Role of Cyclooxygenase-2 in Cytokine-induced β-cell Dysfunction and Damage by Isolated Rat and Human Islets

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Abbreviations: Interleukin-1β (IL-1β); interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), nitric oxide (NO), inducible nitric oxide synthase (iNOS), cyclooxygenase (COX), prostaglandin E2 (PGE2)

Running Title: PGE2 does not mediate cytokine-induced islet dysfunction and damage.
Summary:
Type I diabetes mellitus is an autoimmune disease characterized by the selective destruction of the insulin secreting β-cell found in pancreatic islets of Langerhans. Cytokines such as interleukin-1 (IL-1), interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) mediate β-cell dysfunction and islet degeneration, in part, through the induction of the inducible isoform of nitric oxide synthase (iNOS) and production of nitric oxide (NO) by β-cells. Cytokines also stimulate the expression of the inducible isoform of cyclooxygenase, COX-2, and the production of prostaglandin E2 (PGE2) by rat and human islets; however, the role of increased COX-2 expression and PGE2 production in mediating cytokine-induced inhibition of islet metabolic function and viability is incompletely characterized. In this study, we show that treatment of rat islets with IL-1β or human islets with a cytokine mixture containing IL-1β + IFN-γ +/- TNF-α, stimulates COX-2 expression and PGE2 formation in a time-dependent manner. Co-incubation of rat and human islets with selective COX-2 inhibitors, SC-58236 or Celecoxib, respectively, attenuates cytokine-induced PGE2 formation; however, these inhibitors fail to prevent cytokine-mediated inhibition of insulin secretion or islet degeneration. These findings indicate that selective inhibition of COX-2 activity does not protect rat and human islets from cytokine-induced β-cell dysfunction and islet degeneration and furthermore, that islet production of PGE2 does not mediate these inhibitory and destructive effects.
**Introduction:**

Type I diabetes mellitus is an autoimmune disease characterized by the selective destruction of insulin-secreting β-cells found in pancreatic islets of Langerhans. Although the initiation events leading to the development of disease are not well-characterized, inflammatory cytokines and the free radical, nitric oxide (NO), appear to play an important role. We and others have shown that treatment of isolated rat and human islets with cytokines such as interleukin-1 (IL-1), interferon-γ (IFN-γ) and TNF-α results in the inhibition of glucose-stimulated insulin secretion and islet degeneration. The inhibitory and destructive effects of cytokines on β-cell function and islet viability are mediated, in part, through the expression of the inducible form of nitric oxide synthase (iNOS) and increased production of NO by β-cells (1-5). NO inhibits insulin secretion by targeting iron-sulfur containing enzymes such as aconitase and electron-transporting chain complexes I and II resulting in decreased oxidative phosphorylation and ATP production (6-9). Evidence in support of a role for nitric oxide in mediating cytokine-induced islet damage includes the protective actions of iNOS inhibitors aminoguanidine (AG) or N\(^\text{G}\)-monomethyl L-arginine (L-NMMA) on cytokine-induced inhibition of insulin secretion and islet degeneration ((1) (10) (11) (4)), and the lack of an inhibitory action of cytokines on glucose-stimulated insulin secretion in islets isolated from iNOS-deficient mice ((12)). These results suggest that inflammatory cytokines mediate islet dysfunction and degeneration by inducing iNOS expression and NO formation by β-cells.

Three isoforms of cyclooxygenase (COX) have been characterized to date, two constitutive isoforms, COX-1 and COX-3, and an inducible isoform of the enzyme, COX-2 ((13,14)). The product of the same COX-1 gene, COX-1 is expressed in most tissues whereas splice variant COX-3 appears to be localized primarily to neuronal tissue (13). COX-2 is undetectable in most cells but may be induced in macrophages, fibroblasts, endothelial cells, monocytes and ovarian follicles in response to a number of stimuli including growth factors, bacterial endotoxins and cytokines (15). Nitric oxide has been reported to activate COX-1 and COX-2 isoforms of the enzyme in macrophages, resulting in increased prostaglandin E\(_2\) (PGE\(_2\)) formation ((16)). In islets, cytokines have
also been shown to induce the expression of the COX-2 resulting in increased production of PGE_2 ([17] [18-20]). Similar to macrophages, nitric oxide stimulates COX-1 and COX-2 enzymatic activities in rat and human islets ([18,19]). These results suggest that cytokines stimulate both iNOS and COX-2 expression in islets and that NO contributes to the accumulation of proinflammatory prostaglandins by stimulating the enzymatic activity of COX-1 and COX-2.

