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Sources and functions of prostaglandins in the testis: evidence for their relevance in male (in)fertility

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Abstract

Prostaglandins (PGs) are derived from arachidonic acid by action of the cyclooxygenase (COX) isoenzymes COX1 and COX2. The development of mice deficient in COX1 and/or COX2 has shown that COX2–null female mice are infertile. In contrast, male fertility is not affected in COX1- or COX2-mutant mice from knock-out experiments, suggesting that PGs may not be important for the functioning of the testis. This early general view is being challenged by recent observations. We have reported that whereas COX2 is not detected in normal human testes, it is expressed in testicular biopsies of men with impaired spermatogenesis and male infertility. Moreover, COX is up-regulated in testicular cancer, and COX2 expression is induced in Brown-Norway rat Leydig cells during aging. Several reports describe actions of COX and PGs on steroid hormone production, expression of PGs and their receptors in Sertoli cells, and inhibitory effects of some PGs on spermatogenesis and sperm counts. In brief, this review summarizes the results obtained in the last decades by our laboratory and other groups that point out the impact of COX and PGs in the regulation of testicular function and male fertility.

Keywords: prostaglandins, cyclooxygenase, testis, male infertility.

Introduction

Prostaglandins (PGs) constitute a group of bio-active molecules derived from arachidonic acid. PG biosynthesis is initiated by the action of the cyclooxygenase (COX), which catalyzes the transformation of arachidonic acid into PGG2. Prostaglandin-G2 is later reduced to PGH2 by means of a peroxidation reaction also catalyzed by COX. Prostaglandin-H2 is the common precursor in the synthesis of the remaining PGs through both reactions catalyzed by synthases and ketoreductases and reactions of dehydration and non-enzymatic isomerization (Urade *et al.,* 1995; Smith *et al.*, 2000; Watanabe, 2002; Fig. 1).

Cyclooxygenase is the key regulatory enzyme in the synthesis of PGs. Cyclooxygenase is present in two isoforms: COX1 and COX2. There are processes in which only one isoform is involved, processes in which the two isoforms act in coordination, and physiological events in which usually only one isoform is involved, but which could be compensated by the other in case it was absent (Smith and Langenbach, 2001).

When COX1 and COX2 are expressed in the same cell, their activities are controlled differentially by regulating the intracellular concentrations of arachidonic acid and of the lipids susceptible to peroxidation (Smith *et al.,* 2000). Also, COX2 can utilize esterified fatty acids as alternative substrates, while COX1 lacks this capacity (Kozak *et al.,* 2000).

Cyclooxygenase-1, commonly known as the constitutive isoform, is found in most cell types. The inducible form, COX2, appears to be expressed only in the early stages in the processes of differentiation and cellular replication in response to different stimuli, such as cytokines and mitogenic factors (Katori and Majima, 2000; Smith *et al.,* 2000). The expression of COX2 has been described in physiological and pathological processes, including inflammatory processes, angiogenesis, bone absorption, gastric ulcers, colon cancer, kidney diseases, brain disorders, and female genital tract affections (Katori and Majima, 2000).

The development of COX1- and COX2-deficient mice has been essential in establishing the role exerted by each of these isoforms in the process of reproduction. Female mice deficient in COX1 produce litters with normal weight but have difficulties with parturition, a process dependent on COX1 and PGF2α (Langenbach *et al.,* 1995; Gross *et al.,* 1998), whereas female mice deficient in COX2 are infertile (Lim *et al.,* 1997). It has also been described that COX2 and PGD2 are involved in the ovulation process (Lim *et al.,* 1997; Davis *et al.,* 1999; Sirois *et al.,* 2004).

Unlike the data in females, the data found in the literature in regards to the role of COX and PGs in the male reproductive system are scarce and controversial. It has been reported that fertility is not affected in male mice deficient in COX1 and COX2 (Dinchuk *et al.,* 1995; Langenbach *et al.,* 1999), a fact that led to postulate that PGs might not be relevant in the normal functioning of the testis. However, recent studies appear to contradict this hypothesis.

