ORIGINAL ARTICLE

DIABETES

Effects of cyclooxygenase inhibition on insulin release and pancreatic islet blood flow in rats

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Abstract

Objectives. To examine the effects of inhibition of cyclooxygenase (COX) on islet hormone secretion in vitro and on pancreatic islet blood flow in vivo.

Methods. Insulin release was measured in a static incubation system of islets isolated from Wistar-F rats after inhibition of COX-1 and COX-2 with SC 560 (COX-1), FR 122047 (COX-1), rofecoxib (COX-2), or indomethacin (both COX-1 and COX-2). In other rats organ blood flow values were measured with a microsphere technique during both normo- and hyperglycemia after administration of these enzyme inhibitors.

Results. Serum insulin values were lower after pretreatment with a COX-1 inhibitor or a non-selective COX inhibitor in both control and glucose-injected rats in vivo, whereas COX-2 inhibition had no such effects. However, inhibition of COX had only minor effects on insulin release in vitro. Inhibition of COX affected neither total pancreatic nor islet blood flow in normoglycemic rats. Hyperglycemia caused an increase in both these flow values and in the duodenum. The increase in total pancreatic and duodenal blood flow was prevented by inhibition of COX-2 or non-selective COX inhibition. However, no effects on islet blood flow were seen after COX inhibition.

Conclusion. Inhibition of COX affects insulin release and blood glucose concentrations in vivo. However, COX inhibition has only minor effects on pancreatic islet blood flow, but prevents the glucose-induced increase in total pancreatic blood flow.

Key words: COX inhibition, insulin release, islet blood flow

Introduction

Arachidonic acid can be modified by three major pathways, namely cyclooxygenase (COX), lipoxygenase, and cytochrome P450 into biologically active eicosanoids. These substances have many biological activities, e.g. influencing smooth muscle contraction, platelet aggregation, and inflammatory responses (1-3). The present study focuses on COX, which converts arachidonic acid into various prostaglandins (4). Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the COX activity, but not the peroxidase activity of the enzyme (4).

COX is present in two isoforms of which COX-1 is constitutively expressed in nearly all cells at a constant level, whereas COX-2 is constitutively expressed in the brain and spinal cord. In most cells COX-2 expression is induced by pro-inflammatory cytokines and growth factors (1).

Since a chronic low-grade inflammation is associated with the peripheral insulin resistance in type 2 diabetes (T2D) possible mediators have been extensively studied (5-7), and a clear correlation with COX-mediated inflammation has been found (8). Furthermore, there is also a report on an association between a promoter variant of COX-2 gene with T2D in Pima Indians (9). Also the possibilities to modulate insulin resistance by COX inhibitors have been investigated, and most studies demonstrate beneficial effects of non-specific COX or selective COX-2 inhibition (5,10).

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Isolated pancreatic islets contain, depending on species, COX-1, COX-2, and 12-lipoxygenase, and their products have been shown to affect insulin secretion (11,12). COX-2 is constituatively expressed in pancreatic islets (12-14), and its expression increases glucose-dependently (15). COX products are unlikely to have major effects on islet endocrine function, but modulate insulin secretion (12,13,16). It has recently also been shown that prostaglandins have a regulatory role in crinophagy in pancreatic islet β-cells (14,17).

Prostaglandins are also versatile mediators in blood flow regulation, and the endothelium is the most prominent source of these substances in the peripheral circulation (2,18,19). They play an important role in gastric and duodenal blood flow regulation, where they are crucial for normal mucosal defense (20,21), and they also affect the pathogenesis of acute pancreatitis (22).

Thus, COX products affect β-cell function as well as the vasculature. In view of this we decided to study further how selective and combined inhibition of COX-1 and COX-2 affected islet insulin secretion in vivo and in vitro and how this correlated to the blood perfusion of the pancreatic islets.

Materials and methods

Animals

Male Wistar–Furth rats (Scanbur, Sollentuna, Sweden) weighing 320 ± 2 g (n = 92) with free access to pelleted food and tap water were used. All experiments were approved by the local animal ethics committee at Uppsala University.

