Glucose-Induced Regulation of COX-2 Expression in Human Islets of Langerhans

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Cyclo-oxygenase (COX), the enzyme responsible for conversion of arachidonic acid to prostanoids, exists as two isoforms. In most tissues, COX-1 is a constitutive enzyme involved in prostaglandin-mediated physiological processes, whereas COX-2 is thought to be induced by inflammatory stimuli. However, it has previously been reported that COX-2 is the dominant isoform in islets and an insulin-secreting β-cell line under basal conditions. We have investigated the relative abundance of COX-1 and COX-2 mRNAs in MIN6 cells, a mouse insulin-secreting cell line, and in primary mouse and human islets. We found that COX-2 was the dominant isoform in MIN6 cells, but that COX-1 mRNA was more abundant than that of COX-2 in freshly isolated mouse islets. Furthermore, COX-2 expression was induced by maintenance of mouse islets in culture, and experiments with human islets indicated that exposure of the islets to hyperglycemic conditions was sufficient to upregulate COX-2 mRNA levels. Given that hyperglycemia has been reported to increase human β-cell production of interleukin-1β and that this cytokine can induce COX-2 expression, our observations of glucose-induced induction of COX-2 in human islets suggest that this is one route through which hyperglycemia may contribute to β-cell dysfunction. *Diabetes* 53 (Suppl. 1): S190–S192, 2004

Until recently, type 1 and type 2 diabetes were generally considered to be two related but separate conditions with distinct etiologies. Thus, it is well-established that type 1 diabetes arises through autoimmune destruction of pancreatic β-cells, whereas type 2 diabetes is known to result from a reduced functional capacity of β-cells and/or insensitivity of peripheral tissues to circulating levels of insulin. However, it is clear that individuals initially diagnosed with type 2 diabetes may progress to insulin dependency if the β-cells fail to secrete sufficient insulin to control hyperglycemia (1). There is convincing evidence that the hyperglycemia associated with poorly controlled diabetes has deleterious effects on the β-cell (2,3), and it has been proposed that cytokines, conventional mediators of β-cell destruction in type 1 diabetes, also play a pivotal role in the pathogenesis of β-cell glucotoxicity in type 2 diabetes (4). This recent study indicated that prolonged exposure of human islets to supraphysiological levels of glucose resulted in elevations in interleukin (IL)-1β concentration, and the β-cells themselves were identified as the source of the IL-1β production. In addition, hyperglycemia-induced apoptosis was prevented by an IL-1β receptor antagonist (4).

Cyclo-oxygenase (COX)-2 is known to be induced by proinflammatory stimuli such as cytokines, and there is convincing evidence that prostaglandins generated through COX-2 activation play a major role in proinflammatory reactions (5). Furthermore, selective COX-2 inhibitors have been developed as potential anti-inflammatory agents (6). It has been reported that COX-2, which is normally considered to be an inducible enzyme, is dominant in islets and insulin-secreting cell lines (7), and it is upregulated by IL-1 (7,8). We have now quantified the expression of mRNAs coding for COX-1 and COX-2 isoforms in an insulin-secreting cell line and in mouse and human islets and have examined the effect of hyperglycemia on COX-2 mRNA levels. Our data suggest that COX-2 is not the dominant isoform in freshly isolated islets and that its expression is upregulated by hyperglycemia. These data provide a convincing link between hyperglycemia, as is experienced in poorly controlled type 2 diabetes, and pro-inflammatory events through COX-2 induction.

RESEARCH DESIGN AND METHODS

Tissue. Mouse islets were isolated by collagenase digestion of the pancreas, and human islets, isolated as described previously (9), were provided by Dr. Guo Cai Huang (King's College Hospital, London, U.K.). Mouse islets were used immediately after isolation or after maintenance for 40 h in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FCS, 100 units/ml penicillin, 100 μg/ml streptomycin, and 25 mmol/l glucose. MIN6 insulin-secreting cells were maintained in culture in DMEM (25 mmol/l glucose), and human islets were maintained in culture for 40 h in medium containing 2.5, 5.5, or 25 mmol/l glucose.

Lightcycler RT-PCR. Messenger RNA was extracted from MIN6 cells and islets [Dynabeads Oligo$(dT)_{25}$ kit] and reverse-transcribed, and COX-1, COX-2, and β-actin mRNA levels were amplified by Real-time Lightcycler PCR (10) using the primers and conditions listed in Table 1. COX-2 mRNA levels are expressed either as copies per 2 μl cDNA when being quantified relative to COX-1 in the same sample or as number of copies per 1,000 copies of β-actin mRNA when levels were quantified in different islet populations.