Numerous investigators have studied the effects of nonsteroidal, anti-inflammatory drugs (NSAIDs), which inhibit both COX-1 and COX-2 activities, on insulin secretion both in vivo and ex vivo in isolated islet preparations. Increases in basal insulin, first-phase insulin response, 2nd phase or total insulin and improved glucose tolerance have been observed in normal and diabetic patients following treatment with sodium salicylate, acetasalicylic acid, and ibuprofen ([21]). In addition, PGE_2 has been shown to decrease insulin secretion and glucose tolerance in normal human subjects. Together, these data suggest that COX activity or COX-derived PGE_2 modulates insulin secretion and glucose tolerance in normal and diabetic patients. In vitro, sodium salicylate, acetasalicylic acid, and ibuprofen appear to augment glucose-stimulated insulin secretion by isolated rat islets ([21]). Salicylates have also been shown to attenuate cytokine-mediated inhibition of insulin secretion by rat islets ([22]). Recent reports suggest that selective inhibition of COX-2 attenuates diabetes development in the low-dose streptozotocin (STZ) mouse model and protects rat islets from cytokine-induced inhibition of glucose-stimulated insulin secretion ([23] [24]) implicating COX-2 and COX-2-derived PGE_2 in cytokine-mediated β-cell dysfunction and diabetes development. In contrast, indomethacin, a more potent COX inhibitor than the salicylates, decreases first-phase insulin secretion and glucose tolerance in normal human subjects ([21]) and does not protect against cytokine-mediated inhibition of insulin secretion by rat islets in vitro ([17,24] [25] [11]) calling into question whether COX activity and COX derived PGE_2 mediate these effects. To determine whether COX-2 and COX-2-derived PGE_2 mediate the inhibitory and destructive effects of cytokines on islet function and viability, we have characterized the actions of cytokines on the expression of COX-2 and production of PGE_2 by both rat and human islets and examined whether selective COX-2 inhibition protects islets from cytokine-induced β-cell dysfunction and morphological
damage. Treatment of rat and human islets with cytokines results in the time-dependent expression of COX-2 and formation of PGE₂ that correlates with the inhibitory and destructive effects of cytokines on insulin secretion and islet viability. Selective COX-2 inhibitors, Celebrex and SC-58236 completely prevent cytokine-induced PGE₂ formation by human and rat islets, respectively; however this treatment fails to prevent the inhibitory and destructive effects of cytokine treatment on glucose-stimulated insulin secretion and islet degeneration. Consistent with previous reports, inhibitors of iNOS, AG and NMMA, prevent the inhibitory and destructive effects of cytokines on islet function and viability by both rat and human islets. These findings suggest that, although COX-2 expression and PGE₂ formation by rat and human islets correlate with cytokine-induced islet damage, increased COX-2 expression and PGE₂ production do not appear to mediate this damage.Instead, our findings support a primary role for nitric oxide as the mediator cytokine-induced inhibition of insulin secretion and islet morphological degeneration.
**Experimental Procedures:**

**Materials** -- CMRL-1066 tissue culture medium, L-glutamine, penicillin, and streptomycin were from Gibco BRL (Grand Island, NY). Fetal calf serum was obtained from Hyclone (Logan, UT). Male Sprague-Dawley rats (250-300g) were purchased from Harlan Breeders (Indianapolis, IN). Collagenase type XI was from Sigma (St. Louis, MO). Enhanced chemiluminescence (ECL) reagents were purchased from Amersham Pharmacia Biotech. Human recombinant IL-1β was from R&D Systems (Minneapolis, MN). Human TNF-α and human IFN-γ were purchased from Roche Diagnostics (Indianapolis, IN). SC-58236 and Celebrex were obtained from the Pfizer Research Compound File (Kalamazoo, MI). Rabbit antiserum specific for the C-terminal 27 amino acids of mouse macrophage iNOS, rabbit antiserum specific for human iNOS and recombinant COX-1, COX-2 and human iNOS proteins were obtained from Dr. Thomas Misko (Pfizer, St. Louis, MO). Rabbit antiserum to COX-1 and COX-2 were purchased from Cayman Chemical (Ann Arbor, MI). Horseradish peroxidase (HRP)-conjugated donkey anti-rabbit IgG was obtained from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA). All other reagents were of research grade from commercially available sources.

**Islet isolation and culture:** Islets were isolated from male Sprague-Dawley rats by collagenase digestion as described previously ((26)). Following isolation, islets were cultured overnight in complete CMRL-1066 (CMRL-1066 containing 2 mM L-glutamine, 10% heat-inactivated fetal calf serum, 100 units/ml penicillin, and 100 µg/ml streptomycin) under an atmosphere of 95% air and 5% CO₂ at 37°C. Prior to each experiment, islets were washed 3 times in complete CMRL-1066, counted, and then cultured for an additional 3 h at 37°C. Experiments were initiated by the addition of IL-1β +/- SC-58236, PGE₂, AG or NMMA as indicated followed by culture for the indicated times.

