donor. This also distinguishes direct NO toxicity from IL-1 toxicity. In the latter case, ionic fluxes were noted directly after the addition of the cytokine [15]. In our experiment [Ca\textsuperscript{2+}], started to increase progressively after 30 min until membrane rupture after approx. 3 h (not shown). This progressive calcium ion influx appeared to be closely associated with cell damage because nicotinamide, which has already been shown to prevent islet cell lysis [2], also inhibited cytosolic calcium accumulation (Figure 3b). We assumed that the slow calcium influx was a consequence, rather than the cause, of cell membrane damage. This was supported by our discovery that chelation of Ca\textsuperscript{2+} in the medium by EGTA did not prevent islet cell lysis (Figure 3c).

On the contrary, addition of the calcium ionophore, A23187, suppressed NO toxicity. This indicated that either A23187 or the resulting elevated intracellular calcium levels interfere with a critical step of the NO toxicity pathway in islet cells.

**Conclusions**

Direct toxicity of chemically-produced NO differs from IL-1-induced NO-dependent toxicity in many aspects: NO toxicity does not require de novo protein biosynthesis and NO synthases in islet cells do not contribute to cell death; the mechanism leading to cell lysis requires neither arachidonic acid metabolism nor calcium influx. Incubation of islet cells with the calcium ionophore A23187 prevents DNA damage and cell lysis.

This work was supported by the Deutsche Forschungsgemeinschaft, the Bundesminister für Gesundheit and the Minister für Wissenschaft und Forschung des Landes Nordrhein-Westfalen.


Received 11 October 1993
Insulin Secretion and B-Cell Dysfunction

Toxic effects in various endocrine disorders [4]. In particular, it has been postulated that cytokines can play a crucial role in the development of autoimmune insulin-dependent diabetes mellitus [5, 6]. High concentrations of cytokines, released by inflammatory cells infiltrating the pancreatic islet during the process of insulitis, would cause destruction of the pancreatic B-cells. In this context, most attention has so far been focused on interleukin (IL)-1, since this cytokine appears to have the most deleterious effects on B-cells [7, 8]. However, in the in vivo milieu, there are several cytokines that are most likely to be present simultaneously and additive or synergistic actions of the cytokines must be envisaged.

The exploration of the effects of various cytokines on the function of B-cells has turned out to be a cumbersome effort. Accumulated data show that the B-cell response to a certain cytokine can differ depending on the species of animal examined, method of islet cell preparation (e.g. isolated islets compared with monolayer cultures or purified B-cells), concentration and duration of the cytokine exposure and the functional state of the B-cells [7-11]. In addition, the action of some cytokines, such as interferon-γ (IFN-γ), seems to be species specific, whilst other cytokine preparations, such as human IL-1, are effective on B-cells obtained from different species. Against this background it is not surprising that some reports in the literature dealing with the effects of cytokines on B-cells might appear contradictory (see Table 1). We will describe the effects on B-cell function of some cytokines, namely IL-1β, tumour necrosis factor-α (TNF-α), IFN-γ, IL-6, and IL-10. Since these experiments have also provided insight into molecular and biochemical pathways operating in the B-cells, possible signal transduction mechanisms activated by the cytokines will be discussed.

**IL-1**

Acute exposure of B-cells to IL-1β in vitro stimulates insulin secretion [8] that corresponds to a hyperinsulinaemic effect induced by the cytokine after acute administration to rats [8, 26, 27]. It is conceivable that this represents a physiological response to an elevated concentration of the cytokine. However, it has been difficult to interpret the reported effects on B-cell function of repeated or prolonged administration of IL-1 to animals in vivo. This is probably due to the fact that the in vivo B-cell responsiveness to IL-1 can be modulated by several mechanisms as summarized in Figure I.