Chemicals

Chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless stated otherwise. The specificity of the COX inhibitors is as follows: Indomethacin, non-specific COX inhibitor; SC 560 and FR 122047, COX-1 inhibitors; rofecoxib, COX-2 inhibitor. The rationale for the use of two COX-1 inhibitors is that some doubts have been raised on the specificity of SC 560 for COX-1 (23). As seen below, both alleged COX-1 inhibitors had similar effects.

Islet isolation

Pancreatic islets were isolated from rats by collage-nase digestion (24) and cultured in groups of 150 islets for 3–4 days in 5 mL of culture medium consisting of RPMI 1640 supplemented with L-glutamine, benzylpenicillin (100 U/mL; Roche Diagnostics Scandinavia, Bromma, Sweden), streptomycin (0.1 mg/mL), and 10% (vol/vol) fetal calf serum. To some of the cultures we added SC 560 (3 μmol/L), FR 122047 (5 μmol/L), rofecoxib (10 μmol/L), or indomethacin (10 μmol/L), all of which were dissolved in dimethyl sulfoxide (DMSO) at a final concentration of 0.1% (vol/vol). Culture medium was changed every second day, and COX inhibitors were added during the final 1–2 days of culture.

Glucose-stimulated insulin release and islet insulin contents

Groups of 10 islets, control or pretreated islets, were transferred to vials containing Krebs–Ringer bicarbonate buffer supplemented with 10 mmol/L HEPES and 2 mg/mL bovine serum albumin (BSA; ICN Biomedicals Inc. Aurora, Ohio, USA; hereafter referred to as KR BH buffer). The KR BH buffer contained 1.67 mmol/L D-glucose during the first hour of incubation at 37°C (O2/CO2, 95:5). The medium was then removed and replaced by KR BH supplemented with 16.7 mmol/L glucose, and the islets were then incubated for a second hour. As mentioned above, some islets had been cultured with different COX inhibitors for the final 1–2 days of culture, and these substances were added also during the release experiments. That is, SC 560 (3 μmol/L), FR 122047 (5 μmol/L), rofecoxib (10 μmol/L), or indomethacin (10 μmol/L), all of which were dissolved in DMSO at a final concentration of 0.1% (vol/vol), was added to the release medium throughout the 2-h period. The islets were harvested, following retrieval of medium, and homogenized by sonication in 200 μL redistilled water. DNA and insulin contents were then measured as previously described (24).

Blood flow measurements

The rats were anesthetized with an intraperitoneal injection of thiobutabarbital sodium (120 mg/kg body weight; Inactin®; Research Biochemicals International, Natick, MA, USA). The animals were then placed on a heated operating table to maintain body temperature at approximately 37.5°C. Polyethylene catheters were inserted into the ascending aorta, via the right carotid artery, and into the left femoral artery and vein. The former catheter was connected to a pressure transducer (PDCR 75/1; Druck Ltd, Groby, UK), whereas the latter was used to infuse Ringer solution (6 mL/kg body weight/h) to substitute for fluid losses. When the blood pressure had remained stable for at least 20 min, vehicle (0.03 mg/mL of DMSO in saline; 1 mL/kg body weight), SC 560 (2.5 mg/kg body weight), FR 122047 (1.5 mg/kg body weight), rofecoxib (10 mg/kg body weight), or
indomethacin (5 mg/kg body weight) was injected intravenously. Ten minutes later blood flow values were measured as outlined below. In separate animals an additional intravenous injection of 1 mL D-glucose (300 mg/mL) was given 3 min before the blood flow measurements. A total of $2.5 \times 10^5$ black non-radioactive microspheres (EZ-Trac™; Triton Microspheres, San Diego, CA, USA), with a diameter of 10 µm were injected via the catheter with its tip in the ascending aorta during 10 s and subsequently processed as previously described in detail (25).