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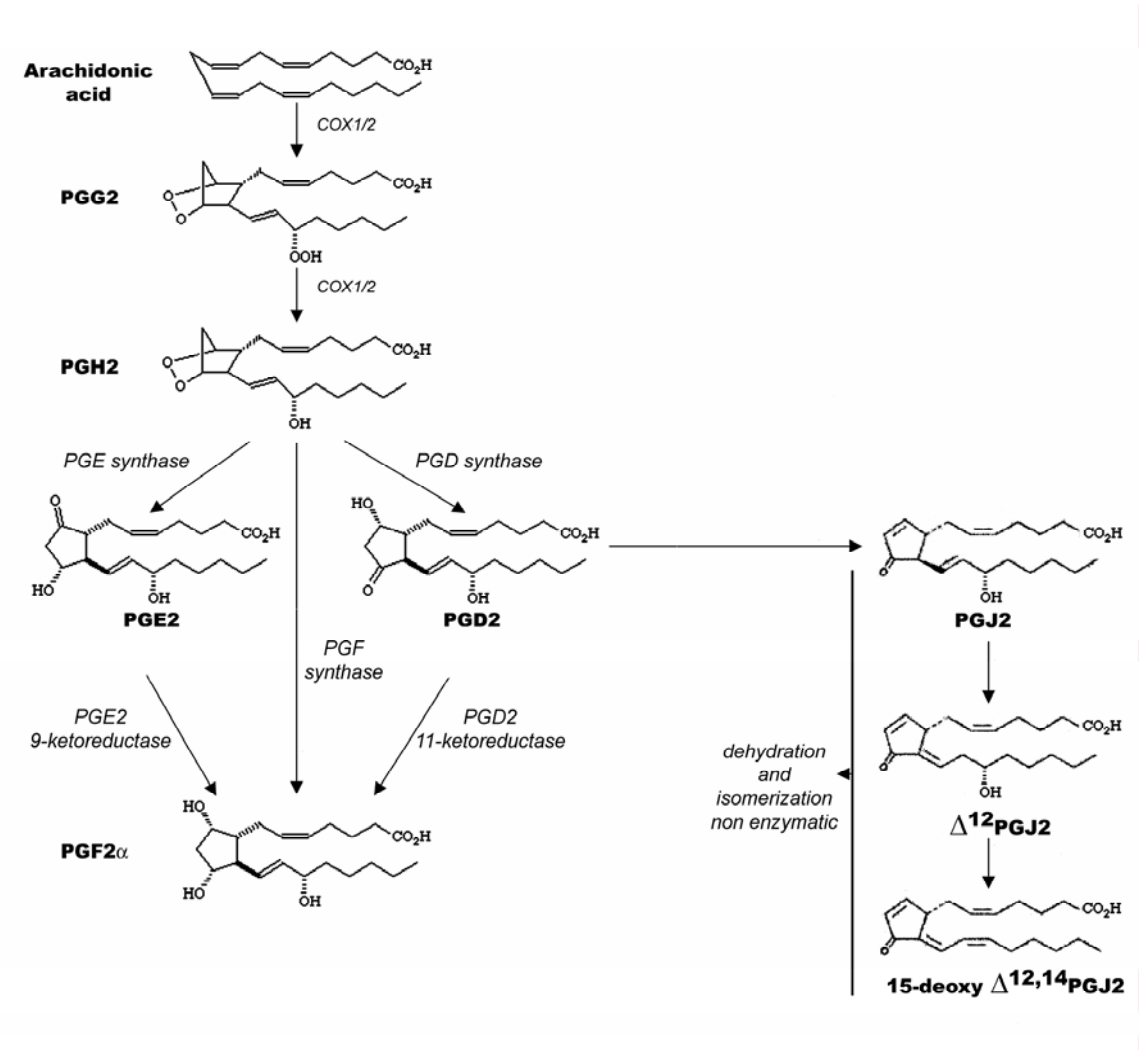

Figure 1. Schematic representation of the COX pathway illustrating the synthesis of the major PGs. The process is initiated by the cyclooxygenase enzyme (COX), which catalyzes both the conversion of arachidonic acid into PGG2, and the subsequent reduction of PGG2 to PGH2, the latter being the common precursor for the synthesis of the remaining PGs.

