

N. Shanmugam · I. T. Todorov · I. Nair · K. Omori ·  
M. A. Reddy · R. Natarajan

## Increased expression of cyclooxygenase-2 in human pancreatic islets treated with high glucose or ligands of the advanced glycation endproduct-specific receptor (AGER), and in islets from diabetic mice

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**Abstract** *Aims/hypothesis:* The cyclooxygenase-2 (PTGS2, previously known as COX2) enzyme and its products, such as prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), have been implicated in the pathogenesis of several inflammatory diseases including islet dysfunction under diabetic conditions. In this study we evaluated whether diabetic conditions in vitro, such as high-glucose (HG) culture or AGE, or in vivo in animal models of diabetes can induce *PTGS2* expression and activity in pancreatic islets. *Materials and methods:* Isolated human pancreatic islets were treated for 24 h with HG (25 mmol/l) or with S100b (5 mg/l), a specific ligand for the AGE-specific receptor. *PTGS2* and cyclooxygenase-1 (*PTGS1*, previously known as *COX1*) mRNA, protein expression and product PGE<sub>2</sub> were analysed by RT-PCR, Western blots and specific enzyme immunoassay respectively. Islet *PTGS2* production in animal models was assessed by immunofluorescence. *Results:* Treatment of human pancreatic islets with HG and S100b led to a three–five-fold induction of *PTGS2* mRNA ( $p < 0.001$ ). *PTGS2* protein and its product PGE<sub>2</sub> (351.4 ± 13.05 fg/ml vs control 39.4 ± 0.11 fg/ml) were also increased ( $p < 0.001$ ). Pretreatment with specific inhibitors demonstrated the involvement of protein kinase C and oxidant stress in S100b- and HG-induced *PTGS2* expression. However, insulin secretion was not significantly altered by S100b. Double immunofluorescent staining showed increased *PTGS2* production in pancreatic islets from diabetic mice relative to corresponding controls. *Conclusion/interpretation:* These results show for the first time that diabetes as well as diabetic conditions such as AGE and HG

in vitro can directly upregulate the expression of the inflammatory *PTGS2* gene in pancreatic islets. This might contribute to the pathogenesis of islet dysfunction in diabetes.

**Keywords** Advanced glycation endproducts · Cyclooxygenase-2 · *db/db* mice · Human islets · Receptor for advanced glycation endproducts · Streptozotocin-induced diabetes

**Abbreviations** AGER: AGE-specific receptor · DAPI: 4',6-diamidino-2-phenylindole · EIA: enzyme immunoassay · GFX: bisindolylmaleimide · HG: high glucose · NF-κB: nuclear factor-kappa B · NAC: *N*-acetyl cysteine · NG: normal glucose · PGE<sub>2</sub>: prostaglandin E<sub>2</sub> · PTGS: cyclooxygenase · PTGS1: cyclooxygenase-1 · PTGS2: cyclooxygenase-2 · STZ: streptozotocin · TFA: thenoylfluoroacetone

### Introduction

Cyclooxygenase (PTGS, previously known as COX) enzymes catalyse the rate-limiting step in the conversion of arachidonic acid to prostaglandins [1–5], which are important mediators of acute and chronic inflammation, development and immune functions [6]. The constitutively expressed cyclooxygenase-1 (PTGS1, previously known as COX1) isoform appears to regulate many normal physiological functions in several cell types, whereas the inducible isoform, cyclooxygenase-2 (PTGS2, previously COX2), is usually expressed at low levels in most tissues and cells, but is significantly induced by a wide range of inflammatory stimuli such as lipopolysaccharide, cytokines and chemicals [1, 2]. *PTGS2* overexpression has been demonstrated in several human inflammatory diseases [2]. In the pancreatic islet *PTGS2* is constitutively and dominantly expressed [7, 8] and here *PTGS2* products such as prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) are believed to play a role in inflammation, islet destruction and inhibition of insulin secretion [7–9]. A

N. Shanmugam · I. T. Todorov · I. Nair · K. Omori ·  
M. A. Reddy · R. Natarajan (✉)  
Gonda Diabetes Centre,  
Beckman Research Institute of City of Hope,  
1500 East Duarte Road,  
Duarte, CA 91010, USA  
e-mail: rnatarajan@coh.org  
Tel.: +1-626-359-8111  
Fax: +1-626-301-8136

recent report showed that *PTGS2* mRNA, however, was present in lower abundance than *PTGS1* in islets [10].