Human islets were obtained from the islet isolation core facility at Washington University School of Medicine (St. Louis, MO), the Diabetes Research Institute at the University of Miami School of Medicine (Miami, FL), and CellzDirect (Tucson, AZ). Isolated human islets were cultured for 3 days at 37°C in complete CMRL-1066 medium.
prior to experimentation. Prior to each experiment, islets were washed 3 times in complete CMRL-1066, counted, and then cultured for an additional 3 h at 37°C. Experiments were initiated by the addition of IL-1β, IFN-γ and TNF-α +/- Celebrex, PGE₂ or NMMA as indicated followed by culture for the indicated times.

**Insulin secretion:** Islets (220/ml of complete CMRL-1066) were cultured for 40 h (rat islets) or 48 h (human islets) with the indicated concentrations of cytokines in the presence or absence of PGE₂, SC-58236 or Celebrex, AG or NMMA. 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, and 0.1% BSA, pH 7.4) containing 3 mM D-glucose and insulin secretion was performed in the presence of either 3 mM or 20 mM D-glucose as described ((4)). Medium insulin content was determined by radioimmunoassay ((27)).

**Islet viability:** Islets (25/500 µl of complete CMRL-1066) were cultured for 96 h in 24-well microtiter plates with the indicated concentrations of cytokines, PGE₂ +/- SC-58236 or Celebrex. Islet degeneration was determined in a blinded fashion by phase-contrast microscopic analysis. Islet degeneration is characterized by the loss of islet integrity as assessed by disintegration and partial dispersion of islets as described previously ((28) (11)).

**Western blot analysis:** Rat or human islets (120/400 µl of complete CMRL-1066), cultured for the indicated times with cytokines, PGE₂, SC-58236 or Celebrex, +/- AG or NMMA, were isolated, lysed, and protein separated by SDS-gel electrophoresis as described ((4)). Detection of rat iNOS (1:5000 dilution), rat COX-2 (1:100 dilution), human iNOS (1:500 dilution), human COX-2 (1:1000 dilution), human COX-1 (1:1000 dilution) were by ECL according to manufacturer’s specifications (Amersham, Arlington Heights, IL).

**Nitrite and Prostaglandin E₂ Determination:** Nitrite production was determined by mixing 50 µl of culture medium with 50 µl of Griess reagent ((29)). The absorbance at
540 nm was measured and nitrite concentrations were calculated from a sodium nitrite standard curve. Prostaglandin \( E_2 \) production was determined by using a monoclonal Prostaglandin \( E_2 \) EIA kit (Cayman Chemical, Ann Arbor, MI) according to the manufacturer specifications.

**Statistical Analyses:** Statistical comparisons were made between groups using a one-way analysis of variance. Significant differences between treatment groups compared with untreated controls (\( p < 0.05 \)) were evaluated using a Least Significant Differences posthoc analysis.

**Results:**

*Time-dependent effects of IL-1\( \beta \) on COX-2 expression and PGE\( _2 \) formation by rat islets.*

Previous studies have shown that IL-1\( \beta \) induces the time-dependent production of nitric oxide and PGE\( _2 \) by rat islets that is maximal following 48 h incubation ([19] and Fig 1a). To characterize the time-dependent expression of COX-2, rat islets were incubated for 0-48 h with 1 unit/ml IL-1\( \beta \) followed by Western blot analysis of COX-2 protein expression. As shown in Fig 1b, IL-1\( \beta \) induces COX-2 protein expression that is first observed following 3 h incubation, maximally expressed following 12 h incubation and remains at detectable levels of expression following 48 h incubation. Consistent with IL-1\( \beta \)-induced COX-2 expression, IL-1\( \beta \)-induces the time-dependent formation of PGE\( _2 \) by rat islets that is first observed following a 6 hr incubation and maximal following 48 h incubation. Similar to COX-2 expression, IL-1\( \beta \)-stimulated iNOS expression is first observed following 6 h incubation, is maximally expressed following a 24 h incubation, and its expression level is sustained for up to 48h (Figure 1b). iNOS expression correlates with the stimulatory actions of this cytokine on NO formation as nitrite production in response to IL-1\( \beta \) is first apparent following 6 h incubation and maximal following 48 h incubation (Figure 1a). These results indicate that IL-1\( \beta \)-induced NO formation and PGE\( _2 \) production correlate with IL-1\( \beta \)-induced iNOS and COX-2 expression by isolated rat islets.
Effects of COX-2 inhibition on IL-1β-induced NO and PGE2 formation by rat islets. To examine the effects of selective COX-2 inhibition on islet PGE2 accumulation and NO formation, rat islets were incubated for 40 h with 1 unit/ml IL-1β in the presence or absence of 25 ng/ml SC-58236 (30). As shown in Figure 2, IL-1β induces a ~7-fold increase in both PGE2 and nitrite production following a 40 h incubation. The COX-2-selective inhibitor SC-58236 (25 ng/ml) prevents IL-1β-induced PGE2 formation (Figure 2a) without affecting IL-1β-induced nitrite production by rat islets (Figure 2b). Consistent with previous studies, the iNOS-selective inhibitor, AG, prevents IL-1β-induced nitrite production (Figure 2b) and significantly attenuates IL-1β-induced PGE2 formation by rat islets ((18) and Figure 2b). SC-58236 alone fails to induce PGE2 or NO formation (Figure 2) by isolated rat islets and does not modulate IL-1β-induced iNOS or COX-2 expression (Figure 2b inset and data not shown). These results indicate that selective COX-2 inhibition by SC-58236 prevents IL-1β-induced PGE2 formation without affecting IL-1β-induced iNOS expression and NO formation by rat islets.

