Prostaglandins: **Critical roles in pregnancy and colon cancer**

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Cyclooxygenase catalyses a key step in prostaglandin biosynthesis, and recent work suggests that one isoenzyme, COX-2, has important roles in early stages of pregnancy; it also appears to be involved in the somewhat analogous process of colon tumor formation and spread.

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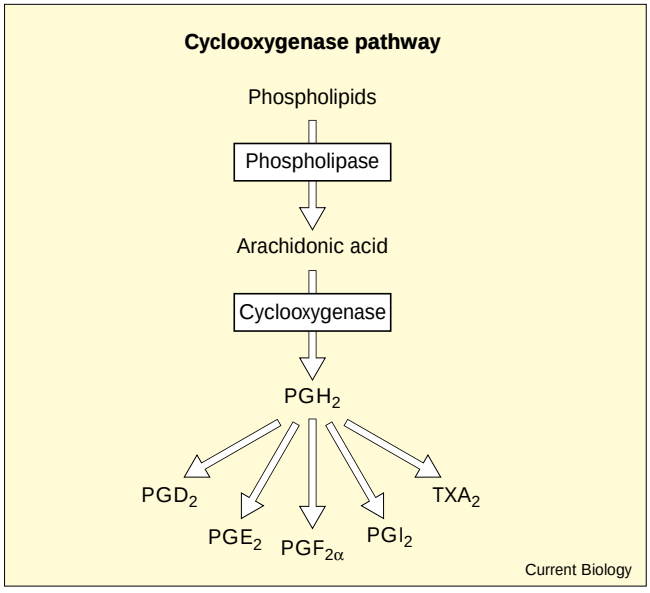
Prostaglandins are best known as mediators of inflammation and hemostasis, hence the popular use of aspirin in the control of pain and inflammation, and as an antithrombotic agent. Prostaglandins are oxygenated derivatives of arachidonic acid, a fatty acid that does not naturally occur free inside cells. Together with other arachidonic acid derivatives — a group collectively known as icosanoids — prostaglandins are produced only when cells are stimulated by agonists such as hormones, cytokines, growth factors and neurotransmitters [1]. Agonists that stimulate prostaglandin production do so by binding to cell-surface receptors, triggering the activation of cellular phospholipases that catalyse the hydrolysis of specific membrane phospholipids, releasing free arachidonic acid. The liberated arachidonic acid is oxygenated by the enzyme cyclooxygenase to form prostaglandin H_2 (PGH_2), the precursor of all prostaglandins (Figure 1). The end products act as hormone-like signalling molecules through G-protein-coupled receptors to control physiological processes such as vascular tone, salt and water balance, gastric acid secretion and platelet activation. Recent studies with knockout mice have now provided evidence that prostaglandins actually have much broader roles than previously thought, controlling complex processes such as pregnancy.

Two distinct cyclooxygenase isoenzymes are known, encoded by different genes and designated COX-1 and COX-2 [1]. COX-1 is produced constitutively by most cells, and so is primarily responsible for the immediate synthesis of prostaglandins in response to agonist stimulation. COX-2, in contrast, is made only in response to agonists, such as inflammatory cytokines, so the prostaglandin synthesis that it catalyses occurs several hours after stimulation. COX-2 turns over rapidly, and transcription of its gene is subject to complex control by the intracellular signalling molecules cyclic (c)AMP, tyrosine kinases and protein kinase C.

Cyclooxygenase inhibitors — the so-called non-steroidal anti-inflammatory drugs (NSAIDs) — are an important group of drugs in the treatment of inflammation. These drugs differ in their selectivity for COX-1 *versus* COX-2, although all of the currently marketed drugs preferentially inhibit COX-1. Several COX-2-selective inhibitors are now in development, as it is thought that COX-2 is more important in inflammation than COX-1. The major toxic side-effects of current NSAIDs are bleeding and gastritis, both of which are thought to result from COX-1 inhibition. Selective COX-2 inhibitors may thus turn out to be efficient anti-inflammatory agents with relatively low toxicity.

The results of numerous studies indicate that COX-1 and COX-2 have discrete functions. Much has recently been learned about these enzymes from a series of gene knockout experiments in mice. The *COX-1* knockout mouse has a mild phenotype, with decreased platelet function — this

Figure 1



The cyclooxygenase pathway. Extracellular agonists bind to cell-surface receptors and thereby activate phospholipases that liberate arachidonate from membrane phospholipids. Cyclooxygenase, either COX-1 or COX-2, then catalyses the formation of prostaglandin H_2 (PGH_2). Other enzymes then form the specific products prostaglandin D_2 (PGD_2), prostaglandin E_2 (PGE_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), prostacyclin (PGI_2) and thromboxane A_2 (TXA_2).

is not surprising, as platelets express only *COX-1* [2]. The *COX-1* mutants are fertile, but homozygous matings tend to yield dead pups for unknown reasons. *COX-2* knockout mice, in contrast, succumb to a nephropathy at about eight weeks of age, and the females are sterile [3]. The basis for the female sterility has been elucidated in a recent study [4] that explored the role of COX-2 in the early stages of pregnancy. COX-2 was found to be important for each stage of pregnancy examined.