Cyclooxygenase expression in the testis

Our group has previously described the absence of COX2 in human testicular biopsies with normal morphology. However, biopsies from testes of both infertile patients with Sertoli-cell-only syndrome (SCO-syndrome) and patients with the syndrome of germinal arrest and hypospermatogenesis (GA-syndrome) express COX2 in the interstitial cells (Fig. 2; Frungieri *et al.*, 2002b). Thus, pathological human testes that present an altered morphology appear to be the only ones capable of synthesizing PGs. Recently, we have isolated COX2-immunoreactive interstitial cells showing the characteristic punctuate chromatin pattern of Leydig cells by laser microdissection. We have found expression of steroidogenic acute regulatory protein (StAR) in isolated COX2-immunoreactive interstitial cells. In contrast, they do not express markers of macrophages and mast cells. These results indicate that Leydig cells express COX2 in human pathological biopsies (Frungieri *et al.*, 2007). Moreover, we have also described that the increased number of testicular macrophages found in biopsies from testes of infertile patients (Frungieri *et al.*, 2002a) appears to be involved in the induction of COX2 expression in human Leydig cells (Frungieri *et al.*, 2007). Hase *et al.* (2003) have not detected COX expression in the normal human testis, but did observe induction of COX1 and COX2 expression in human testicular cancer.

Studies performed in Brown-Norway rats have shown that COX appears to play a role in the decrease of the testicular production of testosterone that takes place during aging (Wang *et al.*, 2005). In addition, Wang *et al.* (2003) have shown that in the MA-10 murine Leydig tumor cell line, the inhibition of COX2 stimulates steroidogenesis and the expression of the

StAR protein, whereas the enhanced expression of COX2 generates opposite effects. Sirianni *et al.* (2007) have recently demonstrated that COX2 inhibitors down-regulate aromatase expression and inhibit proliferation in the R2C rat Leydig tumor cell line. Recently, in our laboratory, we have evaluated the testicular expression of COX2 in different species with the aim of obtaining an animal experimental model that could allow us to perform physiological tests related to the role exerted by COX/PGs in testicular function. Immunohistochemical studies have allowed us to detect expression of COX2 in Leydig cells from reproductively-active Golden hamsters (*Mesocricetus auratus*). We have observed no expression of COX2 in the testes of mice, rats, pigs, or monkeys (Frungieri *et al.*, 2006). However, real-time PCR studies have recently detected COX2 expression in spermatogenic and somatic cells (i.e. Leydig and Sertoli cells) from testes of adult rats (Winnall *et al.*, 2007).

