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

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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 and the production of nitric oxide by β-cells. Cytokines also stimulate the expression of the inducible isoform of cyclooxygenase, COX-2, and the production of prostaglandin E_2 (PGE_2) by rat and human islets; however, the role of increased COX-2 expression and PGE_2 production in mediating cytokine-induced inhibition of islet metabolic function and viability has been incompletely characterized. In this study, we have shown 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 PGE_2 formation in a time-dependent manner. Coincubation of rat and human islets with selective COX-2 inhibitors SC-58236 and Celecoxib, respectively, attenuated cytokine-induced PGE_2 formation. However, these inhibitors failed 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 PGE_2 does not mediate these inhibitory and destructive effects.

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)[1] appear to play an important role. We and others have shown that treatment of isolated rat and human islets with cytokines such as IL-1, 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 NO in mediating cytokine-induced islet damage includes the protective actions of iNOS inhibitors aminoguanidine (AG) or N^G-monomethyl L-arginine (L-NMMA) on cytokine-induced inhibition of insulin secretion and islet degeneration (2, 4, 10, 11), as well as 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 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). NO has been reported to activate COX-1 and COX-2 isoforms of the enzyme in macrophages, resulting in increased PGE_2 formation (16). In islets, cytokines have also been shown to induce the expression of COX-2, resulting in increased production of PGE_2 (17–20). Similar to macrophages, NO 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, second phase or total insulin, and improved glucose tolerance have been observed in normal and diabetic patients following treatment with sodium salicylate, acetosalicylic acid, and ibuprofen (21). In addition, PGE_2 has been shown to decrease insulin secretion and glucose

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[1] The abbreviations used are: NO, nitric oxide; AG, aminoguanidine; IL-1, interleukin-1; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor-α; IKK, inhibitor of IκB kinase; iNOS, inducible nitric-oxide synthase; COX, cyclooxygenase; L-NMMA, N^G-monomethyl L-arginine; NSAID, nonsteroidal anti-inflammatory drug; PGE_2, prostaglandin E_2.

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, acetosalicylic 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 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* (11, 17, 24, 25), 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 resulted in the time-dependent expression of COX-2 and formation of PGE_2, which correlated with the inhibitory and destructive effects of cytokines on insulin secretion and islet viability. Selective COX-2 inhibitors Celebrex and SC-58236 completely prevented cytokine-induced PGE_2 formation by human and rat islets, respectively. However this treatment failed 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 L-NMMA prevented the inhibitory and destructive effects of cytokines on islet function and viability by both rat and human islets (5). These findings suggest that although COX-2 expression and PGE_2 formation by rat and human islets correlate with cytokine-induced islet damage, increased COX-2 expression and PGE_2 production do not appear to mediate this damage. Instead, our findings support a primary role for NO as the mediator of 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 Invitrogen. Fetal calf serum was obtained from Hyclone (Logan, UT). Male Sprague-Dawley rats (250–300 g) were purchased from Harlan Breeders (Indianapolis, IN). Collagenase type XI was from Sigma. ECL reagents were purchased from Amersham Biosciences. Human recombinant IL-1β was from R&D Systems (Minneapolis, MN). Human TNF-α and human IFN-γ were purchased from Roche Diagnostics. 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-conjugated donkey anti-rabbit IgG was obtained from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA). All other reagents were of research grade and obtained 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% CO_2 at 37 °C. Prior to each experiment, islets were washed three 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, PGE_2, AG, or L-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 three 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, PGE_2 or L-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 PGE_2, SC-58236, Celebrex, AG, or L-NMMA. The islets were isolated and washed three times in Krebs-Ringer bicarbonate buffer (25 mM Hepes, 115 mM NaCl, 24 mM $NaHCO_3$, 5 mM KCl, 1 mM $MgCl_2$, 2.5 mM $CaCl_2$, and 0.1% bovine serum albumin, pH 7.4) containing 3 mM D-glucose, and insulin secretion was performed in the presence of either 3 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, PGE_2 ± 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 (11, 28).

