Induction of Monocyte Chemoattractant Protein-1 (MCP-1) Expression by Angiotensin II (AngII) in the Pancreatic Islets and Beta Cells


INTRODUCTION

Type 1 (insulin-dependent) diabetes millitus

Monocyte chemoattractant protein (MCP-1)

The circulating renin-angiotensin system (RAS)

INTRODUCTION

Type 1 (insulin-dependent) diabetes millitus

Type 1 diabetes develops as a consequence of autoimmunity, leading to beta cell destruction

The early stages of insulitis

beta cell dysfunction and death

INTRODUCTION

Monocyte chemoattractant protein (MCP-1)

A protein from the chemotactic cytokines (C-C chemokine) subfamily is expressed in human and rodent islets

MCP-1 attracts monocytes, T-cells, and natural killer cells to the site of inflammation

INTRODUCTION

The circulating renin-angiotensin system (RAS)

- Angiotensinogen
- Critical Zn-dependent metallopeptidase
- Angiotensin I (AngI)
- Angiotensin I-converting enzyme (ACE)
- The bioactive octapeptide AngII

Multiple G-protein-coupled receptor subtypes including AngII receptor 1 and 2 (AT1R, AT2R)
INTRODUCTION

Hypothesis

AngII may contribute to islet inflammation through induction of MCP-1 in the islets and beta cells

MATERIALS AND METHODS

Cell culture

- RINm5F insulinoma cell line
- grown at 37°C under a humidified
- 5% CO₂ atmosphere in RPMI 1640 medium
- supplemented with
- 10% fetal bovine serum
- 2 mM glutamine
- 100 units/ml of penicillin
- 100 μg/ml of streptomycin
- 2.5 μg/ml of amphotericin B

RESULTS

AngII-induces MCP-1 mRNA accumulation and secretion of MCP-1 in cultured beta cells
**MATERIALS AND METHODS**

To examine whether the increase in MCP-1 mRNA levels in response to AngII is associated with MCP-1 production

**DISCUSSION**

- AngII is a potent stimulator of MCP-1 expression.
  - Induced MCP-1 accumulation rapidly in the pancreatic islets with significant magnitude.
  - In beta cells, AngII (10^-7 mol/L) significantly induces MCP-1 mRNA and protein levels.

**RESULTS**

AngII-induced MCP-1 mRNA accumulation and secretion of MCP-1 in cultured beta cells

**MATERIALS AND METHODS**

Rat-specific MCP-1 ELISA kit

**RESULTS**

AngII induces MCP-1 promoter activity in RINm5F cells

**MATERIALS AND METHODS**

RINm5F

**RESULTS**

To determine the receptor that mediates the AngII-induced MCP-1 gene expression in beta cells

**MATERIALS AND METHODS**

Rat MCP-1 promoter

**RESULTS**

AngII-induced MCP-1 promoter activity in RINm5F cells with GAPDH

**MATERIALS AND METHODS**

AngII-induced MCP-1 promoter expression in RINm5F cells is blocked by AT1R antagonist

**RESULTS**

To determine the receptor that mediates the AngII-induced MCP-1 gene expression in beta cells
MATERIALS AND METHODS

transfected cells

AngII (10^{-7} \text{mol/l})

24 hrs

1 & 2 hrs

luciferase activity in the cell lysates was measured

RESULTS

AngII induces MCP-1 promoter activity in RINm5F cells.

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{fold_induction}
\caption{Fold Induction of Luciferase Activity}
\end{figure}

\begin{itemize}
\item control
\item AngII 10^{-7} 1 hr
\item AngII 10^{-7} 2 hr
\end{itemize}

P<0.05

DISCUSSION

- ATIR antagonist prevented the AngII-MCP-1 production
- AngII addition to beta cells results in a concomitant increase in ATIR mRNA expression
- Assume that AngII itself could have a regulatory effect on ATIR in beta cells which could consequently contribute to AngII-MCP-1 induction

MATERIALS AND METHODS

To determine whether an increase in tyrosine phosphorylation induces MCP-1 mRNA accumulation

\begin{itemize}
\item sodium orthovanadate = protein tyrosine phosphatase inhibitor
\end{itemize}

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{sodium_orthovanadate}
\caption{sodium orthovanadate concentrations}
\end{figure}

\begin{itemize}
\item sodium orthovanadate
50 \mu M
100 \mu M
200 \mu M
\end{itemize}

\begin{itemize}
\item sodium orthovanadate
RINm5F
\end{itemize}

3 hrs RT-PCR

DISCUSSION

- MCP-1 promoter was induced significantly as early as after 1 hour of stimulation.
  - acute response to AngII
  - Further studies are required
    - analyze the AngII specific cis-elements on MCP-1 promoter
    - AngII-induced MCP-1 upregulation is mediated through NF-kB

- possible that AngII induces MCP-1 transcripts in the pancreatic islets and beta cells
  - directly through acting on its promoter
  - indirectly through ATIR upregulation

\begin{itemize}
\item -ATIR antagonist prevented the AngII-MCP-1 production
\item - AngII addition to beta cells results in a concomitant increase in ATIR mRNA expression
\item - Assume that AngII itself could have a regulatory effect on ATIR in beta cells which could consequently contribute to AngII-MCP-1 induction
\end{itemize}
**Result**