Effects of SC-58236 on IL-1β-induced islet degeneration and inhibition of glucose-stimulated insulin secretion. The inhibitory and destructive actions of IL-1β on glucose-stimulated insulin secretion and islet degeneration are due in part to β-cell production of nitric oxide. β-cells are the primary islet cellular source of iNOS in response to IL-1β treatment and inhibitors of iNOS prevent IL-1β-induced islet dysfunction and damage ((1) (8) (5) and Figure 3). Recent data has implicated COX-2-derived PGE2 production as a mediator of IL-1-induced inhibition of insulin secretion by rat islets ((23)). To determine whether inhibition of COX-2 activity and PGE2 formation protects islets from the inhibitory and destructive effects of IL-1β on glucose-stimulated insulin secretion and islet viability, islets were incubated with 1 unit/ml IL-1β in the presence or absence of SC-58236 (25 ng/ml) and exogenous PGE2 (1 µM). Treatment of rat islets with IL-1β results in a ~68% inhibition of glucose-stimulated insulin secretion following 40 h incubation (Fig. 3a) and ~98% islet morphological degeneration following 96 h incubation (Fig. 3b). SC-58236, which prevents IL-1β-induced PGE2 formation (Fig. 2a) does not prevent the inhibitory actions of IL-1β on insulin secretion, nor does it prevent
islet degeneration. Addition of exogenous PGE$_2$ (1 $\mu$M) does not impair glucose-stimulated insulin secretion nor does it stimulate islet degeneration. Preincubation of islets with the selective iNOS inhibitor, AG, prevents both the inhibitory actions of IL-1$\beta$ on insulin secretion and the destructive effects of IL-1$\beta$ on islet viability. These results indicate that selective inhibition of COX-2 does not prevent IL-1$\beta$-induced $\beta$-cell dysfunction and damage. These findings also support a primary role for nitric oxide as the mediator of cytokine-induced inhibition of insulin secretion and islet morphological degeneration.

Effects of IL-1$\beta$+IFN-$\gamma$ on COX-2 expression and PGE$_2$ formation by human islets. While IL-1$\beta$ alone is sufficient to induce iNOS and COX-2 expression and NO and PGE$_2$ formation by rat islets, a combination of cytokines is required to induce similar responses in isolated human islets. Previous studies have shown that incubation of human islets with IL-1$\beta$ + IFN-$\gamma$ or IL-1$\beta$ + IFN-$\gamma$ + TNF-$\alpha$ results in iNOS and COX-2 expression and the time-dependent formation of NO and PGE$_2$ that is maximal following a 48 h incubation (Fig 4a and (31) (19) (4)). To further characterize the temporal expression of COX-2 in response to cytokines, human islets were incubated for 0-48 h with the combination of 75 units/ml IL-1$\beta$ and 750 units/ml IFN-$\gamma$. As shown in Figure 4b, IL-1$\beta$ + IFN-$\gamma$ induces the time-dependent expression of COX-2 that is first apparent following 3 h incubation, maximal following an 18-24 h incubation, and remains elevated for up to 48h. The expression of the constitutive isoform of COX, COX-1, is unaltered in human islets by cytokine treatment (Fig 4b). Consistent with COX-2 expression, PGE$_2$ accumulation is first apparent following 12 h incubation and the levels continue to increase to maximal (~11-fold) levels following 40-48h incubation. Similar to COX-2 expression, this combination of cytokines stimulates the time-dependent expression of iNOS that is first apparent following a 6 h incubation and maximal following a 40-48 h incubation (Fig 4b). Cytokine-induced NO formation by human islets is also time-dependent, correlating with cytokine-induced iNOS expression, with maximal levels following 48 h incubation ((32) (5) and Figure 4). These results indicate that IL-1$\beta$ +
IFN-γ induce PGE₂ and NO formation by human islets that is time-dependent and correlates with IL-1β + IFN-γ-induced iNOS and COX-2 expression.

