Reversibility of interleukin-1 β -induced islet destruction and dysfunction by the inhibition of nitric oxide synthase

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We have examined the reversibility of NO-mediated islet dysfunction and destruction induced by interleukin-1 β (IL-1 β). Previous studies have shown that pretreatment of islets for 18 h with IL-1 β results in an inhibition of glucose-stimulated insulin secretion that requires 4 days incubation in the absence of IL-1 β to restore islet secretory function. In this study we use a sequential experimental design in which islets are first exposed to IL-1 β for 18 h, and then treated with the NO synthase inhibitor *N*^G-monomethyl-L-arginine (NMMA). Insulin secretion is inhibited by 98 % after the 18 h incubation with IL-1 β , and this inhibition is reversed in a time-dependent fashion by NMMA, with complete recovery of insulin secretion observed 8 h after the inhibition of NO synthase. Inhibition of NO synthase also restores IL-1 β -induced inhibition of mitochondrial aconitase activity in a time-dependent fashion that mimics the recovery of glucose-stimulated insulin secretion by islets. Ferrous iron and the reducing agents cysteine and thiosulphate accelerate the rate of recovery of insulin secretion, and ferrous iron and thiosulphate stimulate the recovery of islet aconitase activity, suggesting that iron–sulphur-centre reconstitution may be involved in the recovery process.

The recovery process also appears to require mRNA transcription, because the transcriptional inhibitor actinomycin D prevents the recovery of insulin secretion by islets after the inhibition of NO synthase. Although IL-1 β induces the co-expression of NO synthase and cyclo-oxygenase by islets, cyclo-oxygenase is not involved in the recovery of glucose-stimulated insulin secretion. Inhibition of NO synthase also prevents IL-1 β -induced islet destruction, which otherwise occurs during a 96 h continuous exposure to this cytokine. The destructive effects of IL-1 β on islet viability are prevented if NMMA is added to islet cultures during the first 24 h of exposure to IL-1 β , but islet destruction is not prevented if NMMA is added after the first 48 h exposure to IL-1 β . These results show that IL-1 β -induced islet dysfunction is reversed by the inhibition of NO synthase, that recovery of insulin secretion is stimulated by iron and reducing agents, and that the recovery process appears to require mRNA transcription. We also show that it is possible to rescue islets from the destructive effects of IL-1 β if NO synthase is inhibited early after its induction.

INTRODUCTION

NO has recently been implicated as an effector molecule which mediates both cytokine-induced islet dysfunction and destruction, and activates macrophage-induced islet-cell lysis (for reviews see [1–3]). Treatment of islets for 8–24 h with interleukin-1 β (IL-1 β) results in a potent inhibition of glucose-stimulated insulin secretion [4–7]. This effect is prevented by *N*^G-monomethyl-L-arginine (NMMA), *N*^G-nitro-L-arginine methyl ester and aminoguanidine, inhibitors of NO synthase [8–11]. NMMA also prevents activated macrophage-mediated islet-cell destruction [12], and NMMA and aminoguanidine prevent IL-1 β -induced islet destruction [13]. The inhibitory effects of IL-1 β on islet function and viability are the result of NO overproduction by the inducible isoform of NO synthase. Cycloheximide prevents IL-1 β -induced nitrite formation and cyclic GMP accumulation by islets [14] and purified β -cells [15], and IL-1 β -induced iron–nitrosyl complex-formation by islets and RINm5F cells [9,11,14–16]. Recently, Northern-blot analysis has been used to demonstrate the induction of NO synthase mRNA after treatment of hamster insulinoma cells (HIT) with IL-1 β [17,18].

NO mediates the inhibitory effects of IL-1 β on islet function by the destruction of iron–sulphur centres of iron-containing enzymes. E.p.r. spectroscopy has provided initial evidence implicating destruction of iron–sulphur centres as a mechanism by which IL-1 β -induced NO formation results in the inhibition of insulin secretion by islets. IL-1 β induces the formation of a *g* = 2.04 iron–nitrosyl feature in both rat and human islets, and

the formation of this e.p.r. feature is prevented by NMMA, aminoguanidine and cycloheximide [9,11,14–16]. This *g* = 2.04 e.p.r. feature is characteristic of NO complexed with iron–sulphur centres and has been demonstrated in activated macrophages and their tumour target cells [19–22]. The formation of these complexes correlates with an inhibition of the mitochondrial iron–sulphur-containing enzyme aconitase and the electron-transport-chain complexes I and II [23–26]. NO has been shown to inhibit mitochondrial aconitase activity of rat islets and the rat insulinoma cell line RINm5F following IL-1 β treatment, and this effect is prevented by NMMA [10,15].

