Molecular and Cellular Endocrinology 343 (2011) 18–24

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Upregulation of pancreatic derived factor (FAM3B) expression in pancreatic β-cells by MCP-1 (CCL2)

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ARTICLE INFO

Article history:
Received 11 January 2011
Received in revised form 22 April 2011
Accepted 27 May 2011
Available online 1 June 2011

Keywords:
CCL2
Pancreatic derived factor
FAM3B
Pancreatic islet
β-Cell
Signal transduction

ABSTRACT

Pancreatic derived factor (PANDER, FAM3B) is a peptide mainly synthesized and secreted by pancreatic β-cells. PANDER is proposed to be involved in regulation of β-cell function under physiological conditions and impairment of β-cell function under pathological conditions. MCP-1 (CCL2) is expressed by normal pancreatic islets and has been implicated in inflammation related pancreatic disorders. We examined the effect of MCP-1 on PANDER expression by using murine pancreatic β-cell line MIN6 and pancreatic islets. We found that MCP-1 induced PANDER mRNA transcription and protein synthesis in MIN6 cells and islets. By using calcium chelator (EGTA); inhibitors for PKC (Go6976), MEK1/2 (PD98059) or *c-Jun*-N-terminal kinase (JNK) (SP600125); *c-Jun* dominant-negative construct; PANDER promoter luciferase constructs; and islets isolated from *Fos* knockout mice; we demonstrated that MCP-1 induced PANDER gene expression in β-cells through Ca^{2+}–ERK1/2–AP-1 and PKC–JNK–AP-1 signaling pathways. Our findings suggest a new link between the endocrine and immune systems and provide useful information for further investigating the physiological functions of PANDER and its involvement in inflammation-related pancreatic disorders.

1. Introduction

Pancreatic derived factor (PANDER), also named FAM3B, is a cytokine-like peptide identified in 2002 during a search for novel cytokines based on predicted four-helix-bundle structures (Zhu et al., 2002). PANDER is mainly expressed in pancreatic islets (Zhu et al., 2002). In vitro studies showed that recombinant PANDER protein and overexpression of PANDER could induce apoptosis of pancreatic β-cells and pancreatic islets from human and rodent (Cao et al., 2003, 2005), and inhibit insulin secretion induced by carbachol plus glucose or high potassium (Cao et al., 2005). Recent studies showed that PANDER could inhibit insulin signaling in hepatic cell line HepG2 (Yang et al., 2009). PANDER knockout mice exhibit a glucose-intolerant phenotype after a high-fat diet (Robert-Cooperman et al., 2011). Researches with PANDER knockout mice suggest a potential role of PANDER in regulation or facilitation of insulin secretion (Robert-Cooperman et al., 2010). Collectively, these observations suggest that PANDER may have a potential role in regulation of β-cell function and glucose homeostasis and may be involved in β-cell dysfunction under pathological conditions.

Understanding the regulation of PANDER gene expression under physiological and pathophysiological conditions will be helpful for elucidating the biological functions and pathological significance of PANDER. However, there are only few reports regarding the regulation of PANDER gene expression. It has been reported that PDX-1 is involved in PANDER gene expression (Burkhardt et al., 2008). PANDER expression and secretion is enhanced by glucose (Yang et al., 2005; Wang et al., 2008). Our previous studies demonstrated that glucose upregulated PANDER gene expression by β-cells through Ca^{2+}–PKA–ERK1/2–CREB and Ca^{2+}–PKC–CREB signaling pathways (Wang et al., 2008). These results suggest that the expression of PANDER is regulated in a similar manner with insulin. Xu et al. reported that IFN-γ alone or combination with IL-1β and TNF-α could upregulate PANDER mRNA expression in islets and βTC3 cells (Xu et al., 2005), suggesting that PANDER may be involved in IFN-γ-mediated apoptosis of β-cells under pathological conditions, such as type 1 diabetes.

Proinflammatory cytokines, such as TNFα, IL-1β, IL-6 and monocyte chemoattractant protein-1 (MCP-1, CCL2), are involved

Abbreviations: AP-1, activator protein-1; CCL2, chemokine (C–C motif) ligand 2; *c-Jun* DN, *c-Jun* dominant-negative construct; EGTA, ethylene glycol tetraacetic acid; FAM3B, family with sequence similarity 3, member B; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemotactic protein 1; MEK, mitogen-activated protein kinase kinase; PANDER, pancreatic derived factor; PDX-1, pancreatic and duodenal homeobox1; PKC, protein kinase C; JNK, *c-Jun*-N-terminal kinase.

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doi:10.1016/j.mce.2011.05.039

in β-cell dysfunction in acute and chronic inflammation-related pancreatic disorders, including pancreatitis (Granger and Remick, 2005), islet transplant rejection (Narang and Mahato, 2006), insulin resistance and type 2 diabetes (Lee and Pratley, 2005; Tataranni and Ortega, 2005). By using murine pancreatic β-cell line MIN6 and pancreatic islet, we examined the effects of these cytokines on PANDER gene expression. While TNFα, IL-1β and IL-6 had no effect on PANDER gene expression in MIN6 cells (data not shown), we found that MCP-1 could upregulate PANDER expression and further explored the underlying molecular mechanisms.

2. Materials and methods

2.1. Cell culture and treatment

The mouse insulin-secreting cell line MIN6 cells were cultured in DMEM medium (Gibco BRL, Burlinton, ON, Canada) containing 5.6 mM glucose, 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin in a humidified atmosphere at 37 °C with 5% CO_2. MIN6 cells were treated with DMEM containing the indicated concentrations of MCP-1 (Peprotech, Rocky Hill, NJ, USA) for the indicated time periods.