Western Blot Analysis—Rat or human islets (120/400 μl of complete CMRL-1066), cultured for the indicated times with cytokines ± PGE_2, SC-58236, Celebrex, AG, or L-NMMA, were isolated and lysed, and proteins were 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), and human COX-1 (1:1000 dilution) were by ECL according to manufacturer's specifications (Amersham Biosciences).

Nitrite and Prostaglandin E_2 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. PGE_2 production was determined by using a monoclonal PGE_2 enzyme immunoassay kit (Cayman Chemical) 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 post-hoc analysis.

RESULTS

Time-dependent Effects of IL-1β on COX-2 Expression and PGE_2 Formation by Rat Islets—Previous studies have shown that IL-1β induces the time-dependent production of nitric oxide and PGE_2 by rat islets that is maximal following 48 h incubation (Ref. 19 and Fig 1*a*). To characterize the time-dependent expression of COX-2, rat islets were incubated for 0–48 h with 1 unit/ml IL-1β followed by Western blot analysis of COX-2 protein expression. As shown in Fig 1*b*, IL-1β induced COX-2 protein expression, which was first observed following 3 h of incubation, was maximally expressed following 12 h of incubation, and remained at detectable levels of expression following 48 h of incubation. Consistent with IL-1β-induced COX-2 expression, IL-1β induced the time-dependent formation of PGE_2 by rat islets, which was first observed following 6 h of incubation and was maximal following 48 h of incubation (Fig. 1*a*). Similar to COX-2 expression, IL-1β-stimulated iNOS expression was first observed following 6 h of incubation and was maximally expressed following a 24-h incubation; its expression level was sustained for up to 48 h (Fig. 1*c*). iNOS expression correlates with the stimulatory actions of this cytokine on NO formation as nitrite production in response to IL-1β

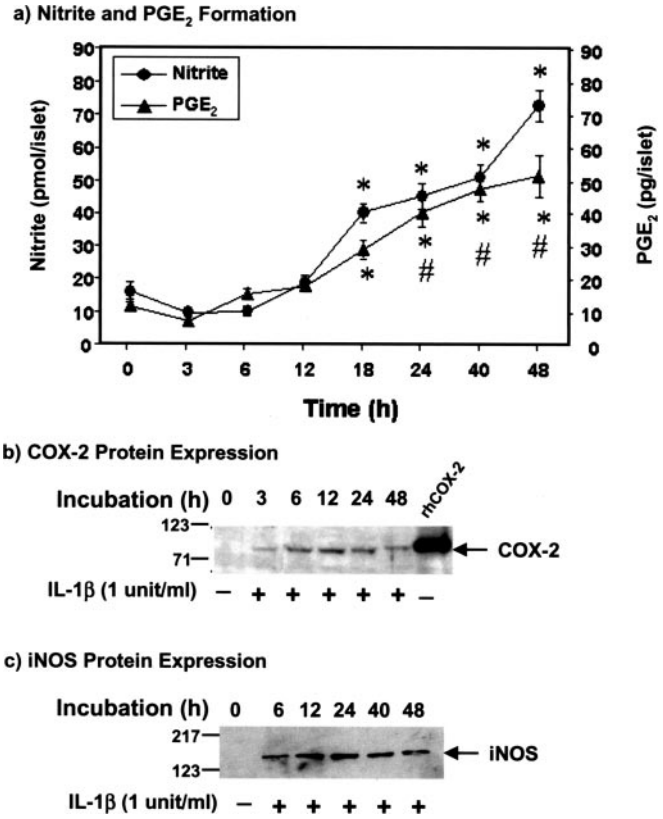

FIG. 1. **Time-dependent effects of IL-1β on NO and PGE_2 formation and iNOS and COX-2 expression by rat islets.** Rat islets (120/400 μl of complete CMRL-1066) were treated with 1 unit/ml IL-1β for the indicated times at 37 °C. After treatment, the medium was removed for nitrite and PGE_2 formation (*a*) and COX-2 and iNOS expression by islets was examined by Western blot analysis (*b* and *c*) as described under "Experimental Procedures." Results for nitrite are the average ± S.E. 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; #, $p < 0.001$ *versus* 18-h IL-1-treated islets.