The diabetic state impairs beta cell function in animals and humans. Type 1 diabetes mellitus is caused by autoimmune and inflammatory processes in the pancreas, leading to selective destruction of the beta cell [11, 12]. Evidence indicates that both hyperglycaemia and AGE are important mediators of beta cell dysfunction [13]. Exposure of human islets to a high glucose (HG) concentration resulted in increased apoptosis of beta cells and this was attributed to the IL1B-nuclear factor-kappa B (NF-κB) pathway [14]. Furthermore, inflammatory cytokines released by infiltrating macrophages have been implicated as effector molecules that participate in both islet inflammation and beta cell destruction during the development of diabetes [8, 15]. For instance, it was shown that when isolated rat islets are incubated with IL1B, IFNG and TNF they synergistically lead to the impairment of beta cell function and cause beta cell death; this appeared to be due the induction of *PTGS2*, oxidant stress and inducible nitric oxide synthase [8]. The molecular mechanisms responsible for the cytotoxic effect of these cytokines, particularly under diabetic conditions, remains to be elucidated. HG could induce IL1B in human pancreatic islets [14], and very recently Persaud et al. [10] showed that HG can induce *PTGS2* in islets. However, it is not clear whether under diabetic conditions in vivo or in vitro, treatment with AGE can directly induce *PTGS2* expression in mouse and human islets.

Chronic hyperglycaemia-mediated diabetic complications are, in part, related to non-enzymic glycation of proteins and lipids, and its sequelae. Early glycated molecules can be further modified by the formation of AGE that act via receptors such as the AGE-specific receptor (AGER, previously known as RAGE) and lead to cellular damage underlying the microvascular and macrovascular complications of diabetes [16]. However, it not known to what extent AGE directly influence pancreatic beta cell function.

In this study we show for the first time that a specific inflammatory ligand of the AGER, S100b, can induce *PTGS2* expression and activity in isolated human pancreatic islets. This was similar to the effects of HG. We have also shown that *Ptgs2* expression is increased in vivo in the islets of mouse models of type 1 and type 2 diabetes mellitus. These studies are significant because early cellular dysfunction and inflammation induced by HG and AGE in islets may play major roles in the pathogenesis of diabetes and its complications.

## Materials and methods

**Materials** RT-PCR reagents were from Applied Biosystems (Foster City, CA, USA) while Quantum RNA 18S Internal Standards were from Ambion (Austin, TX, USA). PGE<sub>2</sub> enzyme immunoassay (EIA) kit was from Cayman Chemical (Ann Arbor, MI, USA). Anti-*PTGS2* antibody was from BD Transduction Laboratories (Palo Alto, CA, USA) or from Cayman Chemical, and anti-insulin antibody was from Linco Immunoresearch (St Charles, MO, USA).

**Cell culture and animal treatments** Normal human pancreatic islets were obtained from the Southern California Islet Consortium (City of Hope Medical Centre, Duarte, CA, USA) and cultured in RPMI-1640 medium supplemented with 10% FCS, glutamine, HEPES, streptomycin (100 µg/ml)-penicillin (100 U/ml) and D-glucose (3.5 mmol/l) (normal glucose, NG) in a 5% CO<sub>2</sub> incubator at 37°C. For experiments, islets were treated with or without D-glucose (25 mmol/l) (HG) or S100b (5 mg/l) (Calbiochem, San Diego, CA, USA).

We purchased *db/db* mice and their genetic control *db/m* mice, and C57BL/6J mice from Jackson Laboratory (Bar Harbor, ME, USA). In addition, diabetes was induced in 11-week-old C57BL/6J mice by injection of 50 mg streptozotocin (STZ)/kg body weight (concentration 7.5 mg/ml) on five consecutive days. Streptozotocin (Sigma Chemical, St Louis, MO, USA) was dissolved in a sodium citrate solution (10 mmol/l) containing 0.9% NaCl, pH 4.5, immediately before administration. Diabetes induction was assessed by measuring glycaemia levels using Accu-chek Advantage (Roche Diagnostics, Indianapolis, IN, USA). STZ-treated mice were killed 4 days after the last injection when blood glucose levels reached >20 mmol/l. *db/db* and *db/m* mice were killed at about 11 weeks of age. Pancreases from all mice were dissected out and fixed in 10% formalin solution. All experimental procedures involving mice were performed in accordance with protocols approved by the City of Hope/Beckman Research Institute Research *Animal Care Committee*.

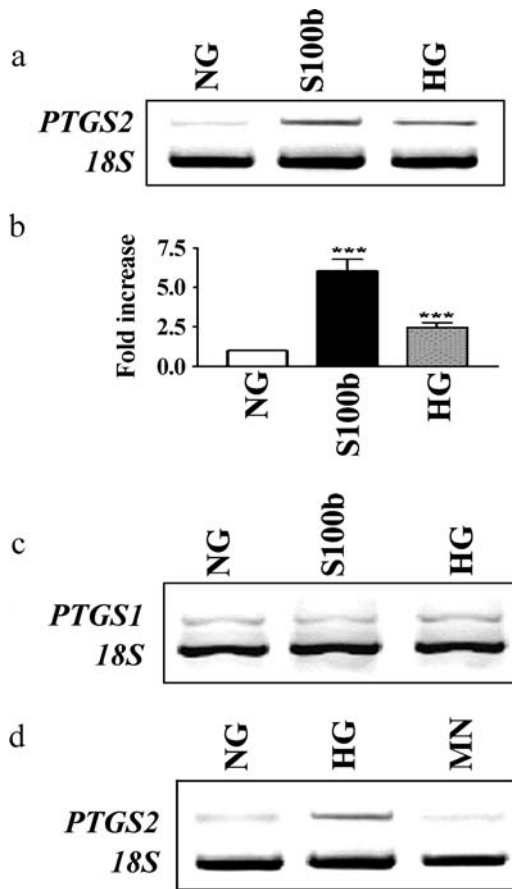
**RNA preparation and relative RT-PCR** Human islets (500 islet equivalents per sample) in 3 ml medium containing 3.5 mmol/l (NG) alone or treated either with S100b (5 mg/l) or 25 mmol glucose/l (HG) or 21.5 mmol mannitol/l (osmolarity control) were cultured in duplicate in six-well dishes for 24 h. Total RNA was isolated by RNA-STAT-60 method [17, 18] and 1 µg used for the RT reaction using Gene Amp RNA PCR kit. Complementary DNA corresponding to 0.05 µg RNA was then used in multiplex PCR reactions as described in [17, 18]. Results are expressed as fold stimulation over NG after normalising with paired 18S RNA levels.

