A Prostaglandin D2 system in the human testis

As shown recently, cyclooxygenase 2 (COX2), the inducible key enzyme for the prostaglandin (PG) biosynthetic pathway, is abundantly present in interstitial cells of testes of men suffering from different forms of impaired spermatogenesis and sub- or infertility, but it is absent in human testes with normal spermatogenesis. Although the spectrum of the downstream products of COX2 action in testis, namely PGs, and their effects are not known, our results show that Prostaglandin D2 (PGD2) likely plays a role. We describe (a) PGD2 synthetases, as well as receptors for PGD2 (DP) in testicular interstitial cells of men suffering from spermatogenic damage and infertility, and report that (b) PGD2 is produced by and can affect Leydig cells of an animal model, which expresses testicular COX2 and DP. (Fertil Steril® 2007;88:233–6. ©2007 by American Society for Reproductive Medicine.)

As shown recently, cyclooxygenase 2 (COX2), the inducible key enzyme for the prostaglandin (PG) biosynthetic pathway, is abundantly present in interstitial cells of testes of men suffering from different forms of impaired spermatogenesis and sub- or infertility, while it is absent in human testes with normal spermatogenesis (1). Although the spectrum of the downstream products of COX2 action in these cases are not known, many different PGs are likely to be produced as a consequence. Provided that further PG synthesizing or modifying enzymes, as well as receptors for PG are present, these PG systems may thus be of unexplored relevance to the cellular events occurring in testes of infertile men with damaged spermatogenesis.

Testicular PGs and their actions in the testis are, however, not well examined. In part, this may be explained by the obvious lack of COX expression in testes of most animal species, which normally neither express COX1 nor COX2 (1, 2). Thus, data about receptors for PGs, namely PGE2 and PGF2α in rat Leydig cells (3), or expression of a so-called lipocalin-type PGD2 synthase by adult-type Leydig cells in rodents (4, 5) have not been viewed in context of functional testicular PG systems. Expression of lipocalin-type PGD2 synthase, for example, changes during development, and has been regarded a developmental marker.

Yet some older reports in rodents and newer studies in MA10 cells imply a role of COX2 and/or PGs in Leydig cell steroidogenesis (6, 7). More recently, it has been observed that COX2 is expressed in aging rat testis (8, 9) and in human testicular tumors (10). Adult golden hamster Leydig cells also express active COX2 linked to production of PGs (2), including PGF2α. This PG inhibits gonadotropin-stimulated testosterone production by altering steroidogenic enzymes, presumably via its FP receptors. This implies a regulatory inhibitory local PGF2α system in testis. Because FP, the receptor activated by PGF2α, is also found in human Leydig cells (2), such a system may be functional in testes of men with impaired spermatogenesis and COX2 expression.

Interestingly, in the study mentioned (2), PGD2 was the only other PG tested, besides PGF2α, which affected the function of Leydig cells. Hamster Leydig cells express DP, the receptor for PGD2 (unpublished observation) and PGD2 stimulated basal testosterone production, but did not alter gonadotropin-stimulated steroid output.

In testes of men with impaired spermatogenesis, PGD2 is likely to be produced by two cell types: mast cells and Leydig cells. Significantly more and activated mast cells are found in testes of infertile men (11). They are thought to express the so-called hematopoetic type of PGD2 synthetase, as do mast cells in other organs of the human body (12), while Leydig cells have been shown to express the so-called lipocalin type PGD2 synthetase (4, 5). We hypothesized that this situation may be of special importance in the interstitial testicular compartment of men with impaired spermatogenesis, where COX2 is abundantly expressed (1), and could provide precursor PGs for PGD2 synthetases (1). We reasoned that identification and localization of the receptor for PGD2, namely the G-protein coupled receptor DP, may hint to a role of PGD2 in the testis.

The present study therefore attempted to elucidate in human testes expression of lipocalin type and hematopoietic type PGD2 synthetases. We then evaluated expression and localization of DP, the receptor for PGD2. In parallel, we used hamster Leydig cells, which, with regard to COX2 expression, resemble human Leydig cells in states of impaired testicular function and spermatogenesis, to explore whether this PG is formed and is active as a regulatory factor in testis.