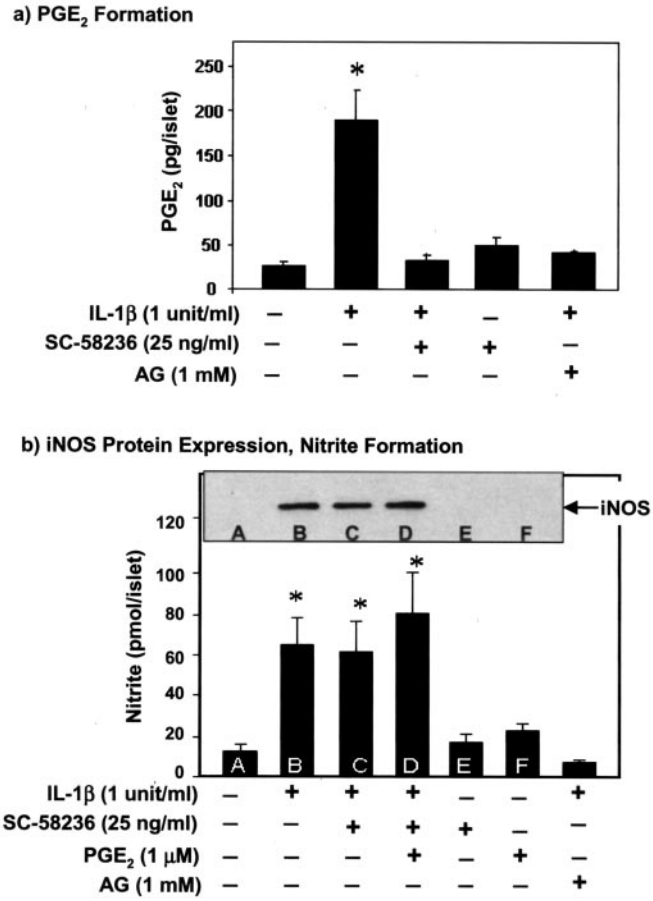

FIG. 2. **Effects of selective COX-2 inhibition by SC-58236 on PGE_2 and NO formation by rat islets.** Rat islets (120/400 μl of complete CMRL-1066) were treated with 1 unit/ml IL-1β for 40 h in the presence or absence of 25 ng/ml SC-58236, 1 μM PGE_2, or 1 mM AG at 37 °C as indicated. After treatment, medium was removed for nitrite and PGE_2 measurements (*a*), and iNOS expression by islets was examined by Western blot analysis (*b*, *inset*) as described under "Experimental Procedures." Results for nitrite and PGE_2 formation are the average ± S.E. of five independent experiments, and iNOS protein expression is representative of three independent experiments. Statistical significance: *, $p < 0.05$ *versus* untreated control.

was first apparent following 6 h of incubation and maximal following 48 h of incubation (Fig. 1*a*). These results indicate that IL-1β-induced NO formation and PGE_2 production correlate with IL-1β-induced iNOS and COX-2 expression by isolated rat islets.

Effects of COX-2 Inhibition on IL-1β-induced NO and PGE_2 Formation by Rat Islets—To examine the effects of selective COX-2 inhibition on islet PGE_2 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 Fig. 2, IL-1β induced a ~7-fold increase in both PGE_2 and nitrite production following a 40-h incubation. The COX-2-selective inhibitor SC-58236 (25 ng/ml) prevented IL-1β-induced PGE_2 formation (Fig. 2*a*) without affecting IL-1β-induced nitrite production by rat islets (Fig. 2*b*). Consistent with previous studies, the iNOS-selective inhibitor, AG, prevented IL-1β-induced nitrite production (Fig. 2*b*) and significantly attenuated IL-1β-induced PGE_2 formation by rat islets (Ref. 18 and Fig. 2*b*). SC-58236 alone failed to induce PGE_2 or NO formation (Fig. 2) by isolated rat islets and did not modulate IL-1β-induced iNOS or COX-2 expression (Fig. 2*b, inset*, and data not shown). These results indicate that selective COX-2 inhibition by SC-58236 prevents IL-1β-induced PGE_2 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 NO. 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, 5, 8). Recent data have implicated COX-2-derived PGE_2 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 PGE_2 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 PGE_2 (1 μM). Treatment of rat islets with IL-1β resulted in a ~68% inhibition of glucose-stimulated insulin secretion following 40 h of incubation (Fig. 3*a*) and ~98% islet morphological degeneration following 96 h of incubation (Fig. 3*b*). SC-58236, which prevents IL-1β-induced PGE_2 formation (Fig. 2*a*), does not prevent the inhibitory actions of IL-1β on insulin secretion nor does it prevent islet degeneration (Fig. 3). The addition of exogenous PGE_2 (1 μM) did not impair glucose-stimulated insulin secretion nor did it stimulate islet degeneration. Preincubation of islets with the selective iNOS inhibitor AG prevented both the inhibitory actions of IL-1β on insulin secretion and the destructive effects of IL-1β on islet viability