**Western blot analysis and PGE<sub>2</sub> EIA** Western blot analysis and PGE<sub>2</sub> EIA were performed using lysed human islets and medium supernatant fractions respectively as described previously [17, 18]

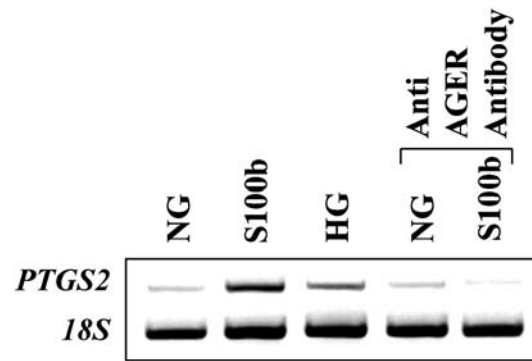
**In vitro insulin release assays** To assess the insulin release function of the islets, a static incubation assay was performed. The static incubation assays were performed on islets pre-treated overnight with S100b, alone or in combination with the inhibitors of oxidant stress (*N*-acetyl cysteine, NAC), mitochondrial complex II inhibitor (thenoyltrifluoroacetone, TTFA) and protein kinase C (bisindolylmaleimide, GFX) in RPMI medium containing low glucose (3.3 mmol/l). Before the static incubation assay, the islets were washed five times with low-glucose medium

(RPMI containing 3.3 mmol glucose/l and 2% FCS) and then incubated in low-glucose medium. After 30 min, the medium was collected for insulin assay (first low-glucose sample) and then replaced by HG stimulation medium (RPMI 1640 containing 19.4 mmol glucose/l and 2% FCS). After incubation for another 30 min, supernatant fractions (HG samples) were collected, islets were washed five times with basal medium and further incubated with basal medium for 30 min to obtain a second low-glucose sample. Collected samples were frozen and stored until measurement of human insulin using an EIA kit (Alpco, Windham, NH, USA). The stimulation index was calculated as insulin released into the HG medium divided by insulin released into the low-glucose medium.

**Analysis of *PTGS2* expression by immunofluorescence microscopy** Formalin-fixed pancreas tissues from mice were processed, and paraffin sections were treated with

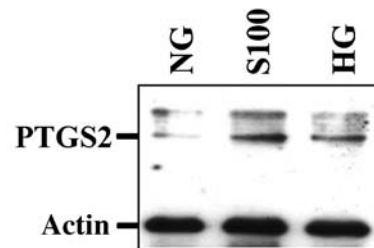


**Fig. 1** Ethidium bromide-stained agarose gels of RT-PCR products and bar graph showing that treatment of normal human donor islets with HG and S100b leads to increased *PTGS2* mRNA expression. Relative RT-PCR with gene-specific primers were performed with total RNA isolated from normal human pancreatic islets treated with HG or S100b for 24 h. 18S RNA primers were included in each PCR reaction as internal control. PCR products were analysed on agarose (2.5%) gels. **a** RT-PCR for *PTGS2*; **b** Results quantitated from six separate experiments ( $n=6$ , \*\*\* $p<0.001$  vs NG); **c** *PTGS1* mRNA; results representative of four similar experiments. **d** *PTGS2* mRNA in cells treated with HG or mannitol (MN); results representative of four similar experiments



**Fig. 2** S100b-induced *PTGS2* mRNA expression is via AGER. Normal human islets were pre-treated with anti-AGER antibody (70  $\mu\text{g/ml}$ ) for 1 h followed by S100b (5 mg/l) for 24 h. *PTGS2* mRNA levels were analysed by RT-PCR using specific primers for *PTGS2* and 18S internal control. Results are representative of four similar experiments