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All methods used have been described previously (1, 2, 11, 13, 14). Human testicular samples (biopsies) were evaluated in this retrospective study. They had been obtained from patients with normal spermatogenesis (including samples from patients with obstructive azoospermia because of prior ligation of the vas deferens or unknown reasons [n = 6]) and impaired spermatogenesis (n = 8; two Sertoli-cell-only, two germ cell arrest, four mixed atrophy syndrome), and are identical to sample pools described previously (14). Data about the general health status of individual patients were not recorded. All participants had granted written Informed Consent to the use of samples and the study had been approved by the local Ethical Committee.

Hamster Leydig cells were isolated as described (2) and enzyme immuno assay for PGD2 (Cayman Chemical, Ann Arbor, MI) were performed following the instructions of the manufacturer. PGD2 (Sigma-Aldrich, Munich, Germany, 2 mM stock solution in 70% ethanol) was used to treat hamster Leydig cells for 3 hours, and testosterone accumulation in the media was measured by RIA, as described (2). A total of four different batches of Leydig cells preparations were studied, consisting of five to six replicates per group. Statistical analysis was done by analysis of variance followed by the Student-Newman-Keuls test.

For immunohistochemistry, the ABC method and a commercial anti DP antibody (Sigma-Aldrich; 1:800) were used. For PCR studies, we used commercial cDNA (pooled testicular samples; Invitrogen GmbH, Karlsruhe, Germany), cDNA obtained from laser microdissected human testicular biopsies (interstitial and tubular areas [13]), as well as deparaffinized testicular sections scratched from glass slides: normal (n = 2) and pathologic samples (n = 4). These biopsies were also used for immunohistochemistry. Oligonucleotide primers for PCR for lipocalin type PGD2 synthetase (accession number Genbank NM_000954) were 5'-GGT GGA GAC CGA CTA CGA C-3' (sense) and 5'-TGT TCC GTC ATG CAC TTA TCG-3' (antisense), for the hematopoietic type PGD2 synthetase (NM_014485) a sense primer, 5'-TGA CTG GCC TGA AAT CAA ATC AA-3' and antisense primer pair 5'-AGT GTC CAC AAT AGC ATC AAC AT-3' were used. Primers for the DP (NM_0009539) were 5'-TGC AAC CTC GGC GCC ATG-3' (sense) and 5'-TCC TGT ACC TAA GAG GTC-3' (antisense). Laser microdissection and treatment of dissected material were peformed as described (1, 14). For laser microdissected material the first set of DP primers was as follows: 5'-TGC AAC CTC GGC GCC ATG-3' (sense) and 5'-TCC TGT ACC TAA GAG GTC-3' (antisense) and nested primers were 5'-CAA CCT CTA TGC GAT GCA-3' (sense) and 5'-CAA GGC TCG GAG GTC TTC-3' (antisense). All PCR products were sequenced to verify their identities (1, 11).

PROSTAGLANDIN D2 SYNTHETASES AND DP IN HUMAN TESTES

Results of reverse transcriptase-polymerase chain reaction (RT-PCR) studies showed expression of PGD2 synthetases, namely lipocalin type and hemaotopoietic type, in the human testis (Fig. 1A). The latter can be assumed to be present in mast cells (our unpublished immunohistochemical studies), while the lipocalin form, according to published data, is likely to be present in Leydig cells (4, 12). RT-PCR also showed that the DP receptor gene is expressed in human testes (Fig. 1A and B). Laser microdissection followed by RT-PCR indicated its expression in interstitial, but not in the tubular compartment (Fig. 1B). This was corroborated by immunolocalization studies, which showed DP in interstitial cell clusters (Fig. 1C).

Together with the documented presence of COX2 in interstitial cells (1), which likely provides precursor PGs for PGD synthesis, these results imply that PGD2 is a product of interstitial cells, both mast cells and Leydig cells of human testes. PGD2 via DP may therefore regulate Leydig cell function in a paracrine/autocrine manner. To explore these possibilities further, additional studies were performed using hamster Leydig cells.

PGD2 IS PRODUCED BY AND ACTS ON LEYDIG CELLS

Freshly isolated hamster Leydig cells constitutively express both COX2 (2), and as we found, secrete PGD2 (150 fmol/10^6 Leydig per 3 hours). In accordance with previous results (2), treatment of hamster Leydig cells with PGD2 (10 μM for 3 hours) resulted in a significant stimulatory effect on basal testosterone production in four experiments ($P<.05$ Student-Neuman-Keuls test; data not shown).