**Effects of Celebrex on cytokine-induced PGE₂ and NO formation by human islets.** To determine the effects of selective COX-2 inhibition on cytokine-induced production of PGE₂ and NO, human islets were incubated with 75 units/ml IL-1β and 750 units/ml IFN-γ in the presence of increasing concentrations of Celebrex. As shown in Figure 5a, Celebrex inhibits IL-1β+ IFN-γ-induced PGE₂ formation in a concentration-dependent fashion with ~91% and ~97% maximal inhibition observed at 1 and 10 µM, respectively. At 10 µM, Celebrex completely prevents PGE₂ formation in response to either IL-1β + IFN-γ or IL-1β + IFN-γ + TNF-α (Figure 5b). This inhibition of PGE₂ production is not due to a decrease in COX-2 expression as Celebrex fails to modulate cytokine-induced COX-2 protein expression (Figure 5c). Celebrex also does not modulate cytokine-stimulated iNOS expression or nitrite production by human islets at concentrations ranging from 0.1-10 µM (Figures 5a and c). At 10 µM, Celebrex appears to slightly increase IL-1β + IFN-γ-induced NO formation (~3.5-fold vs. ~2.5-fold increase for cytokine mix alone), however, this increase was not statistically significant from IL-1β + IFN-γ-induced levels. These results indicate that Celebrex inhibits cytokine-induced PGE₂ formation by human islets in a concentration-dependent fashion without modulating cytokine-induced COX-2 expression. Celebrex also does not modulate cytokine-induced iNOS expression or NO formation by human islets.

**Effects of Celebrex on cytokine-induced islet degeneration and inhibition of glucose-stimulated insulin secretion.** To determine whether PGE₂ formation mediates cytokine-induced inhibition of glucose-stimulated insulin secretion and islet degeneration, human islets were incubated with a mixture of cytokines in the presence and absence of Celebrex. As shown in Figure 6, incubation of human islets with IL-1β + IFN-γ + TNFα, results in a ~79% inhibition of glucose-stimulated insulin secretion following a 48 h incubation and ~96% islet morphological degeneration following 96 h incubation. Co-incubation of islets with 10 µM Celebrex, which prevents cytokine-stimulated PGE₂
formation, fails to prevent the inhibitory and destructive effects of cytokines on glucose-stimulated insulin secretion and islet viability. Alone, neither exogenous PGE\(_2\) nor Celebrex inhibit basal or glucose-stimulated insulin secretion, or induce human islet degeneration (Figure 6 and data not shown). These results indicate that selective inhibition of COX-2 fails to protect islets from the inhibitory and destructive effects of cytokines on glucose-induced insulin secretion and islet viability. These data suggest that cytokine-induced PGE\(_2\) formation does not mediate the inhibitory and destructive effects of cytokines on \(\beta\)-cell function and islet viability by human islets.

**Discussion:**

Insulin-secreting \(\beta\)-cells are destroyed during the development of type I diabetes by an inflammatory reaction in and around islets, termed insulitis. The cellular infiltrate consists of macrophages, CD4\(^+\) and CD8\(^+\) T-lymphocytes, and a limited number of B lymphocytes ((33)). Cytokines released by these macrophages and T-cells, namely IL-1\(\beta\), TNF-\(\alpha\), and IFN-\(\gamma\), are thought to play an important role in the pathogenesis of autoimmune diabetes by inducing the expression of iNOS and the production of NO by \(\beta\)-cells ((5)). The effects of cytokines and the resulting nitric oxide production by \(\beta\)-cells on islet function and viability have been well-characterized and include decreased oxidative phosphorylation, a potent inhibition of glucose-stimulated insulin secretion ((6) (8) (9)), and islet morphological degeneration. Evidence in support of a role for nitric oxide in mediating cytokine-induced islet damage includes the protective actions of iNOS inhibitors AG or L-NMMA on cytokine-induced inhibition of insulin secretion and islet degeneration ((1) (5)) and the lack of an inhibitory action of cytokines on glucose-stimulated insulin secretion in islets isolated from iNOS-deficient mice ((12)).