Although these studies have demonstrated that NO overproduction either by islets or by activated macrophages is destructive to islet function and viability, it is not known whether these effects are reversible. In this report we have investigated the reversibility of NO-mediated islet dysfunction and destruction and present evidence which suggests that reconstitution of iron–sulphur centres of iron-containing enzymes is a key component in recovery from NO-mediated islet damage. We also present evidence which suggests that mRNA transcription is involved in the recovery of insulin secretion by islets.

EXPERIMENTAL

Materials

Collagenase type P was obtained from Boehringer–Mannheim (Indianapolis, IN, U.S.A.). CMRL-1066 tissue-culture media, heat-inactivated fetal-bovine serum, penicillin G (sodium salt)

and streptomycin were from Gibco Laboratories (Grand Island, NY, U.S.A.). IL-1 β was obtained from Cistron Biotechnology (Pine Brook, NJ, U.S.A.). NMMA was obtained from Calbiochem (San Diego, CA, U.S.A.), and aminoguanidine hemisulphate and actinomycin D were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other reagents were from commercially available sources.

Islet isolation, culture, and exposure to IL-1 β

Islets were isolated from male Sprague–Dawley rats (Sasco Inc., O'Fallon, MO, U.S.A.; 200–250 g body wt.) by collagenase digestion as described previously [27]. After isolation, islets were cultured overnight in complete CMRL-1066 culture media (CMRL-1066 supplemented with 2 mM glutamine, 10% heat-inactivated fetal-bovine serum, 100 units/ml penicillin and 100 μ g/ml streptomycin) containing 0.3 mM L-arginine. Experiments were initiated by addition of 5 units/ml IL-1 β (5.7 pM), and islets were incubated at 37 °C under an atmosphere of air/CO₂ (19:1) for 18 h. After exposure to IL-1 β , islets were incubated with NMMA (see the Figure legends for duration of incubation) to examine recovery of insulin secretion.

Glucose-stimulated insulin secretion

Islets (100–200/ml of complete CMRL-1066) were cultured for 18 h with 5 units/ml IL-1 β . After culture, 0.5 mM NMMA was added and the islets were incubated for the indicated periods of time without the removal of IL-1 β . After this treatment the islets were isolated and washed three times in Krebs–Ringer bicarbonate buffer (KRB: 25 mM Hepes, 115 mM NaCl, 24 mM NaHCO₃, 5 mM KCl, 1 mM MgCl₂, 2.5 mM CaCl₂, pH 7.4) containing 3 mM glucose and 0.1% BSA. Groups of 20 islets were counted into 10 mm $\times$ 75 mm borosilicate tubes and preincubated for 30 min at 37 °C with shaking in 200 μ l of the same buffer containing 0.5 mM NMMA. The preincubation buffer was removed and glucose-stimulated insulin secretion was initiated by the addition of 200 μ l of KRB containing either 3 mM or 20 mM glucose and 0.5 mM NMMA. Islets were then incubated for 30 min, the incubation buffer was removed and insulin content was determined by radioimmunoassay [28].

Aconitase activity

For the measurement of aconitase activity, islets isolated from 18 male Sprague–Dawley rats were dispersed into single cells as described previously [15]. The islet cells were then cultured for 18 h in complete CMRL-1066 in the presence or absence of 5 units/ml IL-1 β . NMMA (0.5 mM) was added and the islets were incubated for an additional period of time as stated in the Figure legends. After culture, islet cells were isolated by centrifugation and mitochondrial aconitase activity was determined as described previously [15]. A unit of aconitase activity is defined as 1 pmol of NADPH formed/min per mg of islet protein.

Nitrite formation

Nitrite formation in the culture media after incubations was determined by mixing 50 μ l of culture media with 50 μ l of Griess reagent [29]. The A₅₄₀ was measured with a Titertek Multiskan

MCC/340 plate reader. The lower limit of sensitivity for the Griess assay is 1–5 μ M nitrite.

Islet viability

Islets (25/ml of complete CMRL-1066) were cultured for a total period of 96 h in the presence or absence of 5 units/ml IL-1 β . Islet viability was determined by phase-contrast microscopic analysis using previously described parameters [30]. Islet degeneration is indicated by the loss of islet integrity. Inhibition of IL-1 β -induced islet destruction was determined by the addition of 0.5 mM NMMA at various times after the addition of IL-1 β , followed by continuous exposure of islets to both IL-1 β and NMMA for a total of 96 h. After incubation, islet viability was assessed by phase-contrast microscopy.