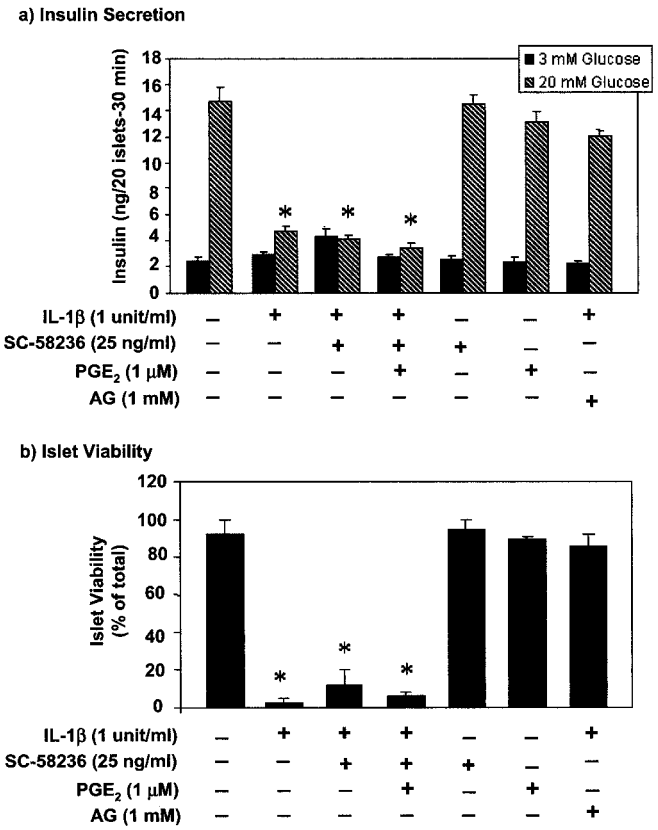

FIG. 3. **Effects of SC-58236 on IL-1β-induced inhibition of glucose-stimulated insulin secretion and islet degeneration by rat islets.** *a*, rat islets (220 islets/ml of complete CMRL-1066) were treated with 1 unit/ml IL-1β for 40 h in the presence or absence of 25 ng/ml SC-58236, 1 μM PGE_2, or 1 mM AG as indicated. Following the incubation period, glucose-induced insulin secretion was examined as stated under "Experimental Procedures." *b*, rat islets (25-islets/500 μl 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 PGE_2, or 1 mM AG as indicated. Islet degeneration was assessed by phase-contrast microscopy in a blinded fashion. Results for insulin secretion are the average ± S.E. of four independent experiments, and islet degeneration are the average ± S.E. of three independent experiments. Statistical significance: *, $p < 0.05$ *versus* untreated control.