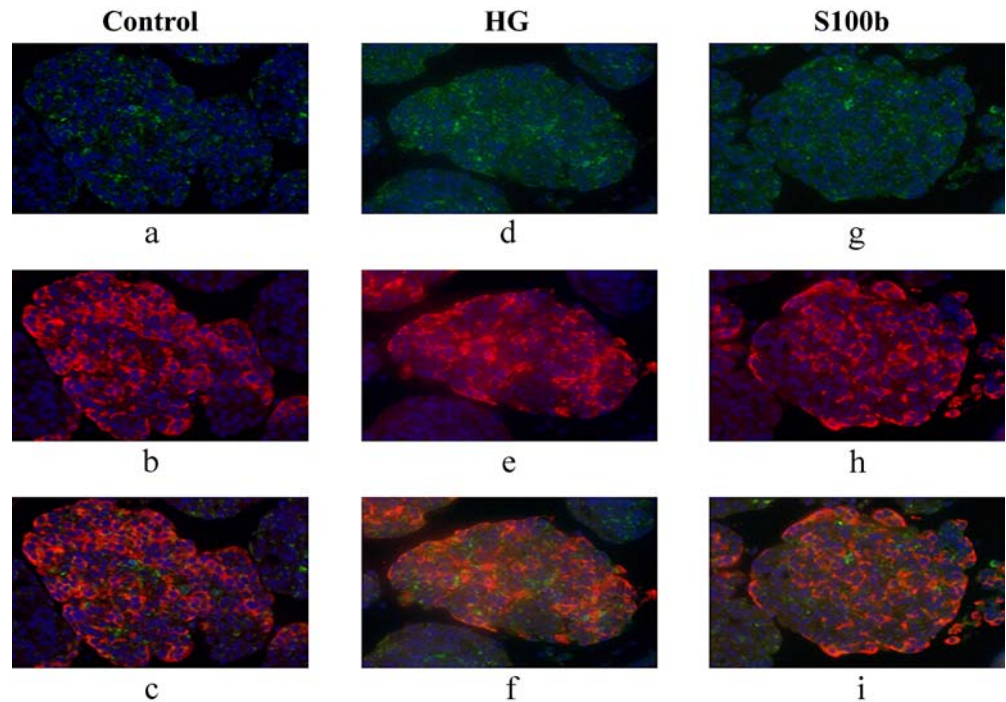
anti-*PTGS2* and anti-insulin antibodies for immunofluorescence analyses. Isolated human islets were treated with S100b (5 mg/l) or HG (25 mmol/l) for 24 h, fixed with formalin and embedded in paraffin. Paraffin-embedded samples were deparaffinised/rehydrated, treated with antigen retrieval solution (Vector Laboratories, Burlingame, CA, USA), according to the manufacturer's protocols, and then processed for double immunofluorescence [19] using rabbit or mouse anti-*PTGS2* antibodies (Becton Dickinson, San Jose, CA, USA) and guinea pig anti-insulin (Linco Immunoresearch) followed by anti-mouse, anti-rabbit or anti-guinea pig Ig antibodies conjugated with fluorescein isothiocyanate or Texas Red (Jackson Immunoresearch, West Grove, PA, USA). After intensive washing the samples were counterstained for DNA with 4',6-diamidino-2-phenylindole (DAPI) (Sigma Chemical), embedded in Vectashield (Vector Laboratories, Burlingame, CA, USA), visualised and documented with an Olympus BX51 microscope (Olympus America, Melville, NY, USA) equipped with a Pixera 600 cooled charged coupled device camera (Pixera, Los Gatos, CA, USA). Fluorescent images were analysed using the ImagePro Plus image analysis software (Media Cybernetics, Silver Spring, MD, USA). The relative fluorescence intensity of the islet areas corresponding to



**Fig. 3** Western blot analysis of *PTGS2* protein in HG- and S100b-treated human islets. Total protein isolated from control untreated human islets or islets treated with HG or S100b for 24 h were resolved by SDS-PAGE, transferred onto Immobilon membranes, and probed with an anti-*PTGS2* polyclonal antibody. As a control for protein loading, the *PTGS2* immunoblot was probed with actin

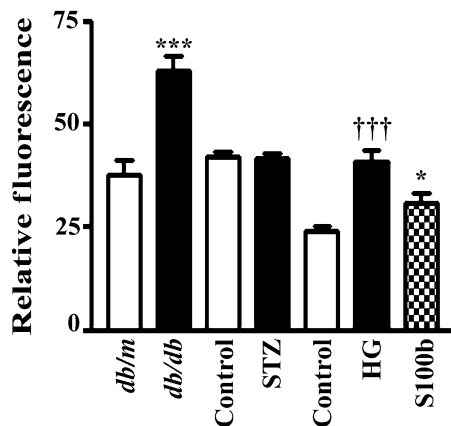


**Fig. 4** PTGS2 protein expression in S100b- and HG-treated human pancreatic islet sections as evaluated by immunofluorescence microscopy. PTGS2 and insulin shown in *green* and *red*, respectively. PTGS2, insulin and merged staining respectively in: **a–c** control human islets; **d–f** islets treated with HG; and **g–i** islets treated with S100b. After fixation, human islet sections were treated with anti-PTGS2 and anti-insulin antibodies, followed by secondary antibodies conjugated with fluorescein isothiocyanate and Texas Red respectively, counterstained for DNA (*blue*) and examined in a fluorescent microscope



PTGS2 staining was measured. The relative fluorescence was generated as mean and SD for each islet area. These were averaged and provided an estimate of relative fluorescence.