Statistics

Statistical comparisons were made between groups using a one-way analysis of variance. Significant differences (* P < 0.05; ** P < 0.01) were evaluated with a *post hoc* analysis using Fisher's PLSD test, as indicated.

RESULTS

Time-dependent reversibility of IL-1 β -induced inhibition of insulin secretion by islets

To examine the recovery of insulin secretion, a sequential experimental design was employed. In these experiments islets were pretreated for 18 h with 5 units/ml IL-1 β in CMRL-1066 tissue-culture medium, 0.5 mM NMMA was then added to inhibit NO production, and the islets were incubated for the indicated time shown on the abscissa. Islets were washed and glucose-stimulated insulin secretion was determined in the presence of 0.5 mM NMMA. As illustrated in Figure 1, treatment of islets for 18 h with IL-1 β results in a potent inhibition of glucose-stimulated insulin secretion. NMMA completely restores the insulin-secretory ability of islets within 8 h. This is a striking result, because 4 days are required to restore completely the

Figure 1 Effects of NMMA on the reversibility of IL-1 β -induced inhibition of glucose-stimulated insulin secretion by islets

Islets were pretreated for 18 h with 5 units/ml IL-1 β , and then 0.5 mM NMMA was added and the islets were incubated for the indicated periods of time as shown on the abscissa. The islets were then isolated and glucose-stimulated insulin secretion was examined as described in the Experimental section. Results are means $\pm$ S.E.M. of 3 individual experiments containing 4 replicates per condition. Also shown is glucose-stimulated insulin secretion from control untreated islets.

Figure 2 Reversibility of IL-1 β -induced inhibition of mitochondrial islet-cell aconitase activity

Islet cells were pretreated for 18 h with 5 units/ml IL-1 β . NMMA (0.5 mM) was added and the islet cells were incubated for the indicated periods of time as shown on the abscissa. Islet cells were then isolated and mitochondrial aconitase activity was determined as described in the Experimental section. Results are means $\pm$ S.E.M. of 3 individual experiments. Also shown is aconitase activity of control untreated islet cells.

insulin-secretory ability of islets when IL-1 β is simply removed from the culture medium after an 18 h exposure [6,7].

Recovery of islet aconitase activity after exposure to IL-1 β

The role of aconitase, a mitochondrial iron-sulphur-containing tricarboxylic-acid-cycle enzyme, in the recovery of insulin secretion was examined by using the experimental protocol outlined for Figure 1. Figure 2 demonstrates that pretreatment of dispersed islet cells for 18 h with IL-1 β results in $\sim 75\%$ inhibition of mitochondrial aconitase activity. Complete recovery of aconitase activity occurs in a time-dependent manner, similar to the time-dependent recovery of insulin secretion (Figure 1) after treatment of islets with NMMA. Linear-regression analysis for both insulin secretion (Figure 1) and aconitase activity (Figure 2) reveals a fractional rate of recovery for insulin secretion of

9.3%/h and for aconitase activity of 8.6%/h. The correlation coefficients (r^2) of the regression analysis for both insulin secretion and aconitase activity were 0.93 and 0.95 respectively.

Role of iron (Fe $^{2+}$) and reducing agents on the recovery of insulin secretion and aconitase activity

The effects of iron alone or in combination with reducing agents (thiosulphate and cysteine) on the rate of recovery of insulin secretion and mitochondrial aconitase activity are shown in Table 1. The addition of FeSO $_4$ further stimulates NMMA-induced recovery of insulin secretion by 23%. The addition of FeSO $_4$ with reducing agents, 1 mM cysteine or 3 mM thiosulphate, further augments the recovery of insulin secretion by $\sim 30\text{--}35\%$ above the level of recovery with NMMA alone. FeSO $_4$, cysteine or thiosulphate exert no effect on IL-1 β -induced inhibition of insulin secretion, or on glucose-stimulated insulin secretion (results not shown). Table 1 also demonstrates that pretreatment of islets for 18 h with IL-1 β followed by an additional 3 h exposure to the combination of 50 μ M FeSO $_4$, 3 mM thiosulphate and 0.5 mM NMMA further stimulates the recovery of mitochondrial aconitase activity by 20% above the level of recovery observed by NMMA treatment. FeSO $_4$ alone also stimulates the recovery of aconitase activity, but this effect did not achieve statistical significance.

