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Localization of cyclooxygenase isoforms in maternal and offspring kidney during pregnancy and lactation

Prostanoids, especially thromboxane A_2, prostacyclin and prostaglandin D_2, E_2 and F_2, play an important role in the physiology of various organs. They are synthesized from arachidonic acid (AA) that is released from cellular membrane phospholipids. The most important step in its metabolic pathway depends on prostaglandin endoperoxide synthase (PGHS) activity also known as cyclooxygenase (COX). This microsomal enzyme has two catalytic sites. AA is initially catalyzed by cyclooxygenase active site to cyclic endoperoxide prostaglandin G_2, that is later converted by peroxidase active site to prostaglandin H_2 (PGH_2). The secondary modification, which leads to final prostanoid synthesis, depends on different isomerises activity or has a non-enzymatic course (11).

The two main COX isoforms are known. COX-1 is involved in physiological processes and therefore is called a constitutive form, whereas, COX-2 (induced form) is responsible for prostanoid synthesis in pathological processes. However, COX-2 activity and immunoexpression were already detected physiologically in various organs, e.g., brain, ovary, uterus, as well as placenta and most fetal tissues (11, 13, 14).

The aim of the present study was to evaluate immunoexpression of the constitutive (COX-1) and inducible (COX-2) isoforms in maternal, fetal and neonatal kidney at the end of the pregnancy in untreated rats.

MATERIAL AND METHODS

Sexually mature albino rats of Wistar CRL:(WI)WUBR strain, obtained from a commercial breeder (Warsaw-Rembertow, Poland) were used. The rats were acclimated for at least 2 weeks, housed and maintained in an animal care facility. On mating days, females weight 200–250 g were placed in cages with males (5:2) for approximately 14 hours. The following morning, a vaginal smear was done to determine if copulation had occurred. The day when sperm was found was designated gestation day 1 (GD1). Sperm positive females were randomly taken for the study group (n=12). No xenobiotics were administered during the study.

On gestation day 21, randomly selected six pregnant females were sacrificed. Fetuses were delivered by caesarean section and examined routinely (1). Both maternal and fetal kidneys were removed and sectioned. The remaining dams were kept until day 7 post delivery and sacrificed. Maternal and pup kidney were also removed and examined.

All the organ samples were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5μm and then stained routinely with hematoxylin and eosin.

Immunohistochemical reactions for both COX isoforms were performed on the 4 μm slides obtained from the paraffin blocks previously used for histological examination. Monoclonal mouse anti-human antibodies against COX-1 and COX-2 (clones 12E12 and 4H12 respectively; Novocastra; Newcastle, UK) and DakoEnvision^{+TM}/HRP kit (DakoCytomation, Glostrup, Denmark) were applied. The details of the method were described previously (2). All slides were evaluated using light microscope (Olympus BX45; Tokyo, Japan).

RESULTS

Immunoexpression of COX-1 was revealed in maternal kidneys during pregnancy and lactation. The strongest cytoplasmic reaction was noted in epithelial cells of proximal convoluted tubules, while less intensive staining was seen in endothelial cells and medullar interstitial cells (Fig. 1A). Occasionally, in some glomeruli a few podocytes were also COX-1 positive. COX-2 was detected in smooth muscle cells of renal blood vessels. Weak expression was revealed in epithelial cells of distal convoluted tubules, as well as in the thick ascending limb of Henle's loop (Fig. 1C). Sporadically, immunoreaction was also observed in single podocytes. The same localization and staining intensivity were revealed at the end of pregnancy and on day 7 of lactation.

Fetal and neonatal kidneys displayed a strong COX-1 reactivity in podocytes and epithelial cells of renal tubules, except for the thin limb of the loop (Fig. 1B). Postive staining was also found in interstitial cells of the cortex and medulla. COX-2 reactivity was seen in podocytes, epithelial cells of the parietal lamina of Bowman's capsule, and most of renal tubulas (Fig. 1D). Similar localization but with low intensity of COX-2 staining was seen in neonatals when compared with fetuses.