In addition to iNOS expression, cytokines have been shown to induce COX-2 expression and PGE\(_2\) production both in isolated rat and human islets; however, the role of COX-2-derived PGE\(_2\) on \(\beta\)-cell function and viability has not been fully characterized. In this study, we have characterized the temporal expression of COX-2 and production of PGE\(_2\) by rat and human islets and determined whether selective COX-2 inhibition prevents cytokine-mediated islet dysfunction and damage. Treatment of rat and human islets with IL-1\(\beta\) and IL-1\(\beta\) + IFN-\(\gamma\) (+/- TNF-\(\alpha\)), respectively, results in the time-
dependent production of PGE$_2$ that is maximal following 24-48 h incubation. Cytokine-induced PGE$_2$ formation by rat and human islets correlates with cytokine-induced COX-2 expression that is first observed following 3 h incubation and remains at detectable levels of expression following 40-48 h incubation. Selective inhibition of COX-2 in rat and human islets by SC-58236 or Celebrex, respectively, at concentrations that completely inhibit cytokine-induced PGE$_2$ formation, does not protect islets from cytokine-induced inhibition of glucose-stimulated insulin secretion or islet morphological degeneration. These results suggest that although cytokine-induced PGE$_2$ production and COX-2 expression correlate with cytokine-induced inhibition of glucose-stimulated insulin secretion, islet production of PGE$_2$ does not mediate these inhibitory and destructive effects.

With the exception of indomethacin, NSAIDs as a class have been shown to increase glucose-stimulated insulin secretion by isolated rat islets in vitro, and to improve glucose tolerance in diabetic and normal human subjects ((21)); however, the mechanism by which NSAIDs mediate these beneficial effects is unclear. Previous reports support the hypothesis that NSAIDs exert their beneficial effects via inhibition of COX-2 activity and COX-2-derived PGE$_2$ production. The selective COX-2 inhibitor, NS-398, has been shown to attenuate diabetes development in the low-dose STZ mouse model and selective COX-2 inhibition by SC-58236 has been shown to attenuate IL-1-induced inhibition of glucose-stimulated insulin secretion by Wistar rat islets ((23) (24)). However, data obtained in the current study indicate that selective COX-2 inhibition by SC-58236 fails to prevent the inhibitory effects of IL-1β on glucose-stimulated insulin secretion by isolated Sprague-Dawley rat islets. The addition of supraphysiological concentrations of PGE$_2$, either alone or in combination with cytokine(s) and COX-2 inhibitor(s), also does not inhibit glucose-stimulated insulin secretion or induce islet damage by rat and human islets. These data are consistent with previous reports in which exogenous PGE$_2$ failed to mimic the inhibitory actions of IL-1 on glucose-stimulated insulin secretion by isolated rat islets ((17)) and in which indomethacin, at concentrations that completely inhibit islet PGE$_2$ production, did not attenuate the inhibitory effects of IL-1 on β-cell function ((11,17)).
To determine whether the strain of rat utilized for these studies could explain these discordant results, we examined whether SC-58236 prevents the inhibitory actions of IL-1 on glucose-stimulated insulin secretion by isolated Wistar rat islets. Similar to islets isolated from Sprague-Dawley rats, SC-58236 fails to prevent the inhibitory actions of IL-1β on glucose-stimulated insulin secretion by Wistar rat islets (data not shown). These data suggest that the discordant results are not due to differences in the strain of rat utilized for experimentation. The studies are otherwise very similar in execution with the exception of the conditions used to culture the islets. Following isolation, rat (Sprague-Dawley and Wistar) and human islets used in the current study were cultured in CMRL-1066 medium containing 5 mM glucose. In contrast, Wistar rat islets were cultured in RPMI-1640 medium containing 11 mM glucose in the study by Tran et al. (23). Previous reports indicate that COX-2 expression is increased in vascular smooth muscle and endothelial cells when cultured in high glucose ((34,35)), and in monocytes isolated from Type I and Type II diabetic individuals ((36)). In addition, Persaud et al. recently reported that, whereas freshly isolated mouse islets express primarily COX-1, COX-2 expression is induced and becomes the primary isoform of COX expressed in islets that are cultured in high glucose ((37)). In a concentration-dependent manner, glucose also induces COX-2 expression in human islets ((37)). Consistent with these data, in a separate report published by the same laboratory as the Tran et al. study, human islets cultured in RPMI-1640 medium containing 11 mM glucose were shown to constitutively express COX-2 as the dominant COX isoform ((20)). Although islets cultured in high glucose constitutively express COX-2, they appear to function normally, secreting insulin in response to a high glucose challenge (23). The addition of exogenous PGE₂ also does not modulate glucose-stimulated insulin secretion ((17), current study) suggesting that increased COX-2 expression and PGE₂ formation do not adversely affect β-cell function. Taken together, these data indicate that islets do not constitutively express COX-2 as the predominant isoform of COX under basal glucose culture conditions, and that increased COX-2 expression and PGE₂ formation do not modulate β-cell function or mediate the inhibitory and destructive effects of cytokines on glucose-stimulated insulin secretion and islet viability.
One mechanism by which salicylates (sodium salicylate, acetylsalicylic acid) appear to exert their beneficial effects on insulin secretion and glucose tolerance is via inhibition of IκB kinase (IKKβ; (38)). IKKα and IKKβ phosphorylate the IκB proteins, which sequester the transcription factor, NF-κB in quiescent cells. Once phosphorylated by IKK, IκB dissociates from the NF-κB:IkB complex allowing NF-κB to translocate to the nucleus and induce gene transcription ((39)). IL-1-induced iNOS and COX-2 expression in islets requires the activation of NF-κB ((40)). Recent data suggests the mechanism by which salicylates attenuate cytokine-induced inhibition of insulin secretion is via inhibition of NF-κB activation and subsequent inhibition of COX-2 expression ((22)). However, since salicylates also inhibit iNOS expression and NO formation by rat islets ((41)), and inhibitors of iNOS activity prevent the damaging actions of cytokines on the function and viability of rat and human islets, it would be reasonable to conclude that salicylates attenuate cytokine-mediated damage by preventing IKK-mediated NF-κB activation and subsequent expression of iNOS by islets. In support of this hypothesis, we have shown that selective inhibition of COX-2 fails to prevent cytokine-induced inhibition of glucose-stimulated insulin secretion and islet degeneration in rat and human islets. However, selective inhibition of iNOS by AG or NMMA, prevents the inhibitory and destructive effect of cytokines on β-cell function and viability in accordance with previous reports ((5)). Taken together, these data provide support for the hypothesis that the mechanism by which salicylates attenuate cytokine-mediated inhibitory and destructive effects on β-cell function and islet viability may be via inhibition of IKKβ activity and subsequent prevention of cytokine-induced NF-κB-mediated expression of iNOS by β-cells.