Effects of actinomycin D on the recovery of insulin secretion by islets

Recovery of insulin secretion and aconitase activity may involve the enzymic or non-enzymic reconstitution of iron-sulphur centres, or NO or IL-1 β may induce the expression of islet proteins that mediate the recovery process. As shown in Table 2, glucose-stimulated insulin secretion is completely inhibited in islets incubated for 18 h with IL-1 β alone, and NMMA restores 33% of the insulin-secretory capacity of islets after a 3 h incubation. Treatment of islets with 1 μ M actinomycin D attenuates NMMA-induced recovery of insulin secretion when added to islet cultures 5 h after addition of IL-1 β . Actinomycin D when added simultaneously with IL-1 β completely prevents IL-1 β -induced inhibition of glucose-stimulated insulin secretion. This result demonstrates that the inhibition of aconitase expression

Table 1 Effects of FeSO $_4$ and reducing agents on the reversibility of IL-1 β -induced inhibition of glucose-stimulated insulin secretion and mitochondrial aconitase activity

Islets (200/ml) were pretreated for 18 h with 5 units/ml IL-1 β and then incubated for a further 3 h with 0.5 mM NMMA, 50 μ M FeSO $_4$, 1 mM cysteine or 3 mM thiosulphate as indicated. Islets were then isolated and glucose-stimulated insulin secretion and aconitase activity were determined. Results are means $\pm$ S.E.M. of 3–4 individual experiments containing 3–4 replicates/condition. Differences between the means of recovery as compared with NMMA-induced recovery were determined by ANOVA: * $P < 0.05$; ** $P < 0.01$.

Conditions	Insulin secretion (ng/30 min per 20 islets)			Aconitase activity	
	Glucose		Recovery (%)	(nmol/min per mg of protein)	Recovery (%)
	3 mM	20 mM			
Control	0.8 $\pm$ 0.2	14.1 $\pm$ 1.2	—	11.7 $\pm$ 0.6	—
IL-1 β	2.2 $\pm$ 0.4	2.5 $\pm$ 0.3	—	3.2 $\pm$ 0.2	—
IL-1 β + NMMA	2.7 $\pm$ 0.3	8.2 $\pm$ 0.9	48	7.2 $\pm$ 0.2	47
IL-1 β + NMMA + FeSO $_4$	3.0 $\pm$ 0.3	10.7 $\pm$ 0.8*	71	7.8 $\pm$ 0.3	54
IL-1 β + NMMA + FeSO $_4$ + cysteine	2.4 $\pm$ 0.4	11.7 $\pm$ 0.9**	79	—	—
IL-1 β + NMMA + FeSO $_4$ + thiosulphate	2.3 $\pm$ 0.4	12.2 $\pm$ 1.3**	84	8.9 $\pm$ 0.4**	67

Table 2 Effects of actinomycin D and indomethacin on NMMA-stimulated recovery of IL-1 β -induced inhibition of insulin secretion by rat islets

Islets (200) were pretreated for 18 h with 5 units/ml IL-1 β . At the initial time of IL-1 β exposure or at 5 h after IL-1 β addition, 1 μ M actinomycin D (Act D) or 10 μ M indomethacin was added and the islets were cultured for an additional 13 h. After the 18 h treatment period islets were incubated for an additional 3 h with 0.5 mM NMMA. Islets were then isolated and glucose-stimulated insulin secretion was examined. Nitrite formation was measured in the incubation media. Results are means $\pm$ S.E.M. of 3–4 individual experiments containing 3–4 replicates per condition. Difference between the mean of recovery with actinomycin D as compared with NMMA-induced recovery was determined by ANOVA: ** $P < 0.01$.

Time of addition (h)...	Conditions			Insulin secretion (ng/30 min per 20 islets)			Nitrite formation (pmol/islet)
				Glucose		Recovery (%)	
	0	5	18	3 mM	20 mM		
	Control			0.9±0.3	17.1±1.3	—	9.3±0.3
	IL-1β	—	—	2.4±0.5	2.4±0.5	—	32.9±0.4
	IL-1β	—	NMMA	2.5±0.5	7.9±0.4	33	23.8±0.2
	IL-1β	Act D	NMMA	1.5±0.4	3.1±0.8**	10	24.5±0.5
	IL-1β+Act D	—	—	1.3±0.3	15.8±0.9	—	11.2±0.2
	Control			1.8±0.4	22.4±1.7	—	3.4±0.8
	Indomethacin	—	—	1.2±0.3	28.5±1.4	—	2.9±0.9
	IL-1β	—	—	0.8±0.2	0.9±0.2	—	28.5±1.8
	IL-1β	—	NMMA	1.2±0.3	7.4±0.5	30	19.3±1.1
	IL-1β+Indomethacin	—	—	1.8±0.5	1.2±0.2	—	33.1±1.9
	IL-1β	Indomethacin	NMMA	1.4±0.3	6.8±0.5	26	19.8±1.6