RT-PCR

MCP-1 / GAPDH

Sodium orthovanadate — 50µM 100µM 200µM

**MATERIALS AND METHODS**

To determine the involvement of protein kinase in AngII-induced MCP-1 expression

Genistein 60 µM AngII 10^{-7} mol/l

RINm5F cells

RT-PCR

AngII 10^{-7} mol/l

RINm5F cells

Genistein: specific tyrosine kinase inhibitor

**Result**

RT-PCR

MCP-1 / GAPDH

AngII 10^{-7} mol/l Genistein

**MATERIALS AND METHODS**

AngII-induced MCP-1 gene expression requires ERK1/2 MAP kinase activity

RINm5F + AngII 10^{-7} mol/l 5 & 10 & 15 & 30 & 60 min

Washing and lysed cells

protein

Western blot

**MATERIALS AND METHODS**

Protein isolation and Western blot analysis

RINm5F cell

modified RIPA lysis buffer

protein

BCA protein assay reagent

BCA = Bicinchoninic Acid

**MATERIALS AND METHODS**

Protein

gel loading buffer at 85°C 5 min

10 % SDS-polyacrylamide slab gels

incubate 4°C overnight

primary antibody diluted in PBS/Tween 20

Transfered
Western blot analysis

- Proteins from beta cells treated with AngII
- The protein bands were visualized with enhanced chemiluminescence reagents
- Analyzed and intensity quantified using EDAS 290

RESULTS

AngII-induced MCP-1 gene expression requires ERK1/2 MAP kinase activity

ERK = extracellular signal regulated kinase

MATERIALS AND METHODS

AngII-induced MCP-1 gene expression requires ERK1/2 MAP kinase activity

MEK1/2 selective inhibitor U0126 30 µM

AngII 10^{-7} mol/l

U0126 = MEK1/2 selective inhibitor

Statistical analysis

Figure 4A, B: Data represent three independent experiments. *p < 0.05 vs. AngII treated cells using one-way repeated ANOVA with subsequent all pairwise comparison procedure by student t-test.
DISCUSSION

- MAP kinases encoded by the extracellular signal regulated kinase (ERK) genes are a family of serine/threonine protein kinases

- ERK referred to as p44 (ERK1), and p42 (ERK2) are activated by phosphorylation of threonine and tyrosine residues by MAP kinase kinase (MEK).

- Using U0126, a selective inhibitor for MEK activation
  - AngII-induced MCP-1 mRNA occurs through a MEK-sensitive mechanism

- Further studies
  - Fully delineate the specific signaling pathway by which AngII ultimately modulates MCP-1 synthesis in the islets

MATERIALS AND METHODS

Mice

Female NOD mice: n=5
ICR mice normoglycemic; n=3
1. Prediabetic (2-4 wks)
2. Diabetic FBG 200-250 mg/dl (13-15 wks)
3. Diabetic FBG >350 mg/dl (20-22 wks)

NOD pancreatic AngII generating system expression correlates with hyperglycemia and progression to severe diabetes

Western blot: ACE protein/Actin
RT-PCR: ACE mRNA, GAPDH

RESULTS

NOD pancreatic Western blot

ACE protein levels (arbitrary units)

RT-PCR

ACE mRNA levels (arbitrary units)
MATERIALS AND METHODS
ICR mice pancreata (3 gr.)

- Clean
- RT-PCR
- Western blot

RESULTS
ICR mice pancreata

Western blot

ACE protein/Ac

20–22 wks
2–4 wks
13–15 wks

MATERIALS AND METHODS
ACE distribution in the diabetic pancreas and colocalization with MCP-1 in beta cells

- An antiserum against ACE
- A goat polyclonal antibody against human MCP-1

RESULTS
ACE distribution in the diabetic pancreas and colocalization with MCP-1 in beta cells.
MATERIALS AND METHODS

Angiotensin II alone and in combination with IL-1β elicit an inflammatory response in the islets by stimulation of MCP-1

Islet cell normoglycemic female NOD mice

digested in shaker-type water bath at 37 °C
culture in RPMI medium

RESULTS

- The first time the presence of an active AngII generating system in the pancreata of NOD mice

- As early as 2 weeks of age
  ACE mRNA and protein were detectable in the non diabetic pancreata

DISCUSSION

- Development of insulitis and progression to hyperglycemia and beta cell destruction

expression levels of ACE

- Suggesting that AngII generation is an active ongoing process with diabetes advancement

DISCUSSION

- ACE is constitutively expressed in most of the islet cells, colocalizing with MCP-1
  - Suggesting an endogenous autocrine / paracrine interaction
  - Using of angiotensin blockade therapies could improve islet function and glucose intolerance in type 2 diabetes patients and in animal models
DISCUSSION

- adding ACE inhibitors in vitro to the islets leads to their protection against glucotoxicity and oxidative stress
- AngII itself affects the islet short-term insulin release
- the long-term effects of AngII on the islet functions are yet to be determined

DISCUSSION

- The novel data about the role of the local pancreatic renin angiotensin system as a source of islet inflammation
- suggest that targeting AngII could be used as a novel therapeutic strategy to
  - reduce MCP-1 levels in the islets
  - inhibit beta cell directed immunity in type 1 diabetes

DISCUSSION

This research showed that

1. AngII elicits an inflammatory response in the islets and beta cells by stimulation of MCP-1 production through an AT1R-ERK1/2-dependent mechanism

2. hyperglycemia and progression to diabetes correlate with upregulation of ACE