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Constitutive and inducible cyclooxygenase isoforms expressed in rat pancreas

Cyclooxygenase (COX) is a key enzyme in thromboxane A_2, prostacyclin and prostaglandins synthesis. Presently, two main isoforms of the enzyme were detected: COX-1 (constitutive form) – involved in regulation of a variety of physiological processes, and COX-2 (induced form) – active mainly in pathological processes (12). The dogma of COX-1 constitutive expression was refuted by Crafford et al. (2) and Schonbeck et al. (9) who detected this isoform in the synovial membrane in rheumatoid arthritis and atherosclerotic plaques, respectively. Simultaneously, the constitutive expression of the COX-2 was found in some organs, e.g., brain, lungs, stomach, intestine, kidney, ovary, testis, skin, placenta, as well as most of the fetal tissues (7, 10–12).

The aim of the present study was to evaluate immunoexpression of the constitutive and inducible isoforms of cyclooxygenase in rat pancreas.

MATERIAL AND METHODS

The study was conducted on sexually mature albino male and female rats of Wistar CRL:(WI)WUBR strain. All the animals were obtained from an accredited breeder (Warsaw-Rembertow, Poland), housed and maintained in an animal care facility. The initial body weight was 200–250 g for females (n=10) and 220–270 g for males (n=10). No xenobiotics were administered during the study. After 4 weeks all the animals were sacrificed by decapitation. Abdominal organs were removed by caesarean section and routinely examined. Pancreatic samples were taken, fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5µm and then stained routinely with hematoxylin and eosin.

Immunohistochemical reaction for COX-1 and COX-2 was performed on the 4 µm slides obtained from the paraffin blocks used previously for histological examination. After dewaxing and rehydration the slides were placed for three cycles of heating in a microwave oven (750W) for 5 min in citrate buffer (0.01 M, pH 6.0) for antigen retrieval. Then endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 5 min, and the slides were incubated for 60 min with the primary monoclonal mouse anti-human antibodies (Novocastra; Newcastle, UK) against COX-1 (clone 12E12, dilution 1:20) and COX-2 (clone 4H12, dilution 1:200). The next step was the incubation with DakoEnvision^{+TM}/HRP, Mouse kit (DakoCytomation; Glostrup, Denmark) according to manufacturer directions. The specific immune reaction was visualized using 3',3-diaminobenzidine tetrahydrochloride (DAB) (DakoCytomation; Glostrup, Denmark) and finally the sections were counterstained with Mayer's hematoxylin. TBS buffer rinsing was used after each step. The whole procedure was performed at the room temperature. In all cases the appropriate positive and negative controls were performed. The sections treated in the same way, but with mouse pre-immune serum except for examined primary antibodies were used as negative controls. For the positive COX-1 and COX-2 controls the human colonic mucosa and osteochondroma were applied, respectively. Before starting the proper immunohistochemical study

the cross-reactivity with rat tissues was verified. All slides were evaluated under the light microscope (Olympus BX45; Tokyo, Japan).

RESULTS

COX-1 immunoexpression was revealed exclusively in acinar secretory cells of pancreas (Fig. 1A). The pancreatic islets were COX-1 negative. Contrary, COX-2 immunoexpression was found in secretory cells of endocrine pancreatic islets, whereas acinar cells were negative. Furthermore, COX-2 was also observed in epithelial cells of pancreatic ducts, as well as in smooth muscle cells of blood vessel of pancreatic stroma (Fig. 1B). Both immunoreactions were limited to the cytoplasm. No differences in COX immunoexpression were found between male and female rats.