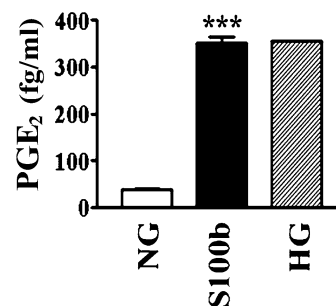
**Data analyses** Data are expressed as mean  $\pm$  SEM of multiple experiments. Paired Student's *t* tests were used to compare two groups and ANOVA for multiple comparisons. Values of  $p < 0.05$  were considered statistically significant.



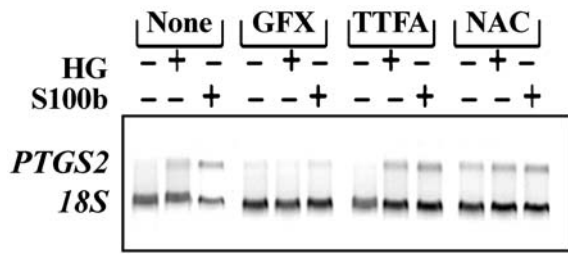
**Fig. 5** Quantitative analysis of PTGS2 immunofluorescence data. The relative fluorescence intensity of PTGS2 staining in the islet areas was quantified using ImagePro Plus software (Media Cybernetics). This was performed in four random islet areas for pancreatic tissue sections from both mouse groups, and from human islets treated with or without HG or S100b. All values are expressed as mean  $\pm$  SEM ( $n=3-6$ ). \* $p < 0.05$  (untreated control vs S100b), \*\*\* $p < 0.001$  (control *db/m* vs *db/db*), ††† $p < 0.001$  (untreated control vs HG)

## Results

**HG and ligation of AGER with S100b increase the expression of PTGS2 but not PTGS1 isoform in human pancreatic islets** Human islets were treated for 24 h either with HG or S100b (5 mg/l), a specific AGER ligand. RNA isolated from these samples was analysed for *PTGS2* mRNA expression by relative RT-PCR with 18S as internal control for normalisation. HG treatment significantly induced a two–three-fold increase, while S100b treatment led to a five–six-fold significant increase in *PTGS2* mRNA expression in human islets compared with NG-grown islets ( $p < 0.001$ ; Fig. 1a,b). Both freshly isolated and frozen stored samples of human islets showed similar increases in *PTGS2* mRNA expression (results not shown). In contrast, neither HG nor S100b had any effect on *PTGS1* mRNA expression (Fig. 1c). Furthermore, Fig. 1d shows that *PTGS2*



**Fig. 6** PGE<sub>2</sub> levels in HG- or S100b-treated islets relative to control NG-cultured islets. PGE<sub>2</sub> was measured by EIA in culture supernatant fractions of human islets treated with or without HG and S100b for 24 h. Values are mean  $\pm$  SEM of three independent experiments ( $n=4$ ). \*\*\* $p < 0.001$  vs S100b. For HG-treated islets the value shown is the mean of two experiments



**Fig. 7** Effects of antioxidant, superoxide and protein kinase C inhibitors on *PTGS2* mRNA expression. Human islets were pre-treated with various inhibitors (protein kinase C inhibitor, GFX; mitochondrial complex II inhibitor, TTFA; antioxidant, NAC) for 1 h before HG or S100b stimulation. Total RNA was isolated post HG and S100b stimulation and *PTGS2* mRNA levels were analysed by RT-PCR as described in Fig. 1. – absence, + presence of S100b or HG, respectively

mRNA was increased only by HG and not by equimolar concentrations of mannitol (osmolality control), indicating the specificity of HG effects. These results suggest that HG and S100b specifically upregulate the inducible inflammatory isoform encoded by *PTGS2*, but do not upregulate *PTGS1* mRNA.

In the next step, we examined whether S100b-induced *PTGS2* mRNA expression occurs via the AGER receptor in islets. Islets were pre-treated with a specific anti-AGER antibody for 1 h before S100b treatment. RT-PCR analyses of RNA from these islets showed complete blockade of the S100b induction of *PTGS2* mRNA levels in antibody-treated islets (Fig. 2), thereby confirming that induction of *PTGS2* mRNA by S100b is via AGER activation.

**HG and AGER ligand increase the production of *PTGS2* protein in human pancreatic islets** Our observations that islet *PTGS2* mRNA levels were upregulated by simulated diabetic conditions in culture, such as HG and AGER ligation, prompted us to examine whether *PTGS2* protein levels were also altered. Western blot analysis with a specific *PTGS2* antibody was carried out using total protein prepared from human islets cultured with NG, HG or S100b. Treatment (24 h) of both HG and S100b showed a clear increase in *PTGS2* protein levels (upper panel of Fig. 3). Equal loading of protein in each lane was confirmed by probing with an anti-actin antibody as internal control (Fig. 3).