### Table 1

**Effects of different cytokines on insulin secretion by B-cells of various species**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Human</th>
<th>Rat</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>+ [12, 13], - [14]</td>
<td>- [7, 8]</td>
<td>- [15]</td>
</tr>
<tr>
<td>IL-6</td>
<td>N [16]</td>
<td>- [17, 18]</td>
<td>n.d.</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>- [14, 16], N [19]</td>
<td>- [20], + [21]</td>
<td>- [22], N*</td>
</tr>
<tr>
<td>TNF-α</td>
<td>- [14], N [16]</td>
<td>N [23, 24]</td>
<td>- [22], +*</td>
</tr>
</tbody>
</table>

* n.d. denotes not determined. *Cetkovic-Cvrlje and D. L. Eizirik, unpublished work.

**Figure 1**

Schematic representation of factors regulating pancreatic B-cell responsiveness to IL-1 at the IL-1 receptor level

When the type I receptor binds IL-1 on the extracellular (EC) side of the cell membrane, an intracellular (IC) signal is conveyed. The type I receptor can also be occupied by the IL-1 receptor antagonist protein (IL-1ra) and by type I receptor antibodies, thereby preventing signal transduction. Circulating IL-1 can be blocked by soluble IL-1 receptors (sol-IL-1-rec) and by IL-1 autoantibodies (IL-1-ab). If IL-1 binds to the type II receptor there is probably no signal generated in the B-cell. The type II receptor then may act as a decoy for IL-1.

The experimental data available suggest that the signal(s) induced by IL-1 is conveyed via an interaction between IL-1 and type 1 receptors on the B-cell surface. Indeed, all effects of IL-1 on B-cells can be blocked by the naturally occurring IL-1.
receptor antagonist protein (IL-1ra) [28]. IL-1ra occupies the IL-1 receptor without transducing any biological signal [29]. In rodent cells IL-1 ra appears to have a preferential affinity for the type I receptor. Moreover, IL-1 can be inactivated in the circulation by binding to IL-1 auto-antibodies [30] and soluble IL-1 receptors [31]. Experimentally generated monoclonal antibodies against the type I receptor also block IL-1 action on B-cells [32], although the presence of IL-1 receptor antibodies in vivo remains to be demonstrated. Finally, it was proposed recently that the expression of IL-1 type II receptors, either membrane bound or secreted, can act as a decoy for IL-1, thereby preventing binding to IL-1 type I receptors [33]. Type II receptors may be expressed on insulin-producing cells and there are experimental data indicating that the mRNA coding for this receptor is increased by IL-1β [34]. This may suggest that B-cells are equipped with a direct cellular negative feed-back mechanism for regulating B-cell sensitivity to the cytokine.

Compared with the available knowledge on the inhibitory action of IL-1 on B-cells (see below), information on the mechanism(s) underlying the acute stimulatory action of IL-1 of insulin secretion is scarce. Certain discoveries, however, suggest that the stimulatory effect requires IL-1 receptor binding to islet cells, expression of unidentified genes and a stimulation of the mitochondrial glucose metabolism [8]. The subsequent inhibitory action, and perhaps the B-cell toxic action, of the cytokine involves a sequence of key events (Figure 2), which probably differs in several aspects from the events mediating early B-cell stimulation. In the rat B-cell, the inhibitory action comprises receptor binding, tyrosine phosphorylation, proteolytic activity, enhanced gene expression (especially of nitric oxide synthase), increased nitric oxide production, binding of nitric oxide to iron-sulphur-containing enzymes (in particular aconitase) of the Krebs' cycle in mitochondria, impaired oxidation of glucose and, finally, defective ATP generation. Eventually this state will cause an inhibition of crucial energy-dependent

**Figure 2**

**Crucial events leading to inhibition of B-cell function by IL-1 in the rat**

IL-1 binding to a receptor is followed by tyrosine phosphorylation and proteolytic activity. This leads to transcription of nitric oxide synthase mRNA and increased NO formation. NO will bind to iron–sulphur (FeS) containing enzymes, in particular aconitase in the mitochondria, which will impair ATP generation. As a result, energy-dependent processes in the B-cell, such as insulin release, are blocked.
processes in the B-cell, such as the synthesis and release of insulin, and may lead to death of the cell [8, 35-37]. It should be noted that the effects of IL-1 on the human pancreatic B-cell might differ in many respects to those outlined for the rat. Thus, in human islets, IL-1 alone does not induce nitric oxide production [13, 38].