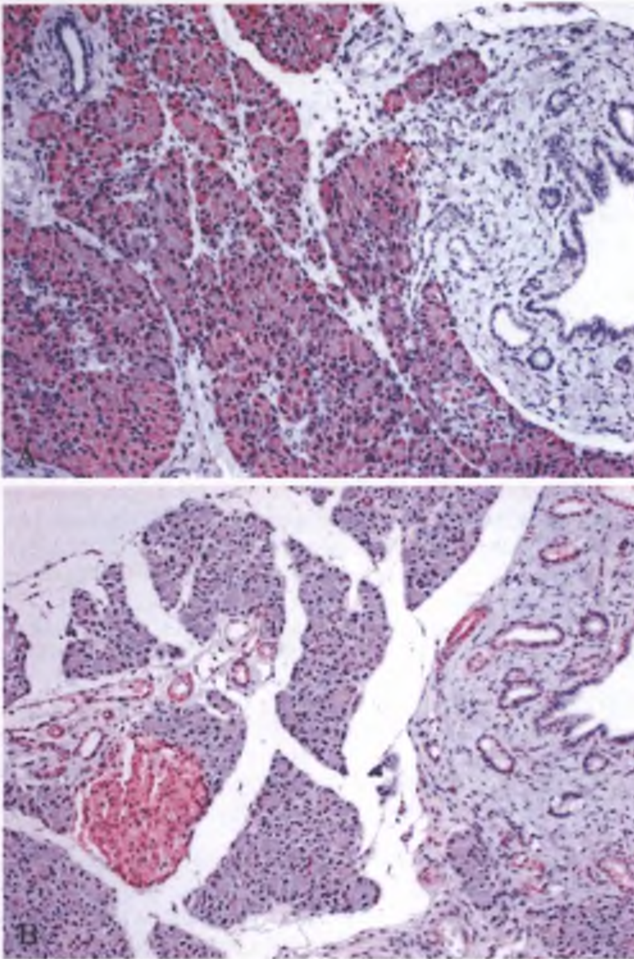

Fig. 1A. Positive immunoreactivity of COX-1 in pancreatic acinar cells. B. Positive immunoreactivity of COX-2 in secretory cells of pancreatic islet, epithelial cells of pancreatic ducts and smooth muscle cells of blood vessels (DakoEnvision^{+TM}/HRP; magn. x100)

DISCUSSION

Our study showed constitutive expression of both COX isoforms in rats' pancreas. Such results confirm previous experimental data (5, 12). Unlike COX-1, dominant expression of gene coded COX-2 was detected in pancreatic islets in humans and hamsters. COX-1 was found in acinar secretory cells. Simultaneously, it was reported that indomethacin – a non-selective COX-inhibitor – decreased basal, glucose-and glucagons-stimulated acute insulin response (12). According to Koliopanos (5) pancreatic islets displayed a variable COX-2 staining pattern, which was associated with the distribution of insulin-positive cells and with the clinical status of diabetes mellitus. Patients with normal insulin production or with latent diabetes showed COX-2 immunoreactivity, whereas in diabetic patients the COX-2 expression was decreased or absent in pancreatic islets.

Unlike our results, Zabel-Langhennig et al. (14) found that both COX-1 and COX-2 isoenzymes are synthesized in rat pancreatic acinar cells. The differences are probably secondary to methodology, since the authors examined both isoenzymes on the mRNA level.

A number of experimental and clinical studies indicate a role of COX-2 in various pancreatic diseases, especially acute and chronic pancreatitis. High expression of COX-2 was reported in rats (4, 13, 14) and mice (3) with acute pancreatitis. Interestingly, the COX-1 expression remained unchanged (14). It was also found that blockade of COX-2 by either pharmacologic inhibition or selective genetic deletion markedly attenuated the severity of the pathological process (4), while indomethacin administration had no effect on the expression of the two isoforms (14). The experimental data were partially confirmed in human by Schlosser et al. (8), who reported overexpression of COX-2 in the atrophic acinar cells, hyperplastic ductal cells, and islets cells but not in normal pancreas in patients with chronic pancreatitis. On the other hand, COX-2 expression was also detected in pancreatic cancer (1). In pancreatic intraepithelial neoplasia (PanIN) – the precursor lesion of pancreatic cancer – COX-2 expression increased with escalating severity of changes. The isoenzyme immunostaining was stronger in PanIN lesions when compared with normal pancreas and pancreatic cancer. Significant differences in COX-2 immunoexpression among different types of intraductal papillary-mucinous tumors (IPMT) (hyperplasia vs. adenoma, noninvasive adenocarcinoma and invasive adenocarcinoma) were also reported (6). Furthermore, the majority of typical ductal pancreatic adenocarcinomas were COX-2-positive. However, duct epithelial cells from normal pancreas and with chronic pancreatitis did not show this isoenzyme.

In conclusion, it was found that both constitutive and inducible cyclooxygenase isoforms are physiologically expressed in pancreas of mature male and female rats.

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SUMMARY

Constitutive (COX-1) and inducible (COX-2) cyclooxygenase isoforms are responsible for prostanoid synthesis. Immunoexpression of both isoforms was evaluated in pancreas of mature male and female CRL:(WI)WUBR Wistar rats. COX-1 immunoexpression was revealed exclusively in acinar secretory cells of pancreas. The pancreatic islets were COX-1 negative, whereas COX-2 immunoexpression was found in secretory cells of endocrine pancreatic islets; acinar cells were negative. Furthermore, COX-2 was also observed in epithelial cells of pancreatic ducts, as well as in smooth muscle cells of blood vessels.

Ekspresja izoformy konstytutywnej i indukowanej cyklooksygenazy w trzustce szczura

Synteza prostanoidów jest inicjowana przez izoformę konstytutywną (COX-1) i indukowaną (COX-2) cyklooksygenazy. Immunoekspresję obu izoform oceniano w trzustkach dorosłych samic i samców szczura szczepu CRL:(WI)WUBR Wistar. Immunoekspresję COX-1 wykazano wyłącznie w komórkach pęcherzykowych trzustki, natomiast ekspresję COX-2 stwierdzono w komórkach wewnątrzwydzielniczych wysp trzustkowych, komórkach nabłonka przewodów oraz miocytach naczyń krwionośnych.