We also examined *PTGS2* protein production by immunofluorescence microscopy using specific anti-*PTGS2* antibodies. Sections from untreated human islets or those stimulated with either HG or S100b were treated with anti-*PTGS2* and anti-insulin antibodies. Fig. 4 shows results of *PTGS2* and insulin immunofluorescence analyses. HG- and S100b-treated islets (Fig. 4d–i) showed clear increases in *PTGS2* expression relative to islets cultured in NG (Fig. 4a–c). Increased *PTGS2* production was detected in both insulin-positive as well as in insulin-negative cells (Fig. 4c,f and i). Similarly, immunofluorescence intensity analyses (Fig. 5) showed a significant increase in *PTGS2*

expression in isolated human islets that were treated with HG and S100b, when compared with control ( $p < 0.05$ ).

***S100b and HG increase *PTGS2* enzyme activity in human pancreatic islets*** To determine whether the induction of *PTGS2* mRNA and protein levels were also associated with increase in *PTGS2* enzyme activity, we next examined the levels of the *PTGS2* product  $PGE_2$  in HG and S100b-treated vs NG-grown normal human islets in vitro.  $PGE_2$  released into the culture supernatant fractions was measured by a specific EIA. As shown in Fig. 6, S100b or HG treatment for 24 h significantly increased  $PGE_2$  levels ( $351.4 \pm 13.05$  fg/ml,  $n=4$ ,  $p < 0.001$  for S100b;  $355$  fg/ml,  $n=2$  for HG) compared with NG ( $39.44 \pm 0.1, 135$  fg/ml,  $n=4$ ). Overall, these results show that diabetic islets have high levels of *PTGS2* mRNA and protein expression and its product  $PGE_2$ , and this might contribute to several adverse events.

**Signal transduction mechanisms involved in S100b and HG-induced *PTGS2* mRNA expression** In order to determine the key signal transduction pathways involved in HG-induced *PTGS2* mRNA in islets, we evaluated the effects of inhibitors of pathways known to be activated by HG and S100b, including signalling kinases and oxidant stress. Islets were pre-incubated with either an antioxidant (NAC), a mitochondrial complex-II inhibitor to block mitochondrial superoxide production (TTFA) or protein kinase C inhibitor (GFX); *PTGS2* mRNA induction by HG or S100b was determined by RT-PCR. The representative RT-PCR blot in Fig. 7 shows that HG- and S100b-induced *PTGS2* mRNA expression was blocked by protein kinase C inhibitor (GFX) and antioxidant (NAC) in islets. However, TTFA had no effect on HG-induced *PTGS2* mRNA expression. These results implicate the involvement of multiple pathways including protein kinase C and oxidant stress in HG- and S100b-induced *PTGS2* mRNA expression in islets.

In parallel experiments, the functionality of islets was analysed by examining glucose-induced insulin secretion after consecutive incubations in low glucose (3.3 mmol/l)

**Table 1** Glucose-induced insulin secretion

Treatment	Insulin (stimulation index)	<i>p</i> value (ANOVA)
Control	2.65±1.51	–
S100b	1.31±0.047	0.103 (control vs S100b)
Control + TTFA	2.02±0.83	–
S100b + TTFA	1.19±0.32	0.073 (control vs S100b)
Control + GFX	1.01±0.25	–
S100b + GFX	1.6±0.76	0.06 (control vs S100b)

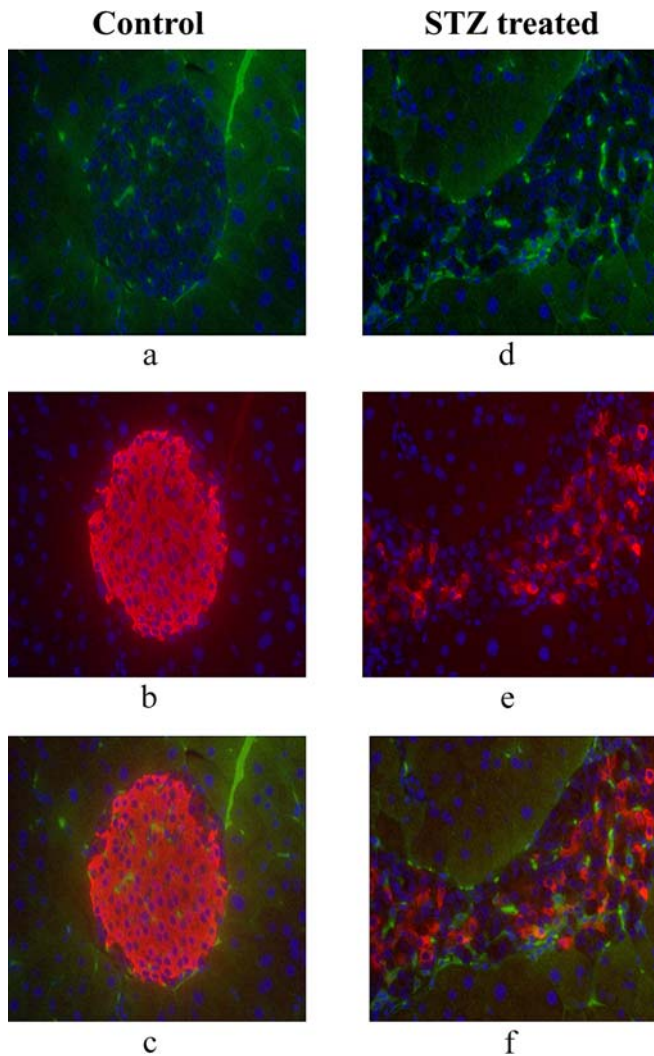
Human pancreatic islets pre-treated with S100b and TTFA or GFX inhibitors and were incubated consecutively for 30 min each in low-glucose medium (3.3 mmol/l) and with a HG (19.4 mmol/l) medium. Insulin secreted in the medium was measured by EIA. Stimulation indexes were calculated by dividing the insulin released in HG medium by in low-glucose medium during the 30 min periods. The results are presented as mean±SD ( $n=4-6$ ).  $p < 0.05$  was considered significant