In conclusion, the results of the current study indicate that selective inhibition of COX-2 activity by SC-58236 or Celebrex fails to protect rat and human islets, respectively, from cytokine-induced inhibition of glucose-stimulated insulin secretion and islet degeneration. These results suggest that the inhibitory and destructive effects of cytokines on islet function and viability are not mediated by cytokine-induced production of PGE2 by islets and provide further support for cytokine-induced iNOS expression and NO formation as mediators of these inhibitory and destructive effects.
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References:


**Figure Legends:**

**Figure 1:**  Time-dependent effects of IL-1β on NO and PGE₂ formation and iNOS and COX-2 expression by rat islets. Rat islets (120/400 µl of complete CMRL-1066) were treated with 1 unit/ml IL-1β for the indicated times at 37°C. After treatment, media was removed for nitrite and PGE₂ formation (a) and iNOS and COX-2 expression by islets was examined by Western blot analysis (b, c) as described in Experimental Procedures. Results for nitrite are the average ± SEM of four independent experiments, and iNOS and COX-2 protein expression are representative of three independent experiments. Statistical significance: *p* < 0.001 versus untreated control (*). Statistical significance: *p* < 0.001 versus 18 h IL-1-treated islets (#).

**Figure 2:**  Effects of selective COX-2 inhibition by SC-58236 on PGE₂ and NO formation by rat islets. Rat islets (120/400 µl of complete CMRL-1066) were treated with 1 unit/ml IL-1β for 40 h in the presence or absence of 25 ng/ml SC-58236, 1 µM PGE₂ or 1 mM AG at 37°C as indicated. After treatment, media was removed for nitrite and PGE₂ measurements (a) and iNOS expression by islets was examined by Western blot analysis (b; inset) as described in Experimental Procedures. Results for nitrite and PGE₂ formation are the average ± SEM of five independent experiments, and iNOS protein expression is representative of three independent experiments. Statistical significance: *p* < 0.05 versus untreated control (*).

**Figure 3:**  Effects of SC-58236 on IL-1β-induced inhibition of glucose-stimulated insulin secretion and islet degeneration by rat islets. a) Rat islets (220 islets/ml of complete CMRL-1066) were treated with 1 unit/ml IL-1β for 40 h in the presence or absence of 25 ng/ml SC-58236, 1 µM PGE₂ or 1 mM AG as indicated at 37°C. Following the incubation period, glucose-induced insulin secretion was examined as stated in
Experimental Procedures. b) Rat islets (25-islets/500 ul of complete CMRL-1066) were incubated with 1 unit/ml IL-1β for 96 h in the presence or absence of 25 ng/ml SC-58236, 1 µM PGE2 or 1 mM AG as indicated. Islet degeneration was assessed by phase-contrast microscopy in a blinded fashion. Results for insulin secretion (a) average ± SEM of four independent experiments and islet degeneration are the average ± SEM of three independent experiments. Statistical significance: \( p < 0.05 \) versus untreated control (*).