RESULTS

Measurements of the relative levels of COX-1 and COX-2 mRNAs in the insulin-secreting MIN6 cell line by real-time PCR indicated that COX-2 was the dominant isoform expressed by these cells, with levels ~30 times higher than those for COX-1 mRNA (Fig. 1*A*). However, this pattern

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COX, cyclo-oxygenase; DMEM, Dulbecco's modified Eagle's medium; IL, interleukin.

TABLE 1
Primers and annealing temperatures for amplification of mouse and human COX-1, COX-2, and β-actin mRNAs

Gene name	Forward primer	Reverse primer	TAnn (°C)
mCOX-1	CTCACAGTGCGGTCCAAC	CCAGCACCTGGTACTTAAG	52
mCOX-2	TTCAAAAGAAGTGCTGGAAAAGGT	GATCATCTCTACCTGAGTGTCTTT	52
m actin	ACGGCCAAGTCATCACTATTG	AGCCACCGATCCACACAGA	58
hCOX-2	TTCAAATGAGATTGTGGAAAAAT	AGATCATCTCTGCCTGAGTATCTT	50
h actin	ATTGGCAATGAGCGGTTCCG	AGGGCAGTGATCTCCTTCTG	60

The primers were used to amplify mouse (m) and human (h) COX-2 and β-actin mRNAs using a Lightcycler rapid thermal cycler system. Annealing temperatures (TAnn) are also indicated.

was not observed in freshly isolated mouse islets, where COX-1 mRNA levels were around 20-fold higher than those of COX-2 (Fig. 1*B*). We investigated the possibility that the enhanced COX-2 expression observed in the MIN6 cells was a consequence of their being maintained in culture, since the mouse islets were freshly retrieved from experimental animals, whereas the MIN6 cells had been continuously maintained in DMEM (25 mmol/l glucose), supplemented with 10% FCS. Maintenance of mouse islets for 40 h under the same culture conditions used for MIN6 cells resulted in a fivefold elevation in COX-2 mRNA levels (Fig. 2*A*). In addition, when COX-2 mRNA levels were expressed relative to COX-1 levels, under these conditions, the situation was reversed compared with that in freshly isolated islets, and COX-2 was now the dominant isoform (Fig. 2*B*).

The observation that islet COX-2 levels were upregulated by maintenance in culture prompted us to examine whether culture per se or the high glucose concentration of the culture medium was responsible for induction of COX-2. We used human islets for these experiments as the most appropriate model for examining the possible role of COX-2 in islet responses to hyperglycemia in type 2 diabetes. Our data indicated that COX-2 mRNA levels after 40 h of exposure of human islets to 5.5 mmol/l glucose were not significantly different from those seen at 2.5 mmol/l glucose, but that increase of the glucose concentration to 25 mmol/l resulted in a sevenfold increase in COX-2 mRNA levels (Fig. 3).

DISCUSSION

The data that we obtained with MIN6 cells indicating that COX-2 mRNA levels are considerably higher than those of COX-1 are in agreement with an earlier study using the HIT-T15 insulin-secreting cell line (7). Given the earlier report that COX-2 is dominant in islets under basal conditions (7), we were surprised to find that COX-2 mRNA was present at a considerably lower abundance than COX-1 mRNA in mouse islets. However, the MIN6 cells in these studies and the HIT-T15 cells in the earlier report (7) had been maintained in culture medium that contained two potential regulators of COX-2 expression. Thus, COX-2 is induced by growth factors at levels that may be obtained in serum-supplemented medium (11), and high concentrations of glucose stimulate the release of IL-1β from β-cells (4), which can upregulate COX-2 (7,8). Our observation of upregulation of COX-2 in mouse islets maintained under the same conditions as MIN6 cells did not discriminate between whether the induction arose from exposure to growth factors or to the hyperglycemic environment. However, our experiments using human islets maintained at a range of glucose concentrations, with unchanged addition of serum, indicated low levels of COX-2 mRNA at sub- and threshold stimulatory concentrations of glucose and significant elevation of COX-2 mRNA after overnight exposure of the human islets to 25 mmol/l glucose. These data indicate that it is the hyperglycemia rather than the exposure to serum that is responsible for COX-2 induction. Interestingly, high levels of COX-2 mRNA have also been observed in human islets maintained for 18 h at 11 mmol/l glucose in 0.2% serum (7).

