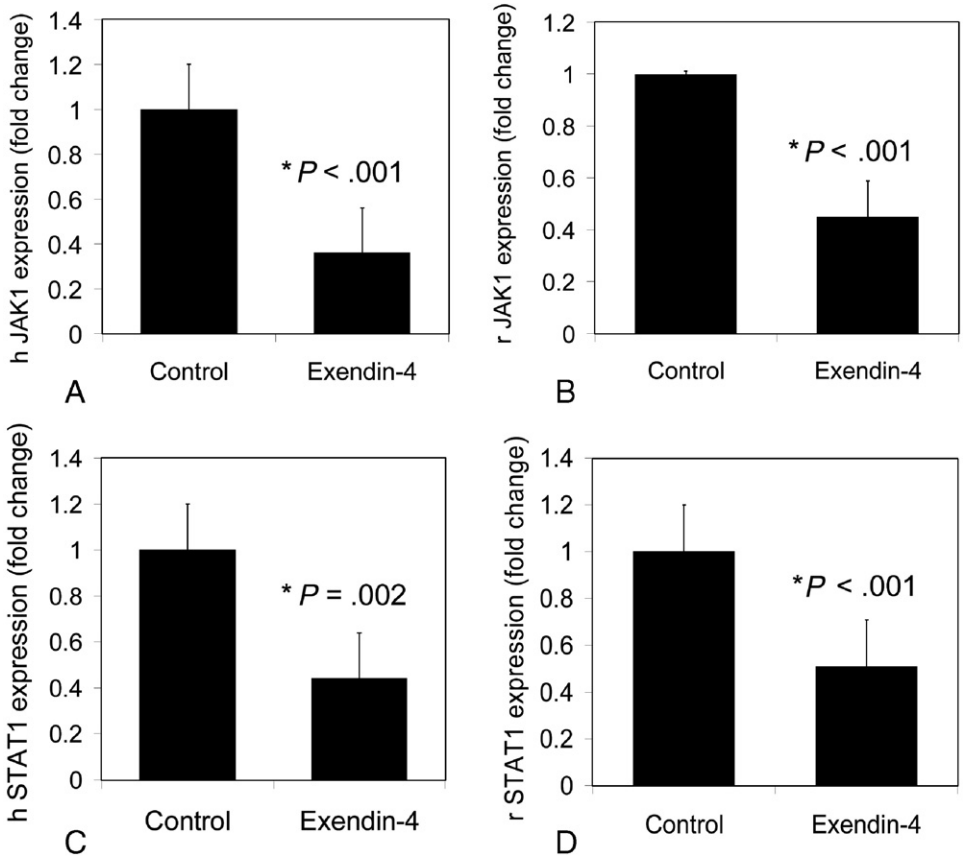

Fig. 1. Exendin-4 effects on JAK1-STAT1 mRNA expression in primary human islet cells and INS-1 cells. JAK1 expression (A, B) and STAT1 expression (C, D) was assessed by quantitative real-time RT-PCR in primary human islets (A, C) and INS-1 cells (B, D) incubated in the presence or absence of 100 nmol/L Ex-4 for 24 hours. Each donor served as its own control. All experiments were analyzed in triplicates; bars represent mean fold change ± SEM. *P* values were calculated by Student *t* tests.

Table 2
Quantitative real-time RT-PCR raw data

	ΔCt[a] (mean ± SEM)	
	Control	Ex-4
Human JAK1	8.97 ± 0.34	10.46 ± 0.19
Human STAT1	8.97 ± 0.46	10.16 ± 0.18
Rat JAK1	10.40 ± 0.15	11.55 ± 0.21
Rat STAT1	11.76 ± 0.12	12.73 ± 0.17

[a] ΔCt = cycle number (sample) − cycle number (18S).

using the primers in Table 1. All primers were tested before use. Dissociation curves were constructed for specificity and all cycle numbers were between 20 and 23 demonstrating efficient amplification. All samples were analyzed in triplicates and corrected for the 18S ribosomal subunit (Applied Biosystems) run as an internal standard.

3. Results and discussion

In this study, we identified 622 differentially expressed probe sets (521 genes) in human islets treated with Ex-4 compared with untreated controls. To further analyze biological interactions and pathways affected by Ex-4, we used the PathwayStudio software and identified the JAK1-STAT1 pathway as a novel target of Ex-4. Both, *JAK1* and *STAT1* were significantly down-regulated by Ex-4 (5-fold and 3-fold, respectively). In contrast, insulin expression, which is known to be up-regulated by Ex-4, was increased more than 40% in response to Ex-4 treatment serving as a positive control.

Quantitative RT-PCR further confirmed the microarray results in human islets (Fig. 1A and C) as well as in INS-1 cells (Fig. 1B and D) (Table 2) revealing a greater than 2-fold reduction in messenger RNA (mRNA) expression of *JAK1* and *STAT1*. Currently, studies are under way to confirm the observed changes in *JAK1-STAT1* mRNA also at the protein level. Although the Ex-4 concentration of 100 nmol/L is widely used for in vitro studies, the inhibition of *JAK1-STAT1* was maintained even at 50 pmol/L (data not shown), which reflects circulating levels found in patients treated with Ex-4.

JAK1-STAT1 is the primary signaling pathway mediating the transcriptional effects of interferon (IFN) γ, which plays a critical role in the beta cell apoptosis of type 1 diabetes mellitus [11]. Although various cytokines are involved [12], IFN-γ is required to prime the beta cell for interleukin 1β– and tumor necrosis factor α–induced apoptosis, and this IFN-γ effect is mediated by STAT1 [11,12]. Interestingly, *STAT1* (−/−) knockout mice are partially resistant to the development of streptozocin-induced diabetes and their beta cells are protected against apoptosis induced by a combination of IFN-γ and interleukin-1β [13]. Together, this suggests that down-regulation of STAT-1 could be a therapeutic target to protect beta cells from autoimmune destruction and our findings raise the possibility that Ex-4 may be an agent capable of conferring this protection.

In fact, studies in INS-1E cells and purified rat beta cells have demonstrated that treatment with Ex-4 causes a significant reduction in cytokine-induced apoptosis [3]. Although an Akt1-dependent pathway has been proposed as the mechanism for this effect [3], our results suggest that Ex-4–mediated down-regulation of JAK1-STAT1 might also be involved. Thus, Ex-4 treatment of patients with newly diagnosed type 1 diabetes mellitus may preserve beta cell mass by blocking the downstream effects of IFN-γ. However, to block the effects of other cytokines additional immunosuppressant therapy may be necessary. This hypothesis is supported by 2 recent studies in diabetic NOD mice [14,15], where Ex-4 treatment only in conjunction with immunosuppression resulted in remission of diabetes.

In summary, Ex-4 reduces *JAK1-STAT1* transcription in primary human islets and INS-1 cells and thereby blocks the key signaling pathway of IFN-γ. These findings suggest that Ex-4 treatment may also be beneficial in type 1 diabetes mellitus, where it may help protect beta cells from cytokine-induced cell death at least in part via *JAK1-STAT1* inhibition.

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