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Interleukin-1β induces the expression of an isoform of nitric oxide synthase in insulin-producing cells, which is similar to that observed in activated macrophages

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The suppressive and cytotoxic effects of interleukin-1β (IL-1β) on rodent insulin-producing cells observed in vitro are probably mediated through formation of nitric oxide (NO). In this study we demonstrate that IL-1-induced NO formation in isolated rat islets and insulin-producing HIT cells is more sensitive to inhibition by N^G-monomethyl-L-arginine than to inhibition by N^G-nitro-L-arginine, thus suggesting that IL-1-exposed insulin-producing cells express an isoform of nitric oxide synthase similar to that present in activated macrophages. Furthermore, IL-1β markedly increased the mRNA levels of the inducible macrophage form of nitric oxide synthase in HIT cells.

Interleukin-1β; Nitric oxide; Nitric oxide synthase; Pancreatic islet; HIT cell; Insulin-dependent diabetes mellitus

1. INTRODUCTION

Following the original description of the suppressive and cytotoxic effects of the cytokine interleukin-1 (IL-1) on rodent pancreatic B-cells [1], there has been a continuous effort to unveil the mechanisms behind these actions of IL-1 [2]. Recent data suggest that the actions of the cytokine initiates with binding to membrane receptors [3,4], induction of gene transcription and protein translation [5,6]. This is followed by a progressive accumulation of the radical nitric oxide [7–10], which may impair the activity of the Kreb's cycle enzyme aconitase [8] and thus lead to a decrease in mitochondrial function and ATP production [11,12].

There are different isoforms of the enzyme NO synthase in distinct tissues [13]. The best characterised isoforms are the ones present in endothelial cells/neurons [14] or in activated macrophages [15,16]. They differ in their sensitivity to inhibition by arginine analogues, cofactor requirements and parts of their deduced amino acid sequence [13–16]. It still remains to be determined which form of NO synthase that is present in pancreatic islets. In the present study we attempted to answere this question by studying the inhibitory effects of two different N^G-substituted arginine derivatives on IL-1-induced nitrite generation, an end product of NO, in rat islets and in the clonal insulin secretory line HIT-T15 cells (HIT cells) [17]. Moreover, we also characterised the expression of mRNA for an inducible form of nitric oxide synthase, recently cloned in murine macrophages [15,16], in IL-1β-exposed HIT cells.

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2. EXPERIMENTAL

2.1. *Materials*

Human recombinant IL-1β (rIL-β) was kindly provided by Dr. K. Bendtzen, Laboratory of Medical Immunology, Righospitalet, Copenhagen, Denmark. The cytokine was produced by Immunex (Seattle, WA) and had a biological activity of 50 U/ng, as compared with an interim international standard rIL-β preparation (NIBSC, London, UK) [18]. The chemicals were purchased from the following sources: N^G-nitro-L-arginine and N^G-monomethyl-L-arginine from Sigma Chemicals Co (St. Louis, MO, USA); FastTrack mRNA Isolation Kit from Invitrogen Corp. (San Diego, CA, USA); Multiprime DNA labelled system and [α-^{32}P]dCTP from Amersham International (Amersham, UK); MagnaGraph Nylon Transfer Membrane from Micron Separations Inc. (Westboro, MA, USA). All other chemicals of analytical grade were obtained from E. Merck (Darmastadt, Germany).

2.2. *Methods*

Pancreatic islets were isolated by collagenase digestion from adult male Sprague–Dawley rats bred in a local colony (Uppsala, Sweden). The clonal hamster insulin-secretory cell line HIT-T15 (HIT cells) [17] was originally obtained from Dr. S.J. Ashcroft, Oxford, UK. The rat islets were cultured free-floating in medium RPMI 1640 containing 11.1 mM glucose and supplemented with 10% (vol/vol) FCS for 4–5 days before exposure to rIL-1β [19]. Growing HIT cells were trypsinized and subcultured in RPMI 1640 supplemented with 10% (vol/vol) FCS, as previously described [20].

Exposure of rat islets or HIT cells to rIL-1β and/or the different arginine analogues was performed for 6, 12 or 24 h in medium RPMI 1640, as described above. Following rIL-1β exposure, aliquots of the culture medium (90 μl) were deproteinized and the nitrite content determined as recently described [8].

For Northern blot analysis, poly(A)$^+$ was isolated from 10^7 HIT cells using a FastTrack mRNA isolation kit. Following isolation, the poly(A)$^+$ samples (5 μg) were electrophoresed on an agarose gel, transferred onto a nylon membrane [21], and hybridized to a ^{32}P-labelled cDNA probe coding for the inducible form of nitric oxide synthase, recently cloned from a murine macrophage cell line [15]. Hybridization and autoradiography were performed as previously described [21].

Data are presented as mean values ± S.E.M., and groups of data were compared using paired or unpaired Student's t-test as appropriate.