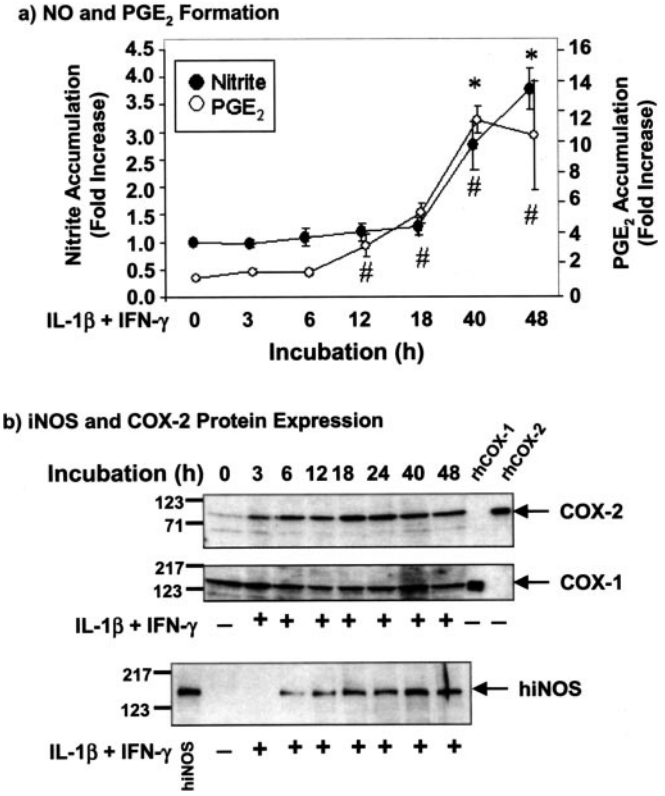

FIG. 4. **Time-dependent effects of IL-1β + IFN-γ on iNOS and COX-2 expression and NO and PGE_2 formation by human islets.** Human islets (120/400 μl of complete CMRL-1066) were treated with 75 units/ml IL-1β + 750 units/ml IFN-γ for the indicated times at 37 °C. After treatment, medium was removed for nitrite and PGE_2 formation (*a*) and iNOS and COX-2 expression by islets was examined by Western blot analysis (*b*) as described under "Experimental Procedures." The results for nitrite are the average ± S.E. 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; #, $p < 0.001$ *versus* untreated control for PGE_2 determinations.

(Fig. 3). These results indicate that selective inhibition of COX-2 does not prevent IL-1β-induced β-cell dysfunction and damage. These findings also support a primary role for NO as the mediator of cytokine-induced inhibition of insulin secretion and islet morphological degeneration.

Effects of IL-1β + IFN-γ on COX-2 Expression and PGE_2 Formation by Human Islets—Although IL-1β 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-1β + IFN-γ or IL-1β + IFN-γ + TNF-α results in iNOS and COX-2 expression and the time-dependent formation of NO and PGE_2, which is maximal following a 48-h incubation (4, 19, 31). 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-1β and 750 units/ml IFN-γ. As shown in Fig. 4*b*, IL-1β + IFN-γ induced the time-dependent expression of COX-2, which was first apparent following a 3-h incubation, maximal following an 18–24-h incubation, and remained elevated for up to 48 h. The expression of the constitutive isoform of COX, COX-1, was unaltered in human islets by cytokine treatment (Fig 4*b*). Consistent with COX-2 expression, PGE_2 accumulation was first apparent following a 12-h incubation, and the levels continued to increase to maximal (~11-fold) levels following 40–48 h of incubation (Fig. 4*a*). Similar to COX-2 expression, this combination of cytokines stimulated the time-dependent expression of iNOS, which was first apparent following a 6-h incubation and maximal following a 40–48 h incubation (Fig 4*b*). Cytokine-induced NO formation by human islets is also time-dependent, correlating with cytokine-induced iNOS expression, with maximal levels following 48 h of incubation (5, 32) (Fig. 4). These results indicate that IL-1β + IFN-γ induce PGE_2 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 PGE_2 and NO Formation by Human Islets—To determine the effects of selective COX-2 inhibition on cytokine-induced production of PGE_2 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 Fig. 5*a*, Celebrex inhibited IL-1 + IFN-γ-induced PGE_2 formation in a concentration-dependent fashion with ~91 and ~97% maximal inhibition observed at 1 and 10 μM, respectively. At 10 μM, Celebrex completely prevented PGE_2 formation in response to either IL-1β + IFN-γ or IL-1β + IFN-γ + TNF-α (Fig. 5*b*). This inhibition of PGE_2 production was not due to a decrease in COX-2 expression, as Celebrex failed to modulate cytokine-induced COX-2 protein expression (Fig. 5*c*). Celebrex also did not modulate cytokine-stimulated nitrite production or iNOS expression by human