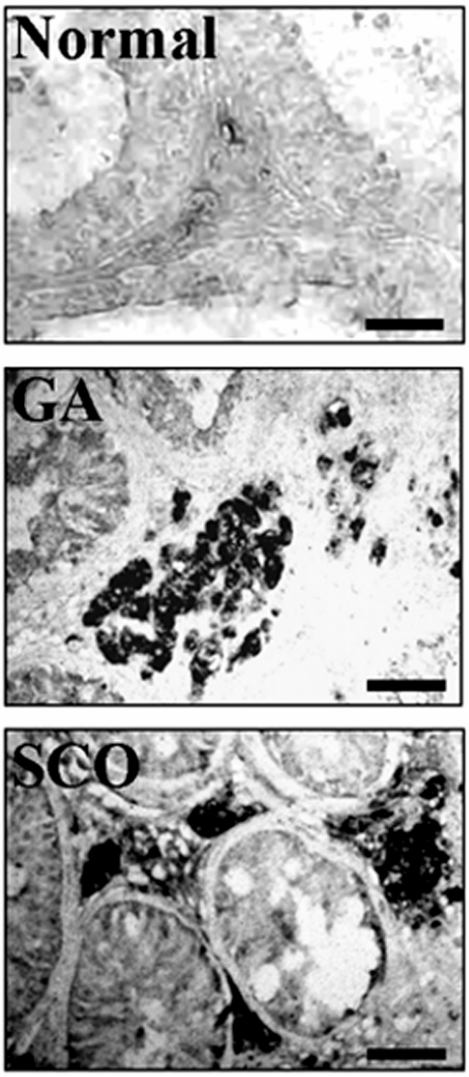

Figure 2. Expression of COX2 in human testicular biopsies. Immunohistochemical staining performed by using a polyclonal rabbit anti-COX2 serum (Oxford Biomedical Research, 1:200) in a human testicular biopsy with normal morphology, in a biopsy from a patient that presents the germ arrest syndrome (GA syndrome), and in a biopsy from a patient that shows the Sertoli cell-only syndrome (SCO-syndrome). Bar: 100 μm.

Prostaglandins and prostanoid receptors in Leydig cells

Data related to testicular expression of prostanoid receptors and to the role exerted by PGs in the testis are very limited. Preliminary studies by Bartke *et al.* (1976) show a significant decrease in the production of testosterone in decapsulated mice testes incubated in the presence of PGA1, PGA2, and PGE1. In addition, Saksena *et al.* (1973) have pointed out that PGE2 and PGF2α decrease the plasmatic levels of testosterone in the male rat. In agreement with these results, it has been described that the repeated administration of PGF2α (Didolkar *et al.*, 1981; Sawada

et al., 1994), as well as the induction of the testicular production of PGF2α by cadmium (Gunnarsson *et al.,* 2004), can cause a significant reduction in the circulating levels of testosterone in the rat. Moreover, Romanelli *et al.* (1995) have demonstrated that PGE2 and PGF2α significantly reduce the secretion of testosterone induced by hCG in rat Leydig cells. Experiments carried out in our laboratory in adult golden hamsters have allowed us to establish that Leydig cells produce PGF2α, which exerts an inhibitory role on the expression of StAR and 17β-hydroxysteroid dehydrogenase, as well as the synthesis of testosterone induced by hCG/LH (Frungieri *et al.,* 2006). In contrast, a single subcutaneous injection of 15 mg PGF2α appears to induce an increase in the circulating concentration of testosterone in Rhesus monkeys (Kimball *et al.,* 1979). Nevertheless, it is important to mention that results obtained by systemic administration of PGs are not necessarily the consequence of a direct action on the testis.

Walch et al. (2003) have described the presence of specific receptors for PGE2 (EP receptors) and for PGF2α (FP receptors) in stem cells of Leydig cells in the rat, whereas those receptors appear to be absent in adult rat Leydig cells. However, immunohistochemical assays performed in Leydig cells purified from adult hamster testes have allowed us to establish the existence of FP receptors (Frungieri *et al.,* 2006).

Prostaglandin-D2 synthase has a hematopoietic and a lipocalin-related isoform. The lipocalin-related isoform of the PGD2 synthase has been found in mice testes (Baker *et al.,* 2001; Ahtiainen *et al.,* 2005), and our group has recently described the presence of PGD2 synthase and DP receptors in the interstitial cells of testicular biopsies obtained from infertile patients (Schell *et al.,* 2007). Studies performed in adult golden hamsters have allowed us to establish that Leydig cells produce PGD2 and that this PG plays a stimulatory role in the basal production of testosterone (Schell *et al.,* 2007).

Prostaglandins and prostanoid receptors in the seminiferous tubules

Expression of the PGD2 synthase has been detected in the cellular lineage that gives rise to the Sertoli cells (Wilhelm *et al.,* 2007), and the Sertoli cells appear to express the PGD2 synthase only during the VI-VIII stages of the spermatogenic cycle, immediately after spermiation (Gerena *et al.,* 2000). The expression of PGD2 synthase in these cells is regulated by progesterone, tri-iodothyronine (T3), and metabolites of vitamin A, and appears to play a role in the differentiation of the Sertoli cells *in vivo* (Samy *et al.,* 2000; Wilhelm *et al.,* 2005).