Figure 4: Time-dependent effects of IL-1β + IFN-γ on iNOS and COX-2 expression and NO and PGE2 formation by human islets. Human islets (120/400 µl of complete CMRL-1066) were treated with 75 units/ml IL-1β + 750 units/ml IFN-γ for the indicated times at 37°C. After treatment, media was removed for nitrite and PGE2 formation (a) and iNOS and COX-2 expression by islets was examined by Western blot analysis (b) as described in Experimental Procedures. Results for nitrite are the average ± SEM of three independent experiments, and iNOS and COX-2 protein expression are representative of three independent experiments. Statistical significance: \( p < 0.05 \) versus untreated control for nitrite determinations (*). Statistical significance: \( p < 0.001 \) versus untreated control for PGE2 determinations (#).

Figure 5: Effects of Celebrex on cytokine-induced NO and PGE2 formation and iNOS and COX-2 expression by human islets. Human islets (120/400 µl of complete CMRL-1066) were treated with 75 units/ml IL-1β + 750 units/ml IFN-γ in the presence of increasing concentrations of Celebrex for 40 h at 37°C. After treatment, media was removed for PGE2 and nitrite (a) formation as described in Experimental Procedures. Human islets (120/400 µl of complete CMRL-1066) were treated with 75 units/ml IL-1β + 750 units/ml IFN-γ + 10 ng/ml TNF-α in the presence or absence of 10 µM Celebrex or
0.1 μM PGE₂ as indicated for 40 h at 37°C. After treatment, media was removed for PGE₂ formation (b) and COX-2 and iNOS expression by islets was examined by Western blot analysis (c) as described in Experimental Procedures. Results for nitrite and PGE₂ formation are the average ± SEM of three independent experiments, and COX-2 protein expression is representative of three independent experiments. Statistical significance: $p < 0.05$ versus untreated control (*) or $p < 0.001$ versus untreated control (#).

**Figure 6:** Effects of Celebrex on cytokine-induced islet degeneration and inhibition of insulin secretion by human islets. Human islets (220/ml of complete CMRL-1066) were treated with 75 units/ml IL-1β + 750 units/ml IFN-γ + 10 ng/ml TNF-α in the presence or absence of 10 μM Celebrex or 0.1 μM PGE₂ as indicated for 48 h at 37°C. Following the incubation period, glucose-induced insulin secretion (hatched bars) was examined as stated in Experimental Procedures. Human islets (25 islets/500 ul of complete CMRL-1066) were treated with 75 units/ml IL-1β + 750 units /ml IFN-γ + 10 ng/ml TNF-α for 96 h in the presence or absence of 10 μM Celebrex or 0.1 μM PGE₂ as indicated. Islet degeneration (black bars) was assessed by phase-contrast microscopy in a blinded fashion. Results for insulin secretion are the average ± SEM of four independent experiments. Results for islet degeneration are the average ± SEM of two independent experiments. Statistical significance: $p < 0.05$ versus untreated control (*) for insulin secretion and (#) for islet viability.
a) Nitrite and PGE$_2$ Formation

![Graph showing nitrite and PGE$_2$ formation over time.](image)

b) COX-2 Expression

<table>
<thead>
<tr>
<th>Incubation (h)</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>12</th>
<th>24</th>
<th>48</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β (1 unit/ml)</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
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</tbody>
</table>

![Western blot showing COX-2 expression.](image)

c) iNOS Expression

<table>
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<th>12</th>
<th>24</th>
<th>40</th>
<th>48</th>
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<tbody>
<tr>
<td>IL-1β (1 unit/ml)</td>
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</tbody>
</table>

![Western blot showing iNOS expression.](image)

Figure 1
Figure 2
Figure 3
Figure 4
c) COX-2, iNOS expression

![Image of Western blots showing COX-2 and iNOS expression levels with different treatments.]

**Figure 5**

<table>
<thead>
<tr>
<th>Cytokine Mix</th>
<th>Celebrex (10 uM)</th>
<th>PGE₂ (0.1 μM)</th>
<th>NMMA (1 mM)</th>
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<tr>
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</table>

**Figure 6**

![Image of a bar graph showing insulin secretion and islet viability with different treatments.]

- Insulin Secretion
- Islet Viability (% of Total)

- Cyto Mix
- Celebrex (10 μM)
- PGE₂ (0.1 μM)
Role of cyclooxygenase-2 in cytokine-induced β-cell dysfunction and damage by isolated rat and human islets
Monique R. Heitmeier, Colleen B. Kelly, Nancy J. Ensor, Kenneth A. Gibson, Karen G. Mullis, John A. Corbett and Timothy J. Maziasz

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