A Role for Tyrosine Kinase Activation in Interleukin-1β Induced Nitric Oxide Production in the Insulin Producing Cell Line RINm-5F

Nils Welsh

Received January 11, 1994; accepted February 10, 1994

The aim of this investigation was to study the putative role of protein phosphorylation in interleukin-1β (IL-1β) induced signal transduction in insulin producing cells. For this purpose, insulin producing RINm-5F cells were exposed to IL-1β for 7 hours with or without different agonists and antagonists to protein kinases and phosphatases and the production of nitrite was subsequently determined. It has been shown earlier that IL-1β will stimulate the production of nitrite in such cells. It was found that EDTA, TPA and staurosporine did not affect IL-1β induced nitrite production. However, the tyrosine kinase antagonist tyrphostin inhibited, whereas sodium orthovanadate, okadaic acid and cyclosporin A, all inhibitors of protein phosphatases, potentiated IL-1β induced nitrite release to the medium. The tyrosine kinase antagonist genistein potentiated at a low concentration and inhibited at a high concentration the IL-1β effect. It is concluded that protein phosphorylation events, mediated either by protein kinases or phosphatases on both tyrosine and serine/threonine residues, may mediate or antagonize IL-1 induced signal transduction in insulin producing cells.

KEY WORDS: interleukin-1; nitric oxide; insulin; tyrosine kinase; phosphatase.

INTRODUCTION

It is today established that interleukin-1β exerts inhibitory and toxic effects on rodent pancreatic β-cells in vitro (1–3) and that this effect is probably mediated by the induction of the enzyme nitric oxide synthase (4). The chain of events is initiated by the binding of IL-1β to its receptor on the β-cell (5), which leads to an altered gene expression and de novo protein synthesis (6, 7). After a lag period of 3–4 hours, the inducible form of nitric oxide synthase (iNOS) is synthesized leading to a high rate of nitric oxide formation (8, 9). Nitric oxide is a short-lived and highly reactive radical, which inhibits β-cell mitochondrial function by
inactivating the Krebs-cycle enzyme aconitase \( (8,10) \) and possibly other Fe-containing enzymes of the respiratory chain. Recently, it has also been described that nitric oxide may act upon the \( \beta \)-cell by inducing DNA strand breaks \( (11) \).

The intracellular signals generated in \( \beta \)-cells by the interaction between IL-1\( \beta \) and its receptor have not been elucidated yet. We have in previous studies not been able to observe any effects of IL-1 on protein kinase C activity and cAMP levels in isolated rat islets \( (12) \), although the induction of these second messenger have been described in other cell types \( (13) \). In the insulin producing cell line HIT-T15, IL-1\( \beta \) induced nitric oxide synthase expression was preceded by an early and transient \( c \)-fos mRNA expression \( (14) \). Induction of this early response gene is thought to be preceded by protein phosphorylation cascades. Moreover, studies performed on mainly T-cells and fibroblasts have recently pointed to protein phosphorylation and dephosphorylation events as mediators of the IL-1 signal \( (15,16) \). In view of this, I have presently studied the effects of different protein (de)phosphorylation modulators on IL-1\( \beta \) induced nitrite production in the insulin producing cell line RINm-5F, and indirect evidence is presented indicating a role of protein phosphorylation, both tyrosine and serine/threonine specific, in IL-1\( \beta \) signal transmission.

**MATERIALS AND METHODS**

**Chemicals**

The chemicals were obtained from the following sources: Flow Laboratories (Irvine, UK): culture medium RPMI 1640, fetal calf serum (FCS) and L-glutamine. Sigma Chemical Co. (St. Louis, MO, USA): staurosporine, 12-O-tetradecanoylphorbol 13-acetate (TPA), ethyleneglycol-bis-(\( \beta \)-aminoethyl ether) N,N,N',N'-tetra-acetic acid (EGTA), tyrphostin 1, tyrphostin 25, sodium orthovanadate, okadaic acid, genistein, naphthylethlenediamine dihydrochloride, sulfanilamide and sulfosalisylic acid. Sandoz (Basel, Switzerland): Cyclosporin A (Sandimmun). Human recombinant IL-1\( \beta \) was kindly provided by Dr. K. Bendtzen (Laboratory of Medical Immunology, Rigshospitalet, Copenhagen, Denmark). IL-1\( \beta \) was produced by Immunex Research and Development Corp. (Seattle, WA, USA) and presented a biological activity of 50 U/ng.

**Cytokine and Test Agent Treatment**

The clonal rat insulin secretory cell line, RINm-5F was cultured in RPMI 1640 + 10% FCS. For experiments, cells were seeded in Falcon multiwell tissue culture plates (Becton Dickinson Labware, Lincoln Park, NJ, USA). When reaching 50–70% confluency \( (5 \times 10^5 \) cells), 25 U/ml of IL-1\( \beta \) and test agents were added as given in the figures. Seven hours later, duplicate samples