The animals were then killed, and the pancreas and adrenal glands were removed in toto, blotted, and weighed. Samples (approximately 100 mg) from the mid-regions of the duodenum, descending colon, and left kidney were also removed, blotted, and weighed. The number of microspheres in the samples referred to above, including the pancreatic islets, was estimated as previously described, and organ blood flows were calculated (25). With regard to islet blood perfusion it was expressed both per gram wet weight of the whole pancreas, and the estimated wet weight of the islets.

**Statistical calculations**

All values are given as means ± SEM. Probabilities ($P$) of chance differences were calculated with Student’s unpaired $t$ test, or one-way repeated measurement ANOVA with Tukey’s correction (SigmaStat™; SSPD, Erfurt, Germany). A value of $P < 0.05$ was considered to be statistically significant.

**Results**

There were no changes in mean arterial blood pressure in any of the groups (Table I). Both SC 560 and indomethacin induced a decrease in hematocrit during hyperglycemia when compared with the corresponding normoglycemic control rats, whereas no effects were seen in the other groups (Table I).

**Insulin release and insulin content**

Basal insulin release at 1.67 mmol/L glucose was similar in all groups, besides islets pretreated with indomethacin, where a lower value was seen. The degree of stimulation of insulin release by high glucose concentrations was approximately three times in the control islets when challenged with the inhibitors only in the release medium (data not shown) or after 1–2 days of culture with the inhibitors (Figure 1A). Glucose increased insulin release in all groups when compared with basal values, but this response was potentiated by inhibition of COX-1 by either SC...
Insulin content was similar in all groups (~40 ng/islet) (Figure 1B).

**Blood glucose and serum insulin**

None of the COX inhibitors affected plasma glucose concentrations in control rats not injected with glucose (Table I). There was a lower blood glucose in glucose-injected animals pretreated with SC 560, FR 122047, or indomethacin, but not rofecoxib (Table I). Serum insulin values 10 min after pretreatment with SC 560, FR 122047, or indomethacin were markedly lower in both control and glucose-injected rats, whereas rofecoxib had no such effects (Figure 2).

**Blood flow values**

Inhibition of COX did not affect total pancreatic blood flow in normoglycemic rats, except for an increase caused by FR 122047 (Figure 3A), whereas none of the pretreatments affected islet blood flow (Figure 3B). Hyperglycemia per se caused an increase of both total pancreatic and islet blood flow.
The increase in total pancreatic blood flow was prevented by administration of rofecoxib or indomethacin (Figure 3A), whereas no effects on islet blood flow was observed after any of the pretreatments given, even though there was a trend ($P = 0.07$) for an increase after administration of FR 122047 (Figure 3B).

Duodenal blood flow changed in concert with that of the whole pancreas in the different experimental groups, whereas colonic blood flow was unaffected (Table I). Rofecoxib administration decreased renal blood flow in normoglycemic rats (Figure 3C). Induced hyperglycemia increased renal blood flow, in vehicle-treated control rats, and such an increase was also seen after rofecoxib administration (Figure 3C).

**Discussion**

Local production of prostaglandins in blood vessel walls constitutes an important system for modulation of local blood perfusion, even though the importance varies considerably between different regional circulations (18,19,26). It is well known that an imbalance between vasoconstrictor and vasodilator prostanoids is present in arteries from diabetic animals, which, at least partially, causes the endothelial dysfunction characteristic of this condition (27,28). However, in the present study all examined inhibitors of COX failed to affect islet blood flow. This confirms the finding in a previous study where the non-selective COX inhibitor indomethacin also failed to affect islet blood perfusion (29). In the present study, glucose administration increased the need for islet insulin release, and, as previously shown (30,31), islet blood flow was also markedly increased. However, there was no change in this glucose-stimulation of islet blood flow after administration of any of the COX inhibitors, once again emphasizing that prostaglandins are of minor importance for islet blood flow regulation.