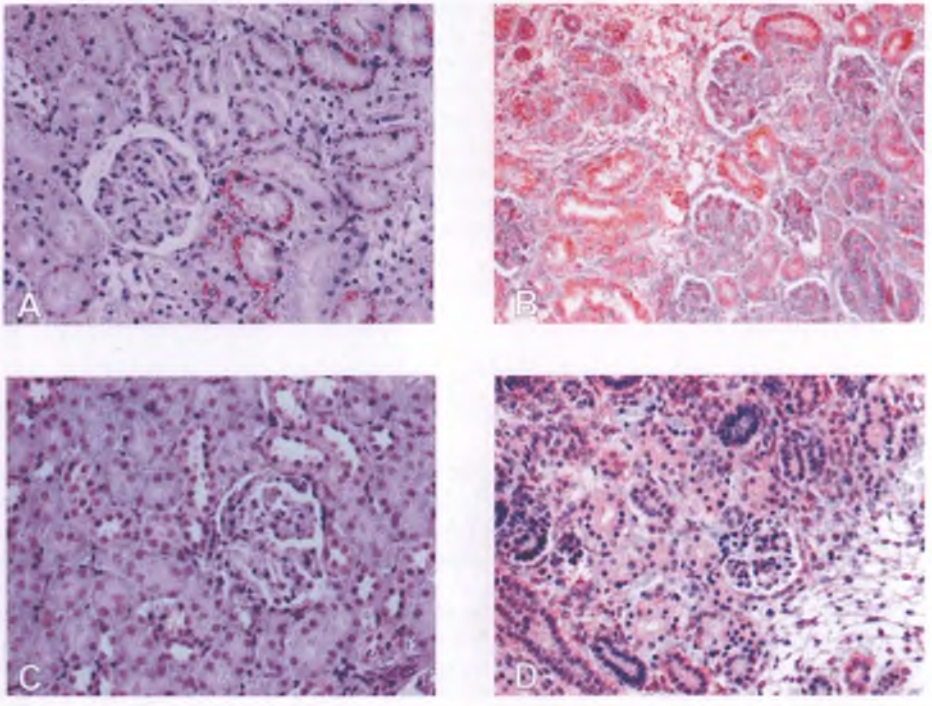

Fig. 1. Immunoreactivity of COX-1 (A, B) and COX-2 (C, D) in breast-feeding mother (A, C) and fetal (B, D) kidney (DakoEnvision^{+TM}/HRP; A, C magn. x200; B, D magn. x100)

DISCUSSION

The present results revealed immunoexpression of both COX isoforms in maternal and fetal kidney. The maternal observations partially confirm previous results obtained from non-pregnant animal (4, 12, 13) and human samples (7, 9). However, immunostaining of COX-1 and COX-2 in macula densa was not found.

The COX-1 immunoexpression was detected in vascular endothelial cells, collecting tubules and medullar interstitial cells in rats, while macula densa, convoluted tubules and Henle's loop did not present any enzyme reactivity. The same observations were also made in cow, guinea pig, rabbit and sheep (12). The COX-1 immunolocalization was confirmed by Harris et al. (4), who also proved the COX-2 expression in the interstitial cells of the papilla, macula densa and adjacent cortical thick ascending limb. It was also stressed that the expression of gene coded COX-2 is strongly regulated by salt intake (4). Such regulation was not revealed for COX-1 isoform.

Using *in situ* hybridization and/or immunostaining methods high COX-1 expression in renal medullary collecting ducts, cortical collecting ducts and in distal convoluted tubule was revealed in rabbits, while COX-2 was detected only in macula densa and medullary interstitial cells (15).