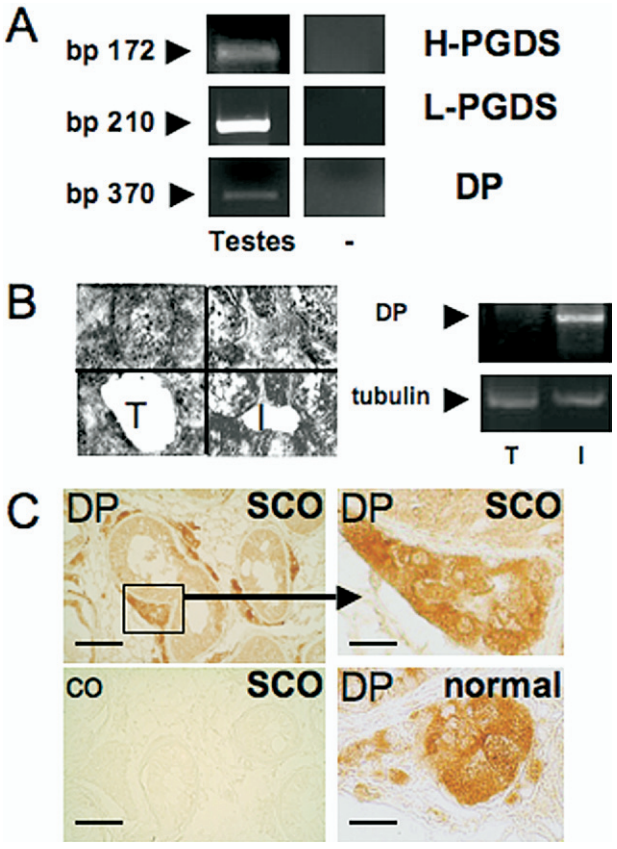
To our knowledge, this is the first report describing a "PGD2-DP system" in human testis. This system appears to be of special relevance to testes of infertile men with deranged spermatogenesis, in which COX2 is also expressed. Thus, COX2 action may initiate a chain of events, which include formation of PGD and its action on Leydig cells.

Although reasons for spermatogenic defects in the patients examined in this study are likely heterogeneous and karyotypes and endocrine profiles were not recorded, COX2 and PG synthesis appear to be common in testes of patients with different types and degrees of derangements of spermatogenesis (1), implying a general mechanism.

Our results indicate two potential sources for PGD2 in human testes: Leydig cells, as evidenced by expression of lipocalin type PGD2 synthetase, and mast cells, which express hematopoietic type of PGD2 synthetase. As COX2, the key enzyme of PG synthesis, and PGD2 synthetases are concomitantly expressed in interstitial cells of men with impaired spermatogenesis, one must assume that formation

FIGURE 1

(**A**) Results from RT-PCR experiments: both hematopoietic-type (H-PGDS) and lipocalin-type (L-PGDS) PGD2 synthetases are found in human testes, which also contain the corresponding DP receptor. Controls (−) without testicular input cDNA are also shown. PCR products were verified by sequencing. (**B**) Detection of DP by laser microdissection and RT-PCR: tubular (T) and interstitial (I) tissue of a Sertoli-cell-only patient biopsy before (*top*) and after (*bottom*) laser microdissection are shown. Samples obtained were subjected to RNA extraction, RT, and nested PCR. DP was found to be present only in interstitial areas. Detection of tubulin in both samples indicates presence of equal amounts of input cDNA. (**C**) Detection of DP by immunohistochemistry: one representative experiment using a biopsy from a Sertoli-cell-only patient is shown (*top* and *bottom left*) and indicates that DP protein is expressed by and restricted to interstitial cells. In the control (co) shown, the primary antibody was replaced by normal serum. DP was also found in interstitial cells of a sample of a testis with normal spermatogenesis (*bottom right*). Bars: approximately 60 μm.



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of PGD2 in these cases occurs in the interstitial compartment. Production of PGD2 was indeed proven in freshly isolated hamster Leydig cells (2), which also respond to PGD2 by increased testosterone production.

In conclusion, the topic of COX2, PGs, and testis is emerging as a field of research, which may be highly relevant to human testicular functions, specifically to men with impaired spermatogenesis and infertility. The currently available data imply that COX2 is present in human testes of men with deranged spermatogenesis, and that at least three PG receptors are expressed in human testes as well, namely FP, PPARγ, and DP (1, 2, and present study). Many commonly used drugs interfere with PG formation. Therefore, deeper insights into these testicular systems are urgently required, and may lead to novel therapeutic approaches in male infertility.

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