When viewing total pancreatic blood flow, on the other hand, a different picture emerges. There was no change during basal conditions, which is in line with other studies suggesting that prostaglandins per se have only minor effects on pancreatic circulation during basal conditions (32). However, both rofecoxib and indomethacin prevented glucose-induced increase in total pancreatic blood flow when compared with control rats, whereas COX-1 inhibition had no such effects. This suggests that prostaglandins derived from the actions of COX-2 are at least partially involved in this blood flow response, which is limited to the exocrine pancreas. It has been shown in other contexts that when increased demands are put upon the exocrine pancreas, such as during the development of acute pancreatitis, prostaglandins can affect pancreatic blood perfusion (22). Thus, the present findings extend these observations and suggest that also the normal physiologic blood flow response to hyperglycemia depends on COX-1-derived products.

Also the duodenal blood flow response was similar to that of the whole pancreas, with no effects seen during basal conditions, but a COX-2-dependent
Figure 3. A: Total pancreatic blood flow in anesthetized rats injected intravenously with 1 mL/kg of vehicle (Veh; 0.03 mg/mL of DMSO in saline), SC 560 (SC; 2.5 mg/kg body weight), FR 122047 (FR; 1.5 mg/kg body weight), rofecoxib (Rof; 1 mg/kg body weight), or indomethacin
inhibition of glucose-induced stimulation. This suggests that the response may be limited to the superior mesenteric artery which provides all of the blood perfusion to the duodenum and approximately two-thirds of that to the pancreas (33). Arguing against this is, however, the findings that renal blood flow reacted similarly. Thus, it may be glucose per se that affects phospholipase A2 (PLA2), which catalyzes the cleavage of arachidonic acid from cellular membranes in different organs, which then is differently processed by COX or lipoxygenase in tissues. There was no systemic vascular effect by any of the COX inhibitors, as evidenced by the unchanged mean arterial blood pressure in all groups. The functional importance of this response in the pancreatic and duodenal circulation is at present unknown.

Arachidonic acid, presumably released through the actions of cytosolic PLA2, is critical for normal islet beta-cell function. Thus, if PLA2 is inhibited, normal glucose-stimulated insulin release is decreased from human islets (12). Arachidonic acid facilitates hormone release through activation of several ionic channels (34,35). Undoubtedly it exerts some of its effects by itself, whereas a major part is mediated by its metabolism through COX, lipoxygenase, or cytochrome P450 (36). Initial reports on the role of prostaglandins formed by COX in insulin release suggested that mainly PGE2 was involved and inhibited insulin release (13,37), primarily through the EP3 receptor (38). However, more recent experiments in human islets have failed to confirm this (12). It was recently suggested that arachidonic acid in beta-cells is metabolized by both COX-1 and COX-2 and their products exert either stimulatory (those derived from COX-1) or inhibitory (those from COX-2) effects on insulin release (36). Our present results on serum glucose and blood insulin concentrations in vivo are well in accordance with the latter view. When examining the effects of the different COX inhibitors in vitro the picture is more complex. If the inhibitors were added to the medium only during the 2-h duration of the experiments, there were no effects on insulin release (data not shown). Also, when the islets had been pre-cultured for 2 days with the inhibitors present, we observed only minor effects on insulin release and no effects at all on islet insulin content. There was, however, a slight increase in basal insulin secretion when indomethacin was added and a slight increase of glucose-stimulated insulin secretion in the presence of SC 560 and FR 122047, i.e. when COX-2 activity was maintained. It should be noted that the studies by Keane and Newsholme (36) were performed on different cell lines, and this may explain some of the differences.

Of more interest is that our in-vitro findings are opposite to the effects on insulin secretion seen in vivo. The reasons for these differences are unknown, but COX exists in most tissues, and a general, systemic inhibition of one or both isoforms in the whole body is naturally associated with many local changes, which then may affect the pancreas and its insulin release. However, the exact nature of these signals is unknown, but is certainly worthy of further studies.

The present study demonstrated that inhibition of COX affects insulin secretion and blood glucose concentrations in vivo, whereas the effects observed in vitro were more modest. Furthermore, COX inhibition has only minor effects on pancreatic islet blood flow, but prevents glucose-induced increase in total pancreatic blood flow.

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