3. RESULTS AND DISCUSSION

As previously demonstrated [7–10], nitrite production by rat pancreatic islets was strongly induced by rIL-1β (Table I). The islet nitrite production in the absence of IL-1β was low, and was not affected by exposure to either 0.1, 1.0 or 2.0 mM N^G-monomethyl-L-arginine (CH_3-A) or N^G-nitro-L-arginine (NO_2-A) (data not shown). NO_2-A, in concentrations up to 2 mM, failed to significantly decrease nitrite production induced by rIL-1β (Table I). On the other hand, the cytokine-stimulated nitrite accumulation was dose-dependently inhibited by CH_3-A. When the effects of the N^G-inhibitors were assessed by recalculating the data as % of control (100% being nitrite production by rat islets exposed to rIL-1β alone), CH_3-A showed a significantly higher inhibitory action than NO_2-A (Fig. 1). It has been previously shown that NO_2-A displayed a striking selectivity for inhibition of brain and endothelial cell NO synthesis [22,23], while CH_3-A was mostly effective against NO production by stimulated macrophages [22,23]. Thus, the above described data suggested that the IL-1β-responsive form of NO synthase present in rat islets was similar to that observed in cytokine-activated macrophages.

The islets of Langerhans contains an heterogeneous cell population. Even considering that the prolonged preculture (4–5 days) before rIL-1β treatment may eliminate most of the non-endocrine cells in the islets, it can not be excluded that rIL-1β induces NO generation by non-endocrine islet cells. Indeed, it has been recently suggested that the main source of cytokine-stimulated NO generation in pancreatic islets are endothelial cells, fibroblasts and/or resident macrophages [10]. To further study this issue, we tested the effects of rIL-1β and/or different NO synthase inhibitors on insulin-producing HIT cells. This cell line has been previously shown to posses IL-1 surface receptors and to be sensitive to the inhibitory effects of the cytokine [3]. We observed that 1 ng/ml rIL-1β induced a 40–50% decrease in insulin accumulation into the medium of HIT cells after 12–24 h (data not shown). As observed with rat islets, rIL-1β induced a marked increase in nitrite accumulation over 12 h (Table II). This observation, together with recent data showing that rIL-1β also induces nitrite production by the rat insulinoma cell line RINm5F [24], suggest that insulin-producing cells can

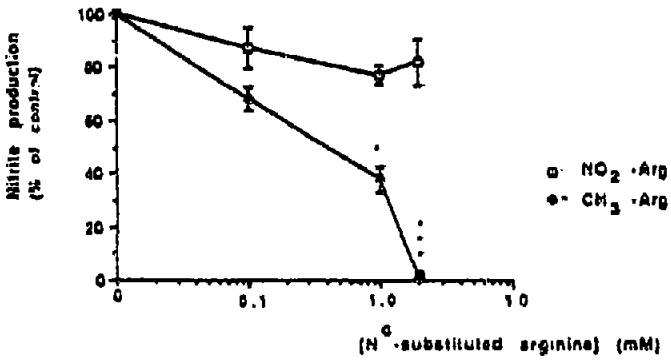

Fig. 1. Inhibition of nitrite accumulation by N^G-substituted arginine analogs in rat pancreatic islets exposed to rIL-1β. Islets were co-treated with rIL-1β and the indicated concentrations of arginine analogs, and the nitrite accumulation into the medium measured after 12 h. Results are means ± S.E.M. of 4–8 experiments. $^*P < 0.05$ and $^{***}P < 0.001$ when comparing to islets to NO_2-A.

be, at least in part, the source of islet NO generation following exposure to rIL-1β. The basal production of nitrite was not affected by NO_2-A or CH_3-A (data not shown), but both drugs inhibited in a dose-dependent way IL-1β-induced nitrite production (Table II). As observed in rat islets, CH_3-A was more potent than NO_2-A in inhibiting the effects of rIL-1β on nitrite accumulation (Fig. 2).

It has been previously suggested that rIL-1β-induced NO generation by pancreatic islets was secondary to activation of gene transcription [5,6]. Thus, we determined whether rIL-1β could induce gene expression of the macrophage form of NO synthase [15,16] in HIT cells. As shown in Fig. 3, 6 or 24 h exposure of HIT cells to the cytokine induced a marked expression of this mRNA. The transcript was 5.0 kb in length, similar to observations in activated macrophages [15]. The finding that after 4 h (not shown) or 6 h (Fig. 3) of rIL-1β

Table I

Effects of rIL-1β and/or two different arginine analogues on rat islet nitrite production

rIL-1β (ng/ml)	N^G-monomethyl-L-arginine (mM)	N^G-nitro-L-arginine (mM)	Nitrite production (pmol islet × 12 h)
0	0	0	0.72 ± 0.24***
1.0	0	0	9.06 ± 1.04
1.0	0	0.1	9.58 ± 1.27
1.0	0	1.0	8.46 ± 0.91
1.0	0	2.0	5.72 ± 1.09
1.0	0.1	0	7.40 ± 0.62
1.0	1.0	0	4.04 ± 0.30*
1.0	2.0	0	0.13 ± 0.08**

Rat islets in groups of 100 were cultured for 12 h with or without rIL-1β and different arginine analogues, and the medium subsequently taken for measurements of nitrite production. Results are expressed of mean ± S.E.M. of 4–8 experiments. * and ** denote $P < 0.01$ and $P < 0.001$ when comparing vs. islets exposed to rIL-1β alone.