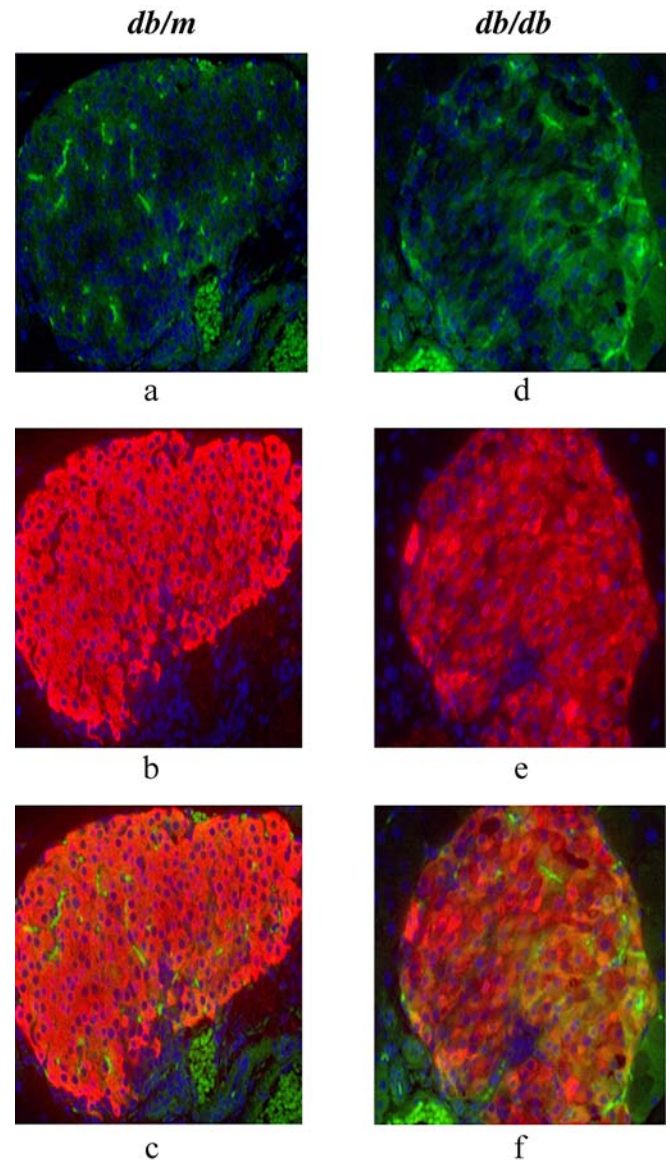
and HG (19.4 mmol/l) glucose. The stimulation index was calculated as the ratio between the insulin secreted at HG and insulin secreted at low glucose. This was tested in islets pre-treated with S100b alone and in combination with the protein kinase C inhibitor, GFX or TTFA. Results shown in Table 1 show that the insulin stimulation index in islets treated with S100b alone or in the presence of inhibitors was slightly decreased relative to control, but this effect was not statistically significant, thus indicating that S100b had no significant effect on insulin secretion.

*PTGS2 expression is increased in vivo in pancreatic islets of mouse models of type 1 and type 2 diabetes mellitus* To further analyse the in vivo relevance of elevated PTGS2 expression, pancreatic tissue sections from STZ-induced diabetic mice (model of type 1 diabetes mellitus), from *db/db* diabetic mice (model of type 2 diabetes mellitus) and

from their respective control groups were studied. PTGS2 protein production was analysed by immunofluorescent staining using specific anti-PTGS2 and anti-insulin antibodies to identify insulin-producing beta cells. PTGS2 staining was detected in both insulin-positive (beta) cells, as well as in insulin-negative cells. Our results in Fig. 8 show that there was a slight increase in PTGS2 protein production in the islets of STZ-induced diabetic mice (Fig. 8d–f) compared with saline-injected control C57BL/6 mice (Fig. 8a–c). However, quantitative image analysis (Fig. 5) shows that this was not statistically significant. On the other hand, PTGS2 protein production was greater in islets of diabetic *db/db* mice (Fig. 9d–f) compared with their genetic