(a)

Figure 3 Reversibility of IL-1 β -induced islet destruction by NMMA

(a) Islets were treated for 96 h in the absence or presence of 5 units/ml IL-1 β . After culture, photographs were taken by phase-contrast microscopy. Original magnification was $\times 20$. (b) Islets were treated for 96 h with 5 units/ml IL-1 β . At the indicated times (abscissa) 0.5 mM NMMA was added (IL-1 β was not removed) and the islets were then cultured for the remaining time totalling 96 h. Islet degeneration was determined as described in the Experimental section. Results are means $\pm$ S.E.M. of 3 individual experiments containing 25 islets per condition.

(or the turnover of enzymes required for insulin secretion) cannot explain the inhibitory effects of actinomycin D on the recovery of insulin secretion, because under these conditions actinomycin D would inhibit protein turnover.

IL-1 β -induced formation of nitrite, an oxidative product of NO, is not affected by the incubation of islets with actinomycin D when added 5 h after the addition of IL-1 β , whereas actinomycin D completely inhibits nitrite formation when added to islet cultures at the same time as IL-1 β (Table 2). Complete inhibition of nitrite formation by actinomycin D demonstrates that IL-1 β -induced expression of NO synthase is prevented, and the lack of an actinomycin D effect on nitrite levels when added 5 h after the addition of IL-1 β shows that the inhibitory effects of actinomycin D on the recovery of insulin secretion are not attributable to suppression of NO synthase expression.

Effects of cyclo-oxygenase on recovery on insulin secretion

IL-1 β has been shown to induce the expression of a number of proteins, and we have recently demonstrated that IL-1 β induces the co-expression of both cyclo-oxygenase and NO synthase by islets [36]. The effects of the cyclo-oxygenase inhibitor indomethacin on NMMA-induced recovery of insulin secretion are shown in Table 2. Indomethacin has no effect on the recovery of glucose-stimulated insulin secretion, the inhibitory effects of IL-1 β on insulin secretion, or the formation of nitrite by islets, suggesting that cyclo-oxygenase activity is not involved in the recovery of glucose-stimulated insulin secretion by islets.

Reversibility of IL-1 β -induced islet degeneration

The reversibility of IL-1 β -induced islet destruction was examined by incubating islets for 96 h with IL-1 β , or NMMA was added at the indicated times after IL-1 β addition and the islets were cultured for a total of 96 h. As shown in Figures 3(a) and 3(b), incubation of islets for 96 h with IL-1 β results in significant islet degeneration. Incubation of islets for 96 h with both IL-1 β and NMMA or aminoguanidine completely prevents the destructive effects of IL-1 β on islet degeneration [13]. The addition of

NMMA 24 h after IL-1 β treatment prevents the destructive effects of IL-1 β on islet degeneration, whereas NMMA added 48 or 72 h after the addition of IL-1 β does not prevent IL-1 β -induced degeneration of islets.

DISCUSSION

It has been previously shown that treatment of islets for 24 h with IL-1 β results in complete inhibition of glucose-stimulated insulin secretion and that recovery of insulin secretion by islets requires a 4–6-day incubation period after removal of the cytokine [6,7]. In this paper, we show that glucose-stimulated insulin secretion is completely restored in IL-1 β -treated islets in a time-dependent fashion within 8 h after the inhibition of NO synthase with NMMA. NO-mediated destruction of iron–sulphur centres of mitochondrial iron-containing enzymes appears to be the mechanism of IL-1 β -induced inhibition of insulin secretion by islets. This is reflected by IL-1 β -induced inhibition of glucose oxidation by islets and purified β -cells, and by the inhibition of mitochondrial aconitase activity of islets and RINm5F insulinoma cells [10,14,15,31]. All of these effects are prevented by NMMA [10,14,15]. Aconitase is exquisitely sensitive to NO-induced damage, because it contains an iron–sulphur centre required for enzymic activity. In this study, the recovery of mitochondrial aconitase activity in IL-1 β -treated islets occurs with a time course that is temporally identical with the recovery of insulin secretion. This finding provides further evidence implicating destruction of iron–sulphur centres of mitochondrial aconitase (as well as other iron–sulphur-containing mitochondrial enzymes) by NO as a mechanism by which IL-1 β inhibits islet function. These results also demonstrate that recovery of insulin secretion coincides with the recovery of islet oxidative metabolism.