The production of PGE2, PGF2α, and PGI2, as well as the expression of prostacyclin receptors (specific for PGI), EP1-4 receptors (specific for PGE), and FP receptors have been described in Sertoli cells from prepubertal and adult rats (Cooper and Carpenter, 1987; Ishikawa and Morris, 2006).

The information related to the role played by PGs in the spermatogenic process is also scarce. Our group has detected the expression of EP3 receptors, FP receptors, and the nuclear peroxisome proliferator-activated receptor γ (PPARγ) in the seminiferous tubules isolated from normal and pathological human testicular biopsies by employing the laser microdissection technique (data not published; Fig. 3).

Preliminary studies point out that the administration of PGE1, PGE2, PGF1α and PGF2α produces a marked inhibition of spermatogenesis and a dramatic decrease in the number of sperm (Abbatiello *et al.,* 1976; Kreider *et al.,* 1981; Dev and Mangat, 1982; Singh and Dominic, 1986; Moskovitz *et al.,* 1987). It has been described that ejaculated human sperm has functional receptors for PGE (Schaefer *et al.,* 1998) and that PGs appear to be associated with sperm quality (Barkay *et al.,* 1984; Knuth *et al.,* 1989). Recently, Winnall *et al.* (2007) detected the expression of COX2 and the production of PGE2 in spermatogenic cells in the rat. In addition, RT-PCR assays have allowed the determination of PGD2 synthase expression in germ cells (Sorrentino *et al.,* 1998).

In our laboratory, we have established that 15-deoxy-$\Delta^{12,14}$-PGJ2 (15d-PGJ2), by acting through the PPARγ receptor, induces the proliferation of human fibroblasts (Frungieri *et al.,* 2002b, 2005). In addition, by employing the laser microdissection technique we have isolated the wall of the seminiferous tubules from human testicular biopsies and determined that it expresses PPARγ receptors (Frungieri *et al.,* 2002b). Thus, we concluded that 15d-PGJ2 may play a role in the generation or maintenance of the fibrosis that occurred in the tubular wall of the testes from infertile patients (Frungieri *et al.,* 2002b).

In summary, investigations carried out in the last years in relation to the role played by COX and PGs in the testis have established that they may play a role in testicular physiopathology. As a consequence, testicular PGs appear to be a promising field of research and particularly relevant in the study of the alterations of male fertility. Therefore, further advances in the knowledge of the role played by COX and PGs in reproductive physiopathology could lead to the development of new therapeutical approaches in idiopathic male infertility. Drugs acting on the biosynthetic pathway of PGs or antagonizing their specific receptors should be tested as preventive or blocking treatments in reduced spermatogenesis and germinal arrest conditions. In this context, it is important to point out that certain drugs currently used in clinical medicine for the treatment of diverse inflammatory processes interfere with the production of PGs. Such drugs are easily available and their degree of toxicity and side

effects in humans have already been established. Thus, the potential therapeutical role of such drugs in the treatment of disorders of male infertility associated with the action of PGs at testicular level should also be investigated.