**Fig. 8** Immunofluorescence analyses of PTGS2 expression in pancreatic sections from control and STZ-treated diabetic mice injected with citrate buffer (Control) or treated with STZ. Blood glucose levels were >20 mmol/l in STZ-treated mice. Pancreatic sections were treated with (a, d) anti-PTGS2 (green), (b, e) and anti-insulin (red). (c, f) Merged stains. The staining was performed as in Fig. 4 (see Materials and methods)



**Fig. 9** Immunofluorescence analyses of PTGS2 expression in pancreas tissue from control (*db/m*) and *db/db* mice. Pancreatic tissue was fixed and treated with anti-PTGS2 (green) and anti-insulin antibody (red) as in Fig. 4 (see Materials and methods). (a–c) Immunofluorescent staining for PTGS2, insulin and merged staining, respectively in control mice, and (d–f) in *db/db* mice

control, *db/m* mice (Fig. 9a–c) and this was statistically significant ( $p < 0.001$ , Fig. 5).

## Discussion

In this study we have demonstrated that treatment of normal human donor pancreatic islets with diabetic stimuli such as AGER ligands or HG can lead to a significant increase in *PTGS2* mRNA and protein expression and product formation. In contrast, HG or S100b did not alter the expression of *PTGS1* mRNA. Furthermore, human islets maintained at the equimolar concentration of mannitol did not induce *PTGS2* mRNA, thereby suggesting that increased *PTGS2* mRNA is specific to HG. The effects of the AGER ligand, S100b, on *PTGS2* mRNA induction was blocked by an anti-AGER antibody, thereby demonstrating the involvement of AGER in *PTGS2* induction.

*PTGS2* protein expression was also increased in vitro in normal human islets treated with HG or S100b. Furthermore, we also noted increased *PTGS2* in vivo in islets of mouse models of type 1 and type 2 diabetes mellitus. Increased *PTGS2* expression/staining was present in beta cells (insulin-positive cells) as well as in non-beta cells (insulin-negative cells). We noted similar increases in *PTGS2* immunostaining compared with controls in the islets of diabetic *db/db* mice and STZ-induced diabetic mice, and with the same mosaic pattern in beta and non-beta cells. Expression levels of *PTGS2* in beta cells were greater in *db/db* mice relative to STZ-injected mice. This might be a reflection of the fact that the STZ-injected mice have greatly reduced numbers of islets and extensive beta cell destruction. Interestingly, in a preliminary unpublished study we similarly noted a marked increase in *PTGS2* expression (by RT-PCR and immunofluorescence) in the islets of a human donor with type 2 diabetes relative to normal non-diabetic donors (results not shown).

Although *PTGS2* and its products have been implicated in beta cell destruction and islet dysfunction, the exact source of *PTGS2* in the islet was not verified in these earlier studies. Cytokines produced by infiltrating macrophages have been shown to clearly mediate beta cell apoptosis and dysfunction. Our study suggests that, in addition, diabetic conditions can also directly increase *PTGS2* expression in islet cells, including beta cells, and this may also contribute to islet dysfunction. Increased *PTGS2* expression could be either direct or via induction of cytokines, such as IL1B, that are potent inducers of *PTGS2*. Furthermore, *PTGS2* expression is known to be regulated by both transcriptional and post-transcriptional mechanisms [20] and it is possible that both are in operation in islets as demonstrated in monocytes [17]. While our demonstration of increased *PTGS2* expression in vitro and in vivo under diabetic conditions does not directly implicate *PTGS2* in the pathogenesis of diabetes, it is highly likely—given the fact that *PTGS2* is induced by inflammatory cytokines and that *PTGS2* products have inflammatory properties—that increased *PTGS2* expression can mediate islet dysfunction and the development or progression of diabetes. This is

supported by studies showing the beneficial effects of *PTGS2* inhibitors in a model of diabetes [21].

Our studies with inhibitors showed that an oxidant NAC and a protein kinase C inhibitor GFX blocked HG- or S100b-induced *PTGS2* expression in islets. In contrast, TFA, which inhibits mitochondrial superoxide production, did not have any effect. These results suggest that multiple signalling pathways including oxidant stress and protein kinase C are involved in HG- or S100b-induced *PTGS2* expression in islets. Furthermore, treatment of islets with S100b alone or in the presence of these inhibitors did not significantly affect glucose-induced insulin secretion. These results are consistent with published results showing that cytokine-induced *PTGS2* did not affect insulin secretion in islets [22, 23] and further support observations that *PTGS2* inhibitors failed to prevent cytokine-induced beta cell dysfunction [23]. In addition, a very recent study [24] showed that *PTGS2* inhibition enhances anti-tumour immunity. These authors suggest that *PTGS2* and its product  $PGE_2$  can modulate immune responses by upregulating the activity of T-lymphocytes. In the context of islets and our results, it is possible that S100b-, HG- and diabetes-induced *PTGS2* expression may modulate autoimmunity and thereby cause islet dysfunction. More extensive studies are needed, including the evaluation of *PTGS2*-deficient mice, specific *PTGS2* inhibitors or similar anti-inflammatory agents, to fully determine the role of *PTGS2* in the pathogenesis of diabetes and its complications.

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