The time-dependent recovery of mitochondrial aconitase activity in islets after the inhibition of NO synthase is similar to the recovery of aconitase activity of hepatocytes after treatment with authentic NO. In these studies, treatment of rat hepatocytes with a 20% solution of NO for 5 min resulted in 80% inhibition of mitochondrial aconitase activity [32,33] that was reversed within 6 h after the removal of NO [33]. The recovery of aconitase activity appears to result from reconstitution of iron–sulphur centres, since iron and reducing agents stimulate the recovery of aconitase activity in tumour cells exposed to activated macrophages [24] under conditions in which these cells produce NO [23]. In this study we have investigated the effects of FeSO₄ and the reducing agents cysteine and thiosulphate on the rate of recovery of both insulin secretion and aconitase activity of islets, which occurs after the inhibition of NO synthase. FeSO₄ alone stimulates NMMA-induced recovery of insulin secretion by ~25%, and either cysteine or thiosulphate with FeSO₄ further stimulates recovery of insulin secretion (~30–35%). FeSO₄ and thiosulphate also stimulate the recovery of islet mitochondrial aconitase activity; however, the level of recovery of aconitase activity was lower than that observed for the recovery of insulin secretion. This result suggests that NO may inhibit other metabolic enzymes required for insulin secretion, and that the recovery of insulin secretion may reflect a combination of recovery of aconitase and other NO-sensitive enzymes. NO-mediated inhibition of the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase has recently been shown in RINm5F cells [34].

The cellular mechanisms responsible for the recovery of islet function also appear to require mRNA transcription, and potentially new protein expression, because actinomycin D prevents NMMA-induced recovery of insulin secretion when added 5 h after the addition of IL-1 β (or under conditions in

which actinomycin D has no effect on NO synthase expression). The effects of actinomycin D on recovery are not associated with an inhibition of total protein turnover, because actinomycin D completely prevents IL-1 β -induced inhibition of insulin secretion when added at the same time as the cytokine. Also, IL-1 β stimulates the expression of inducible cyclo-oxygenase [35,36], but this enzymic activity is not involved in the recovery of insulin secretion by islets.

Inhibition of recovery by actinomycin D suggests that constitutively expressed proteins with a short half-life may participate in islet recovery; however, we favour the possibility that NO may directly regulate iron metabolism. Cytokine-induced NO production has been shown to stimulate iron release from cells [23,37] and to mobilize iron from ferritin [38]. Recently interferon- γ and lipopolysaccharide have been shown to induce the accumulation of ferritin mRNA in J774 cells independently of NO production, and NO appears to influence the translation rather than transcription of ferritin and transferrin receptor mRNA [39,40]. Ferritin and transferrin receptor mRNA translation is regulated by the binding of the iron-regulatory factor (IRF) to specific sequences of ferritin and transferrin receptor mRNA (iron-response elements, IREs). IRF contains aconitase activity which is sensitive to NO. Inhibition of IRF aconitase activity results in binding of IRF to specific sequences of ferritin and transferrin receptor mRNA, and the inhibition of translation. The ability of actinomycin D to prevent recovery of insulin secretion may be due to the inhibition of ferritin mRNA transcription which would decrease the cellular pool of ferritin mRNA. We are currently investigating this potential mechanism, as it relates to recovery of insulin secretion by islets.

Insulin-dependent diabetes mellitus is an autoimmune disease that over time results in selective destruction of insulin-secreting β -cells. Treatment of rat islets for 96 h with IL-1 β results in islet degeneration that is completely prevented by NO synthase inhibitors NMMA and aminoguanidine [13]. In this study we demonstrate that a 'window' of time exists where the destructive effects of IL-1 β on viability can be prevented. Islet destruction is prevented if NO synthase is inhibited within 48 h after its induction by IL-1 β . Inhibition of NO synthase 48 h after its induction, however, does not prevent islet destruction, suggesting that islets are irreversibly injured after a 48 h exposure to IL-1 β . These results suggest that it may be possible to reverse the time-dependent destructive effects of NO on islet function and viability by early intervention with inhibitors of NO synthase.

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