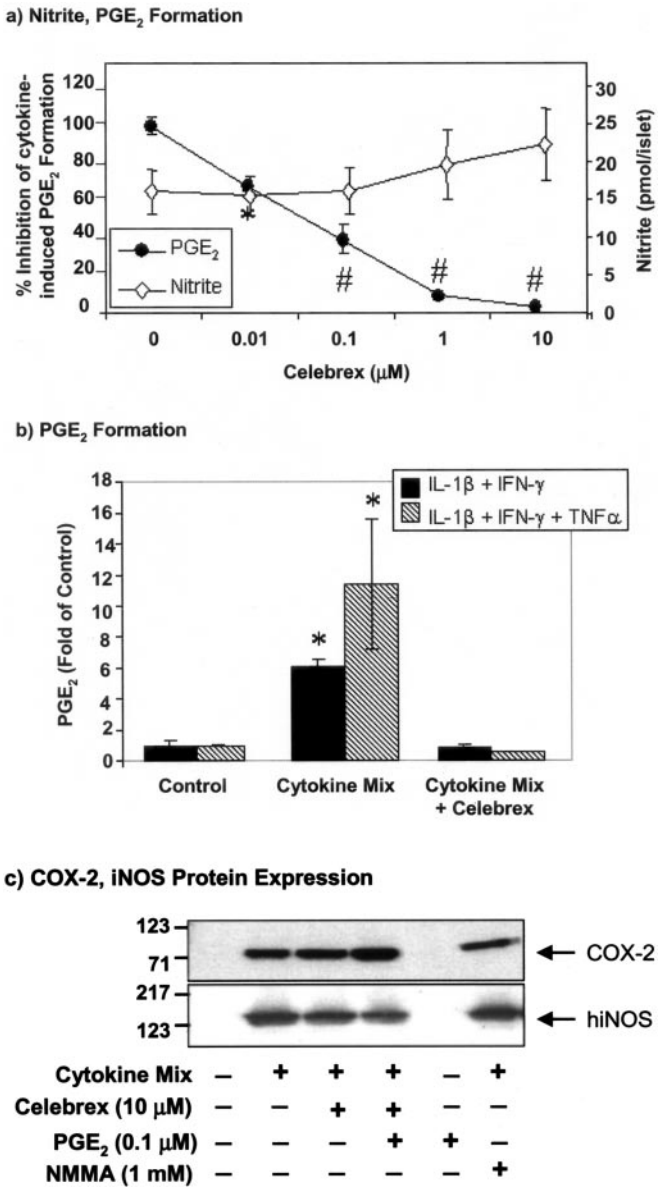

FIG. 5. **Effects of Celebrex on cytokine-induced NO and PGE_2 formation and iNOS and COX-2 expression by human islets.** Human islets (120/400 μl of complete CMRL-1066) were treated with 75 units/ml IL-1β + 750 units/ml IFN-γ in the presence of increasing concentrations of Celebrex for 40 h at 37 °C. After treatment, medium was removed for PGE_2 and nitrite formation (*a*) as described under "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 PGE_2 as indicated for 40 h at 37 °C. After treatment, medium was removed for PGE_2 formation (*b*), and COX-2 and iNOS expression by islets was examined by Western blot analysis (*c*) as described under "Experimental Procedures." Results for nitrite and PGE_2 formation are the average ± S.E. of three independent experiments, and COX-2 protein expression is representative of three independent experiments. Statistical significance: *, $p < 0.05$ *versus* untreated control; #, $p < 0.001$ *versus* untreated control.

islets at concentrations ranging from 0.1 to 10 μM (Fig. 5, *a* and *c*). At 10 μM, Celebrex appeared to slightly increase IL-1β + IFN-γ-induced NO formation (~3.5- *versus* ~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 PGE_2 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.