2.2. Isolation of mouse islets

Animal experiments were in accordance with the National Institutes of Heath Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use committee, Institute for Nutritional Sciences, Chinese Academy of Sciences. Pancreatic islets were isolated from C57/BL6 mice (Shanghai SLAC Laboratory Animal Co., Shanghai, China) or *Fos* knockout mice by type V collagenase (Sigma–Aldrich, St. Louis, MO, USA) digestion, followed by Ficoll 400 (Amersham Pharmacia Biotech, Piscataway, NJ, USA) gradient separation, as described previously (McDaniel et al., 1983). Islets were maintained in DMEM containing 5.6 mM glucose, 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin.

2.3. RNA extraction and real-time PCR

Total RNA was extracted from MIN6 cells or mouse pancreatic islets using Trizol reagent (Invitrogen, Carlsbad, CA) and depleted of contaminating DNA with RNase-free DNase. cDNA was synthesized from 2 μg RNA with M-MuLV reverse transcriptase and random hexamer according to the manufacturer's instructions (Fermentas, Burlington, Ontario, Canada). Quantitative real-time PCR with Power SYBR Green PCR master Mix was performed on an ABI Prism 7500 sequence detector (Applied Biosystems Inc., Foster City, CA, USA). Briefly, reverse-transcribed cDNA in triplicate samples were checked for PANDER and β-actin mRNA expression. Primers for murine PANDER were: 5′-TGCTCGCGGAGCTCATTC (sense), and 5′-CCAATGCTTCGGATGTTGTAGA (antisense). Mouse β-actin primers were: 5′-CAACG AGCGG TTCCG ATG (sense), and 5′-GCCAC AGGAT TCCAT ACCCA (antisense). Amplification of the PANDER cDNA was normalized to β-actin expression. Relative levels of PANDER mRNA expression were calculated with $2^{-\Delta\Delta C_T}$ method.

2.4. Western blotting assay

After stabilization for 2 h at 37 °C in KRB buffer (Benes et al., 1998) with or without inhibitors, MIN6 cells were stimulated with MCP-1 for indicated time periods. Phosphorylated ERK1/2 (p-ERK1/2) and *c-Jun* N-terminal kinase (p-JNK) were examined by Western blot as described previously (Wang et al., 2008). To ensure equal loading, levels of ERK1/2 and JNK were also examined. PANDER protein levels were examined by Western blot in the indicated time intervals with antibody against mouse PANDER protein (R&D Systems Inc., Minneapolis, MN) as previously described (Wang et al., 2008). Immunoblot results were quantified by using Gel-Pro Analyzer software (Media Cybernetics Inc., Silver Spring, Maryland, USA).

2.5. Construction of the PANDER promoter luciferase plasmids and luciferase reporter assay

The construction of mouse PANDER promoter luciferase plasmids was described previously (Wang et al., 2008). Briefly, regions of the PANDER promoter spanning from 338 bp upstream to 491 bp downstream (−338/+491), and from 1 to 491 bp downstream (+1/+491) of the transcriptional start site were amplified by PCR and cloned into the pGL3-basic luciferase reporter plasmid (Promega Corp., Madison, WI) between the KpnI and MluI sites. Both constructs were confirmed by restriction enzyme digestion and sequencing. In the promoter region of PANDER gene, there is a binding site for the transcription factor AP-1 at +96/+105 (GCTTGAGCCA) predicted by AliBaba2. A mutant +1/+491 luciferase plasmid (+1/+491 M) with mutations at the AP1 binding site (GCTCTGGCCA) was constructed by Generay Biotechnology Corporation (Shanghai, China) and verified by sequencing. MIN6 cells were plated in a 24-well tissue culture plate. PANDER promoter luciferase plasmids were cotransfected with the pRL-TK Renilla plasmid with a 40:1 ratio using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Thirty-six hours after the transfection, cells were cultured in DMEM with or without 100 ng/ml MCP-1 for another 6 h. Inhibitors were added 2 h before harvesting the cells for luciferase assay. Luciferase activity of the promoter constructs and the pRL-TK construct was measured sequentially using the Dual-Luciferase Reporter Assay System (Promega). Variation in transfection efficiency was normalized by dividing the promoter construct activity by the respective cotransfected pRL-TK luciferase activity.

2.6. Statistical analysis

Data are presented as means ± SD. Statistical significance of differences between groups was analyzed by Student's *t* test or ANOVA.

3. Results

3.1. MCP-1 induces murine PANDER expression at both mRNA and protein levels

To examine the effect of MCP-1 on PANDER gene expression, MIN6 cells were stimulated with different concentrations of MCP-1 for different lengths of time, and the mRNA levels of PANDER were detected by quantitative real-time PCR. As shown in Fig. 1A and B, MIN6 cells cultured in DMEM containing 5.6 mM glucose expressed transcripts for PANDER and MCP-1 significantly enhanced this expression in time and dose-dependent manners. The minimal concentration of MCP-1 to significantly induce PANDER gene expression was 1 ng/ml. The maximal inductive effect of MCP-1 on PANDER gene expression was obtained after 11 h stimulation. We also examined the effect of MCP-1 on PANDER expression in murine pancreatic islets. Consistently, MCP-1 upregulated PANDER mRNA levels in islets (Fig. 1C). Interestingly, the inductive effect of MCP-1 on PANDER expression is more potent in primary islets than in transformed β-cell line MIN6.

To determine whether MCP-1-induced increase of PANDER mRNA levels was due to the increase of PANDER mRNA stability,