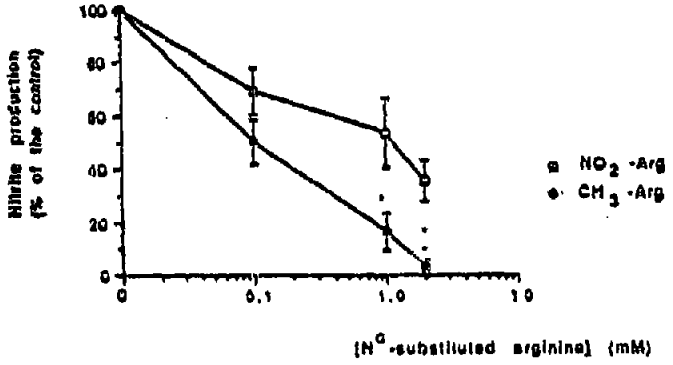

Fig. 2. Inhibition of nitrite accumulation by N^G-substituted arginine analogs in HIT cells exposed to rIL-1β. HIT cells were co-treated with rIL-1β and the indicated concentrations of arginine analogs, and the nitrite accumulation into the medium measured after 12 h. Results are means ± S.E.M. of 4–6 experiments. $^*P < 0.05$ and $^{**}P < 0.01$ when comparing to HIT cells exposed to NO_2-A.

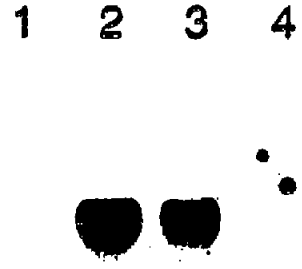

Fig. 3. Effects of rIL-1β exposure on mRNA contents of the macrophage form of nitric oxide synthase in HIT cells. HIT cells were exposed for 6 or 12 h to 1 ng/ml rIL-1β, or for 6 h to 100 ng/ml TPA and then harvested for poly(A)$^+$ extraction. Lane 1, control; lane 2, IL-1 6 h; lane 3, IL-1 24 h; lane 4, TPA 6 h. The figure is representative of 2 separate experiments.

exposure there was already induction of the mRNA, correlates well with the time-course of IL-1β-induced nitrite production in isolated rat islets [8]. Control islets, or islets exposed to a non-specific stimulus (12-*O*-tetradecanoylphorpbol 13-acetate, TPA), did not show detectable levels of the NO synthase mRNA. In another series of experiments (not shown), we also hybridized 5 μg of poly(A)$^+$ obtained from HIT cells or from rat brain tissue with a cDNA encoding the brain form of nitric oxyde synthase [17] (the cDNA probe was a kind gift of Dr. S.H. Snyder, John Hopkins Medical Institutions, MA, USA). In these experiments we observed a clear expression of the mRNA in the brain, but not in the HIT cells. As a whole, this and the above described data suggest that rIL-1β induces in insulin-producing cells the expression of a form of nitric oxyde synthase similar to that observed in activated macrophages. The subsequent NO accumulation will lead to impairment in β-cell mitochondrial function [11,12], and eventually to cell damage [2].

Table II

Effects of rIL-1β and/or two different arginine analogues on HIT-cells nitrite production

rIL-1β (ng/ml)	N^G-monomethyl-L-arginine (mM)	N^G-nitro-L-arginine (mM)	Nitrite production (pmol/10^6 islet × 12 h)
0	0	0	2.26 ± 0.29***
1.0	0	0	15.64 ± 2.02
1.0	0	0.1	10.18 ± 1.10*
1.0	0	1.0	6.96 ± 0.90**
1.0	0	2.0	4.68 ± 0.27**
1.0	0.1	0	7.27 ± 0.92**
1.0	1.0	0	2.79 ± 1.26***
1.0	2.0	0	0.52 ± 0.24***

HIT cells (10^4 cells) were cultured for 12 h with or without rIL-1β and different arginine analogues, and the medium subsequently taken for measurement of nitrite production. Results are expressed as means ± S.E.M. of 4–6 experiments. *, ** and *** denote respectively $P < 0.05$, $P < 0.01$ and $P < 0.001$ when comparing vs. HIT cells exposed to rIL-1β alone.

Recent reports suggest that inhibition of nitric oxide generation may prevent induction of streptozotocin-induced diabetes in mice [25,26]. The present characterisation of the NO synthase form present in pancreatic β-cells may raise new possibilities for an early treatment for insulin-dependent diabetes mellitus (IDDM). This could be achieved by the development of specific inhibitors of the macrophage/β-cell form on NO synthase, able to decrease cytokine-induced β-cell damage without interfering with blood pressure control or neurotransmission [24]. Clearly, extensive studies on the effects of currently available inhibitors of NO generation on the evolution of spontaneous IDDM in rodents must be performed, in order to validate this hypothesis.

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