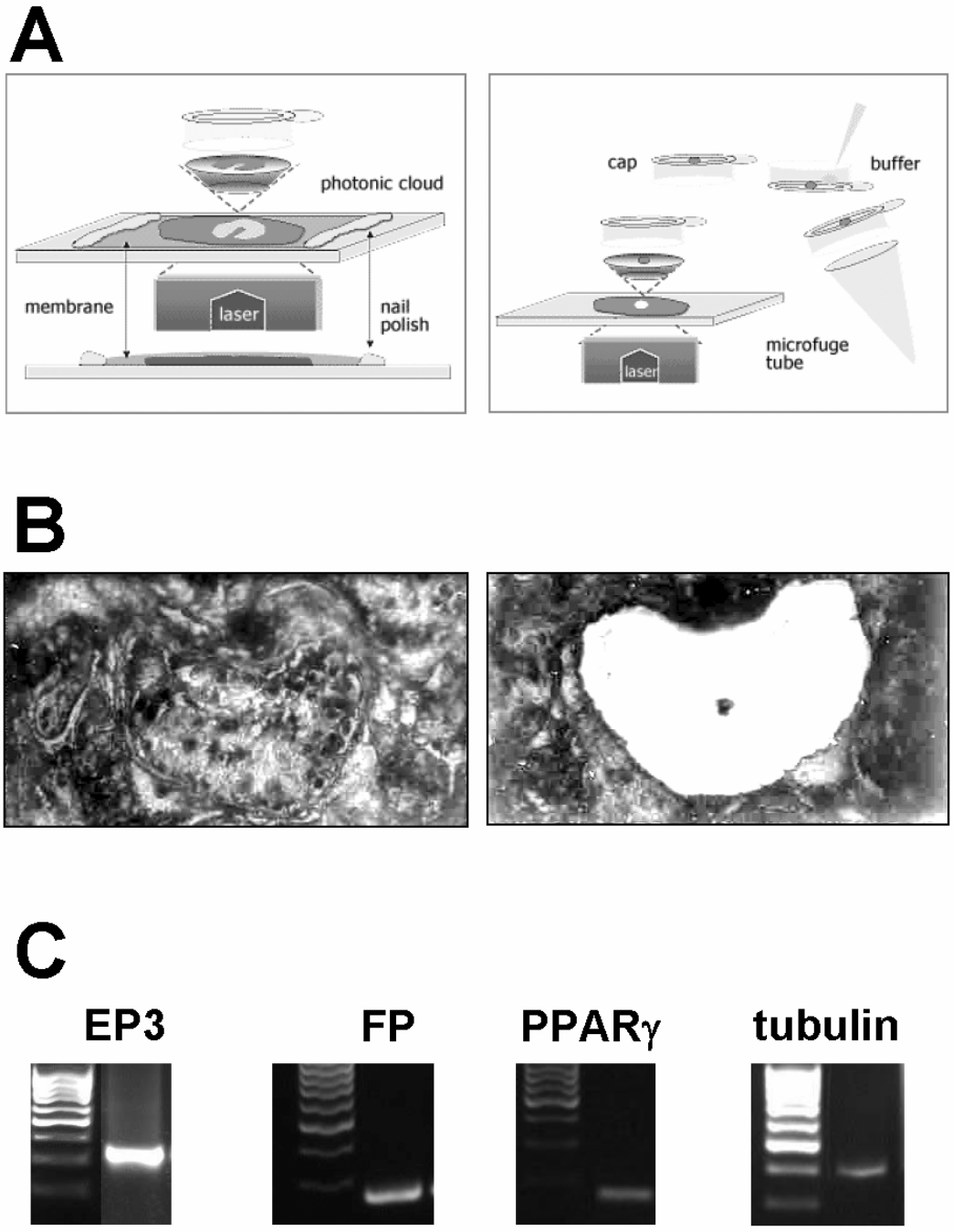

Figure 3. Expression of EP3, FP, and PPARγ receptors in human seminiferous tubules. **A:** Illustrative scheme of the laser microdissection technique employed for the isolation of the seminiferous tubules from normal and pathological (Sertoli cell-only syndrome and germ arrest syndrome) human testicular biopsies. The histological sections have been mounted on a slide covered with a membrane. The high precision laser is employed to initially delimit the area of interest and later catapult the sample inside the lid of an Eppendorf tube. Finally the sample is processed for its evaluation through the RT-PCR technique. **B:** Histological sections stained with hematoxylin-eosin before and after the isolation of a seminiferous tubule from a human testicular biopsy by employing the laser microdissection technique. **C:** Agarose gels stained with ethidium bromide showing cDNA fragments corresponding to the EP3, FP and PPARγ receptors that have been obtained by the laser microdissection technique and RT-PCR from isolated seminiferous tubules of human testicular biopsies.

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