Similar results were obtained in humans. High expression of *COX-1* on both mRNA and protein levels was observed in collecting duct cells, intestinal cells of renal cortex and medulla, as well as endothelial cells and smooth muscles of renal vessels. COX-2 was detected in podocytes and similarly to COX-1 also in the wall of the renal arteries and veins (7). Such observations in vessels were confirmed by Therland et al. (15).

The expression of the constitutive and inducible forms was also seen in fetal kidneys since both cyclooxygenase and prostanoids are important in renal development. However, unlike COX-1, which was detected throughout the whole embryonic and fetal period, COX-2 expression was found in some fetal organs at the end of pregnancy. For example, the inducible isoenzyme was detected in cartilage, kidney, heart, and skin in rat fetuses of age $\geq$16GD (11, 12). Contrary to adult kidney, COX-1 was also detected in fetal human podocytes (7). The results on COX-2 are incompatible. Basing on different reports the COX-2 expression is physiologically detected just in podocytes (6), podocytes and the wall of blood vessels (6), while Nantel et al. (8) find it also in ascending limb of the Henle's loop, macula densa and medullar interstitial cells. Such observations were later proved also by Khan et al. (5) and Therland et al. (15).

Our study reveals that at the end of pregnancy (GD21) and at the day 7 of lactation both constitutive and inducible cyclooxygenase isoenzymes were detected in kidney of untreated pregnant rat females, as well as in their fetuses.

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SUMMARY

Cyclooxygenase (COX) plays an important role in renal physiology in both mature and developing organisms. Immunoexpression of constitutive (COX-1) and inducible (COX-2) cyclooxygenase isoenzymes was evaluated in maternal and offspring kidney on day 21 of gestation and day 7 of lactation in rats. The cytoplasmic reaction for COX-1 was noted in epithelial cells of proximal convoluted tubules, endothelial cells and medullar interstitial cells. COX-2 was detected in smooth muscle cells of renal blood vessels, epithelial cells of distal convoluted tubules and the thick ascending limb of the Henle's loop. Fetal and neonatal kidneys displayed a strong COX-1 reactivity in podocytes and epithelial cells of renal tubules, except for the thin limb of the loop. Positive staining was also found in interstitial cells of the cortex and medulla. COX-2 reactivity was seen in podocytes, epithelial cells of the parietal lamina of Bowman's capsule, and most of renal tubulas. It could be concluded that at the end of pregnancy and early lactation both constitutive and inducible cyclooxygenase isoforms were detected in kidney of untreated rat females, as well as in their fetuses.

Lokalizacja izoenzymów cyklooksygenazy w nerkach matek i potomstwa w okresie ciąży i laktacji

Cyklooksygenaza (COX) odgrywa ważną rolę w fizjologii nerek, zarówno u osobników dorosłych, jak i rozwijających się. Immunoekspresję izoformy konstytutywnej (COX-1) i indukowanej (COX-2) oceniano w nerkach matczynych, płodowych i noworodkowych w 21 dniu ciąży i 7 dniu laktacji u szczura. Cytoplazmatyczny odczyn z COX-1 stwierdzono w komórkach nabłonka kanalików krętych I rzędu, śródbłonku naczyń oraz komórkach śródmiąższowych rdzenia nerki. Natomiast COX-2 wykryto w komórkach mięśniowych gładkich naczyń krwionośnych, nabłonkowych kanalików krętych II rzędu oraz części grubej ramienia wstępującego pętli nefronu. W nerkach płodów i noworodków immunoekspresję COX-1 obserwowano w podocytach, komórkach nabłonkowych kanalików nerkowych, z wyjątkiem części cienkiej pętli nefronu. Odczyn obserwowano także w komórkach śródmiąższowych kory i rdzenia nerki. COX-2 wykryto w podocytach, komórkach nabłonkowych blaszki ściennej torebki kłębuszka i większości kanalików nerkowych. Należy stwierdzić, że nerki matek, płodów i osesków szczurzych wykazują ekspresję zarówno formy konstytutywnej, jak i indukowanej cyklooksygenazy.