In conclusion, the current data indicate that COX-2 mRNA levels are increased by maintenance of human islets in a hyperglycemic environment. It seems likely, given earlier reports (4,7,8), that glucose stimulates COX-2 induction in human islets through IL-1β production. The concomitant upregulation of IL-1β and COX-2 by human

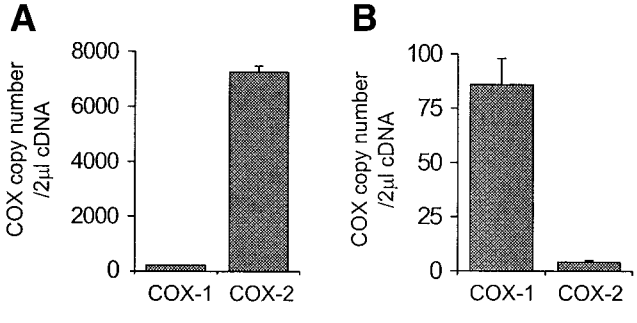

FIG. 1. Relative expression of COX-2 and COX-1 mRNAs in MIN6 insulin-secreting cells and freshly isolated mouse islets. COX-2 mRNA expression in MIN6 cells (*A*) was 32-fold higher than that of COX-1 mRNA, but in freshly isolated mouse islets (*B*), COX-1 mRNA was predominant. Data are means ± SE. $n = 6$ for MIN6 cells, and $n = 12$ for mouse islets. $P < 0.0001$ COX-1 vs. COX-2 for both groups.

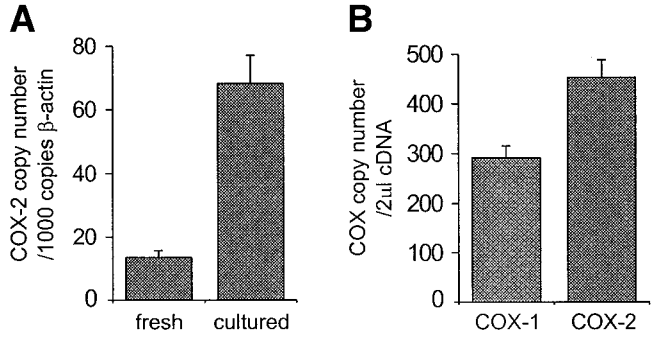

FIG. 2. COX-2 mRNA levels in mouse islets maintained in culture (25 mmol/l glucose, 10% FCS) for 40 h. COX-2 mRNA levels were increased fivefold ($P < 0.01$) by maintenance of mouse islets in culture (10% FCS, 25 mmol/l glucose) for 40 h (*A*). Whereas COX-1 was the predominant COX isoform in freshly isolated mouse islets (Fig. 1), COX-2 mRNA levels were significantly ($P < 0.01$) higher than those of COX-1 after maintenance of mouse islets in culture (*B*). Data are means ± SE; $n = 4$ for both *A* and *B*.

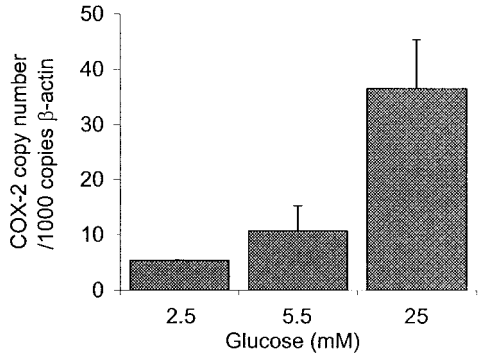

FIG. 3. Glucose-dependent upregulation of COX-2 mRNA levels in human islets. Exposure of human islets to 5.5 mmol/l glucose for 40 h did not significantly elevate COX-2 mRNA levels above those seen at 2.5 mmol/l glucose ($P > 0.2$), but a 20-fold increase in glucose concentration (from 2.5 to 25 mmol/l) resulted in a sevenfold increase in COX-2 mRNA levels ($P < 0.01$). Data are means ± SE; $n = 5$.

islets under conditions of hyperglycemia may contribute to the progressive dysfunction of β-cells in type 2 diabetes that culminates in the requirement of insulin therapy.

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