0013-7227/97/$03.00/0
Endocrinology

Vol. 138, No. 12
Printed in U.S.A.

Irreversible Inhibition of Metabolic Function and Islet Destruction after a 36-Hour Exposure to Interleukin-1β*

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ABSTRACT

The purpose of this study was to identify the duration of exposure of islets to interleukin 1β (IL-1β) that results in irreversible damage. Treatment of rat islets for 18 h with IL-1β results in an inhibition of glucose-stimulated insulin secretion, mitochondrial aconitase activity, and total protein synthesis. The addition of N^G-monomethyl-L-arginine (NMMA) or aminoguanidine to islets preincubated for 18 h with IL-1β, followed by continued culture for 8 h (with both NMMA and IL-1β), results in the recovery of islet secretory function, aconitase activity, and protein synthesis. However, islet metabolic function is irreversibly inhibited after a 36-h incubation with IL-1β, as an additional 8-h incubation with NMMA or aminoguanidine does not stimulate the recovery of insulin secretion, aconitase activity, or protein synthesis. The irreversible inhibition of metabolic function correlates with the commitment of islets to destruction. Treatment of islets for 96 h with IL-1β results in islet degeneration. NMMA, added to islets 24 h after the addition of IL-1β, followed by continued culture for 72 h (with NMMA and IL-1β), prevents islet degeneration. However, NMMA added to islets 36 h or 48 h after the addition of IL-1β, followed by continued culture for a total of 96 h, does not prevent islet degeneration. New messenger RNA expression appears to be required for islet recovery from IL-1β-induced damage as actinomycin D prevents the recovery of islet aconitase activity. Lastly, treatment of human islets with a combination of IL-1β and interferon-γ (IFNγ) results in a potent inhibition of mitochondrial aconitase activity. NMMA, when cocultured with IL-1β + IFNγ, completely prevents cytokine-induced inhibition of human islet aconitase activity. NMMA, when added to human islets pretreated for 18 h with IL-1β + IFNγ, stimulates the recovery of mitochondrial aconitase activity after an additional 8 h incubation. These findings indicate that nitric oxide-induced islet damage is reversible; however, prolonged production of nitric oxide (after a 36-h exposure to IL-1β) results in the irreversible inhibition of islet metabolic and secretory function. (*Endocrinology* **138:** 5301–5307, 1997)

INSULIN-DEPENDENT diabetes mellitus is an autoimmune disease characterized by a local inflammatory reaction in and around the pancreatic islets of Langerhans that leads to the selective dysfunction and destruction of insulin-producing β-cells. Cytokines, released during insulitis, have been proposed to participate in β-cell destruction during the development of autoimmune diabetes (1, 2). Previous reports have shown that islets incubated with the cytokine interleukin-1β (IL-1β) express the inducible isoform of nitric oxide synthase (iNOS) and produce high levels of nitric oxide (3–7). The β-cell appears to be the islet cellular source of iNOS in response to IL-1β (6, 7). The increased production of nitric oxide by IL-1 treated islets correlates with a potent inhibition of glucose-stimulated insulin secretion that is prevented by NOS inhibitors, aminoguanidine (AG), and N^G-monomethyl-L-arginine (NMMA; Refs. 3–7). Nitric oxide, which targets iron-sulfur centers, inhibits the electron transport chain at complexes I and II and the Krebs cycle enzyme, aconitase (8, 9). IL-1 has been shown to inhibit islet aconitase activity and the oxidation of glucose to CO_2, resulting in a reduced cellular level of ATP (6, 10, 11). The reduction in mitochondrial function appears to be one mechanism by which nitric oxide mediates the inhibitory effects of IL-1 on insulin secretion by islets.

Although IL-1 stimulates islet and β-cell damage, the destructive effects of this cytokine on islet metabolic function are reversible. Rat islets incubated with IL-1β for 15 h require a 4-day incubation in cytokine-free media to restore normal glucose-stimulated insulin secretion (12, 13). This window of functional recovery can be reduced to 8 h by the inhibition of iNOS (14). The addition of NMMA to islets preincubated for 18 h with IL-1β, followed by an 8-h incubation in the presence of both NMMA and IL-1, results in a complete recovery of insulin secretion (14). Recovery of insulin secretion function is paralleled by a simultaneous recovery of mitochondrial aconitase activity (14). The aim of this study was to identify the length of exposure of islets to IL-1 that leads to irreversible inhibition of insulin secretion and to determine whether the irreversible inhibition of insulin secretion correlates with an irreversible inhibition of islet metabolic function.

Materials and Methods

Materials, cells, and animals

Rat insulinoma RINm5F cells were obtained from the Washington University Tissue Culture Support Center (St. Louis, MO). Male Sprague-Dawley rats (250–300 g) were purchased from Harlan (Indianapolis, IN). Two preparations of human islets were obtained from the Diabetes Research Institute at the University of Miami (Miami, FL). The donors were a 25- and 16-yr-old male and female, respectively. Collagenase type XI and AG were purchased from Sigma Chemical Co. (St. Louis, MO). RPMI medium 1640 containing 1× L-glutamine, CMRL-1066, MEM, penicillin, streptomycin, and human recombinant interferon-γ (IFNγ) were from GIBCO Laboratories (Grand Island, NY). FCS was obtained

Received June 3, 1997.
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* This work was supported by research grants from Alteon Inc. and The Tobacco Research Council.
† Supported by a Career Development Award from the Juvenile Diabetes Foundation International.