The female wild-type mice used by Lim *et al.* [4] produced seven ova per cycle and when mated, two-thirds of the ova were fertilised. Female *COX-2* mutants, in contrast, produced just three ova per cycle, and none of these was fertilised when mating was attempted. The ova produced by the *COX-2* mutants were abnormal in that the first polar body was not extruded in most cases. These defects were not due to a deficiency of pituitary or ovarian hormones, as the same defects were observed when females were subjected to superovulation with exogenous gonadotropins.

Further experiments showed that blastocyst implantation is also defective in *COX-2* mutant mice [4]. After intrauterine injection of blastocysts into pseudopregnant females, implantation was observed in one-half of the wild-type mice but in only 1% of the *COX-2* mutants. Controls using ovariectomised mice treated with progesterone and oestrogen showed that the defect does not result from inadequate hormones. Implantation was found to be normal in *COX-1* mutant mice; when *COX-1* mutants were treated with high doses of a selective COX-2 inhibitor, however, there was complete failure of implantation. Implantation was also inhibited in wild-type mice by high doses of the selective COX-2 inhibitor, but not in those given aspirin at a dose that primarily inhibits COX-1.

Lim *et al.* [4] also studied the formation of the specialised uterine lining required to support embryos, a process known as decidualisation. They used an assay in which oil was infused into the uterus during pseudopregnancy: by day eight, the uterus of wild-type mice increased 15-fold in weight, but the *COX-2* mutants showed no change in uterine weight. Although these effects appear to be due to the absence of a product of COX-2 in the mutant mice, infusion of PGE_2 or a stabile analogue of prostacyclin did not restore decidualisation in pseudopregnant *COX-2* mutant mice. Perhaps the timing or dose of the exogenously supplied prostaglandin was incorrect, or perhaps another, as yet unknown, icosanoid product of COX-2 is required.

The pattern and timing of *COX-2* expression also supports the view that its product has a role in pregnancy. Injection of rats with gonadotropin results in transient *COX-2* expression in granulosa cells prior to ovulation [5]. Immunohistochemistry of ova on days one and two of pregnancy showed strong *COX-2* expression in the cumulus cells that surround the ovum and are required for development of fertilisation-competent eggs [4]. There was no uterine *COX-2* expression on day four of pseudopregnancy until oil injection into the uterine horn; this caused a marked increase in *COX-2* expression at two hours that was gone by eight hours with no change in *COX-1* expression. Immunohistochemistry indicated that *COX-2* expression was greatest in cells directly adjacent to the implanted blastocyst.

COX-2 products thus appear to be required for every step of early pregnancy, including ovulation, fertilisation, implantation and decidualisation. The particular prostaglandins that are involved in these processes, and the detailed ways that they act, remain to be determined. The results suggest that potent, selective COX-2 inhibitors may interfere with pregnancy in humans. In some cases this effect will be undesirable, but the possibility that these agents might provide a safe and non-toxic 'morning after pill' is intriguing.

An even more intriguing consideration comes from the similarity between the early events of pregnancy and those of tumor spread and metastasis. In both cases, cells must escape from their original environment and implant at a new site where a new vasculature must develop. Several retrospective studies of aspirin use in man suggest that COX inhibitors alter the natural history of colon cancer [6]. The risk of disease appears to be reduced in subjects who take aspirin regularly for years. More surprisingly, the risk of fatal or metastatic colon cancer appears to be reduced by 50% in chronic aspirin users. This may result from COX-2 inhibition, as most colon tumors and tumor cell lines express high levels of COX-2, so products of this enzyme may be important in tumor formation and metastasis [7]. And sulindac, a relatively non-selective COX inhibitor, has been shown to decrease the development of intestinal polyps in patients with familial polyposis [8].

Here again, knockout mice have been informative. Mice in which the tumor suppressor gene *adenomatous polyposis coli* (*APC*) has been inactivated develop polyps in large numbers by 10 weeks of age. Recently, *APC*, *COX-2* double mutant mice were bred by mating *COX-2* mutant mice to the *APC* mutants [9]. The double mutants showed an 85% decrease in polyp production, compared with the single *APC* mutants, directly demonstrating a role for COX-2 in tumorigenesis. It would be interesting to treat these animals with a COX-1 inhibitor to see if tumor development can be further suppressed. Trials of COX-2 inhibitors in patients with familial polyposis are currently under way, and the results should be available soon. It seems likely that these agents will affect the natural history of the disease, given the results of studies using sulindac [8].

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