TNF-α
TNF-α only fails to inhibit insulin secretion in rat and human B-cells, although some studies report inhibition of murine B-cells (Table 1). However, TNF-α in combination with IL-1 has an additive inhibitory effect in rat B-cells [23, 24], which probably reflects a potentiated inhibition of the glucose metabolism.

IL-6
In rat B-cells, IL-6 stimulates insulin secretion at low concentrations but, after prolonged exposure to a high concentration of the cytokine, the insulin secretion is inhibited [17, 18]. The latter effect appears to be independent of any inhibition of the mitochondrial function, thus ruling out the possibility that IL-6 is a second messenger for IL-1 in B-cells. Human B-cells were not affected in vitro by exposure to IL-6 alone [16].

INF-γ
The experiments with IFN-γ have perhaps given the least consistent results (Table 1). As noted above, species specificity of this cytokine seems to be of great importance. The mechanism by which IFN-γ affects B-cell function is unclear but, in human [16, 38] and rat (J. Sternesjö, K. Bendtzen and S. Sandler, unpublished work) pancreatic islets, IFN-γ effects are not related to nitric oxide generation.

IL-10
IL-10 has been reported to counteract the effects of IL-1 in macrophages, owing to a blockage of nitric oxide generation [39]. However, in experiments with rat B-cells, IL-10 failed to protect against nitric oxide production and inhibition of insulin secretion induced by IL-1 [25]. On the other hand, IL-10 supplementation alone promoted an increase in the medium insulin accumulation, and the islet insulin content in culture, without exhibiting any clear inhibitory or toxic actions. The stimulatory effect seems to be dissociated from the islet glucose metabolism.

Cytokine combinations
Using combinations of cytokines, both B-cell inhibitory actions and cytotoxic actions have been reported regularly [14, 19, 20, 23, 38, 40]. Thus several cytokine combinations seem to act in an additive or synergistic manner. In particular, the presence of IFN-γ appears to contribute to such an effect. When IL-1β has been added at high concentrations in combination with other cytokines it can, however, be difficult to distinguish how much this particular cytokine is contributing to the observed effects. One important observation, in relation to insulin-dependent diabetes mellitus, is that combinations of cytokines [16, 38] can be inhibitory and even cytotoxic to human B-cells [14].

More detailed studies on the actions of cytokine combinations, on both human and rodent B-cells, are clearly required. It is warranted that such investigations aim at elucidating cytokine effects on gene expression, e.g. nitric oxide synthase, putative genes involved in cell protection and genes involved in the regulation of insulin secretion and growth of the B-cell.

The authors’ research cited in this review was supported by grants from the Swedish Medical Research Council (12P-1051, 12P-10739, 12X-109, 12X-9237, and 12X-9886), the Swedish Diabetes Association, the Juvenile Diabetes Foundation International, the Nordic Insulin Fund, CFN, the Swedish Society of Medicine, the Family Ernfors Foundation and the Hoechst Diabetes Fund.

Effects of cytokines and nitric oxide donors on insulin secretion, cyclic GMP and DNA damage: relation to nitric oxide production

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Introduction

We have proposed that the effects of cytokines, such as interleukin-1ß (IL-1ß) and tumour necrosis factor ß (TNF ß), on islet insulin secretion involve the generation of nitric oxide [1]. The extent to which nitric oxide can reproduce cytokine effects, and particularly whether it is cytotoxic for B-cells, still remains an open question. In this article, we discuss the use of four different nitric oxide donor compounds and the mechanism and relative potency of their nitric oxide generation. We have measured the effects on insulin secretion, cell signalling via cyclic nucleotides and the DNA-damaging effects of donors versus the effects of IL-1ß, on rat islets of Langerhans and on two insulin-containing cell lines. We discovered that the effects of IL-

Abbreviations used: IL-1ß, interleukin-1ß; TNF ß, tumour necrosis factor ß; t-NAME, Nω-nitro-1-arginine methyl ester; t-NMMA, Nω-monomethyl-l-arginine; SNAP, S-nitroso penicillamine; SIN-1, 3-morpholinosydnonimine; SOD, superoxide dismutase; GSNO, S-nitrosoglutathione.

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