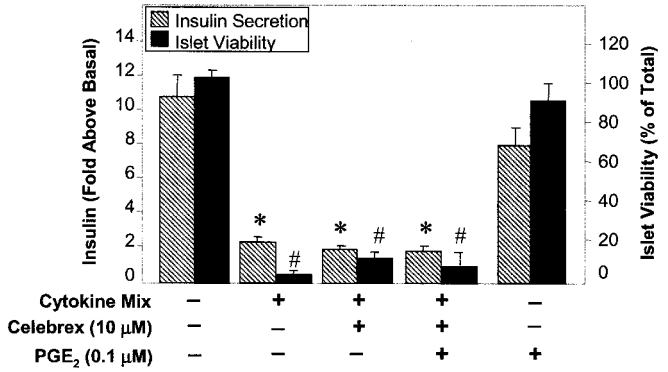

FIG. 6. **Effects of Celebrex on cytokine-induced islet degeneration and inhibition of insulin secretion by human islets.** Human islets (220/ml of complete CMRL-1066) were treated with 75 units/ml IL-1β + 750 units/ml IFN-γ + 10 ng/ml TNF-α in the presence or absence of 10 μM Celebrex or 0.1 μM PGE_2 as indicated for 48 h at 37 °C. Following the incubation period, glucose-induced insulin secretion (*hatched bars*) was examined as stated under "Experimental Procedures." Human islets (25 islets/500 μl 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 PGE_2 as indicated. Islet degeneration (*black bars*) was assessed by phase-contrast microscopy in a blinded fashion. Results for insulin secretion are the average ± S.E. of four independent experiments. Results for islet degeneration are the average ± S.E. of two independent experiments. Statistical significance: $p < 0.05$ *versus* untreated control for insulin secretion (*) and islet viability (#).

Effects of Celebrex on Cytokine-induced Islet Degeneration and Inhibition of Glucose-stimulated Insulin Secretion—To determine whether PGE_2 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 Fig. 6, incubation of human islets with IL-1β + IFN-γ + TNFα, resulted in a ~79% inhibition of glucose-stimulated insulin secretion following 48-h incubation and ~96% islet morphological degeneration following 96 h of incubation. Co-incubation of islets with 10 μM Celebrex, which prevented cytokine-stimulated PGE_2 formation, failed to prevent the inhibitory and destructive effects of cytokines on glucose-stimulated insulin secretion and islet viability. Alone, neither exogenous PGE_2 nor Celebrex inhibited basal or glucose-stimulated insulin secretion or induced human islet degeneration (Fig. 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 β-cell function and islet viability by human islets.

DISCUSSION

Insulin-secreting β-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-1β, TNF-α, and IFN-γ, 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 β-cells (5). The effects of cytokines and the resulting NO production by β-cells on islet function and viability have been well characterized and include decreased oxidative phosphorylation, a potent inhibition of glucose-stimulated insulin secretion, and islet morphological degeneration. Evi-

dence in support of a role for NO in mediating cytokine-induced islet damage includes the protective actions of the 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 in isolated rat and human islets; however, the role of COX-2-derived PGE_2 on β-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-1β and IL-1β + IFN-γ ($\pm$TNF-α), respectively, resulted in the time-dependent production of PGE_2 that was maximal following 24–48 h of incubation. Cytokine-induced PGE_2 formation by rat and human islets correlates with cytokine-induced COX-2 expression, which was first observed following 3 h of incubation and remained at detectable levels of expression following 40–48 h of 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, did 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 streptozotocin 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 did 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 failed 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, used in the study by Tran *et al.* (23), were cultured in RPMI 1640 medium containing 11 mM glucose. 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.* (37) 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. 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 PGE_2 also does not modulate glucose-stimulated insulin secretion (Ref. 17 and current study), suggesting that increased COX-2 expression and PGE_2 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 PGE_2 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, acetosalicylic 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·IκB 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 suggest that 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, because 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 L-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 the 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 cyto-

kine-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 PGE_2 by islets and provides further support for cytokine-induced iNOS expression and NO formation as mediators of these inhibitory and destructive effects.

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