**CFTR gene and male fertility**

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**Introduction**

As early as the 1930s, it was noted that testicular spermatozoa are incapable of fertilization until they have gone through a certain length of the epididymis. Opinions were divided as to whether the process of maturation is intrinsic to the spermatozoon or whether it requires specific secretion by the epididymis *per se*. It was not until 30 years later that Orgebin-Crist first put these arguments to vigorous experimental test. Orgebin-Crist (1967) then reported that sperm maturation in the rabbit appeared to rely on the special environment created by the corpus epithelium. This paper has led to an explosion of work on other species. The results show that for the few animals studied, spermatozoa taken from the more distal part of the epididymis display greater fertilizing capacity than if taken from the more proximal region (for review see Turner, 1995). This principle seems to hold for humans also, although there has been some controversy regarding the human epididymis since men who have undergone anastomosis of the vas to the caput, efferent duct or the testis to overcome obstructions have been found to produce fertile semen. However, in their reviews, Cooper (1990) and Turner (1995) defended the human epididymis by reassessing this information. They cautioned against the use of the pathological tissues in reaching this conclusion. The collective view now is that the human epididymis has the same essential sperm maturation role as in the few animals studied.

Spermatozoa deposited into the female tract have to undergo various prerequisite steps before fertilization can occur. For example, they have to be capacitated, to recognize the egg, to undergo an acrosome reaction, to bind and penetrate the zona pellucida and to fuse with the vitellus. The ability to accomplish all these steps is acquired in the epididymis. Recently, our knowledge of sperm-associated macromolecules and their role in sperm maturation has advanced by leaps and bounds. Advances in molecular biology techniques have made possible the study of these molecules at the gene level. Interested readers will find this topic in other review articles.

**Adrenergic control of electrolyte/liquid secretion**

The epididymis is highly innervated with sympathetic nerves. The distribution of adrenergic nerves in the rat epididymis has been studied using immunohistochemical techniques (Dun et al., 1996). It was found that these nerves penetrate into the interstitial space between the tubules. They also form an extensive plexuses surrounding the epithelium. There have been reports that disruption of the sympathetic nerves to the rat epididymis, either by chemical means (Wen and Wong, 1988) or surgical procedure (Ricker et al., 1996), leads to changes in the sperm concentration, viscosity and protein composition of the luminal fluid, indicating that these nerves play an important role in the formation of the sperm micro-environment.

We have studied how adrenergic agents affect electrolyte secretion by the epididymides of the rat, mouse and man by culturing epididymal epithelial cells on porous supports so that electrolyte transport can be studied using conventional electrophysiological techniques. When grown on polycarbonate filters, epididymal cells form a continuous monolayer of uniform cells which retain the polarized characteristics of epithelial cells. When confluent, the monolayers were clamped in a modified Ussing chamber for the measurement of transepithelial potential difference and the short-circuit current. It was found that the addition of noradrenaline (NA) to the basolateral side of the epithelium stimulated chloride secretion (blood to lumen) by the epithelium. This response was shown to be mediated by the simultaneous activation of both α- and β-adrenergic receptors transduced by intracellular calcium and cAMP respectively (Leung and Wong, 1994).

We used patch-clamp techniques to elucidate the secretory process of chloride secretion by the epididymides of the rat. The use of the pathophysiological techniques has made possible the study of these molecules at the gene level.
mechanism at a subcellular and membrane level (Chan et al., 1994). Under the whole cell-patch clamp condition, NA added to the epididymal cell caused an increase in chloride (Cl⁻) current, consistent with an effect of NA on transepithelial Cl⁻ secretion in cultured epithelia. The increase in current was shown to involve both α-adrenergic and β-adrenergic receptor components. By analysing the kinetics of the current activation, we found that the α- and β-receptor-activated currents have quite distinct characteristics. α-adrenergic agonists were shown to stimulate a current which indicates outward rectification and is time- and voltage-dependent. The β-adrenergic agonists were shown to stimulate a current which displays a linear current/voltage (I/V) relationship and is independent of time and voltage.

Based on this observation, we propose that sympathetic nerves release NA which has dual effects. When interacting with α-adrenergic receptors, it stimulates fluid secretion via a Ca²⁺-activated pathway ending on a distinctive Cl⁻ channel, and when interacting with the β-receptors, it stimulates secretion via the cAMP pathway ending on a cAMP-dependent Cl⁻ channel. Normal secretion requires the simultaneous activation of the two parallel pathways. In cystic fibrosis (CF), the cAMP pathway is disrupted and secretion is reduced resulting in blockage of the glandular lumen with thick mucous materials. These changes underlie the pathophysiology of CF (see below).

**Paracrine control of secretion**

For some time now, we have been interested to know whether epididymal spermatozoa can interact with epididymal epithelial cells. This cross-talk between spermatozoa and epididymal cells may provide a mechanism for the spermatozoa to regulate the fluidity of their own environment. The local renin–angiotensin system (RAS) has been shown to fulfill this role. According to our model, the basal cells generate angiotensin II (Ang II) which is released to the peritubular space. Ang II then acts on the basolateral AT1 receptors on the basolateral membrane of the principal cells to stimulate chloride and fluid secretion. Spermatozoa are known to possess high angiotensin II and its antagonists and immunohistochemical localization of Ang II and AT1 receptor proteins in the epididymis (Wong et al., 1990; Zhao et al., 1996; Leung et al., 1997).

Apart from the angiotensins, various vasoactive peptides have been shown to stimulate secretion via the cAMP signalling pathway converging on the cAMP activated Cl⁻ channel in the apical membrane of the principal cells. This channel protein which is the key element in secretion is encoded by the cystic fibrosis transmembrane conductance regulator (CFTR) gene.

**CFTR gene and male infertility**

Cystic fibrosis (CF) is the most prevalent genetic disease. It affects 1 in 2000 live births in the Caucasian population. The gene responsible for CF comprises a 250 kb sequence of DNA located on the long arm of chromosome 7. A total of 27 coding regions have been sequenced. The normal CFTR gene encodes a 1480 amino acid glycoprotein known as CFTR. The protein has two 6-membered membrane spanning domains linked by a polypeptide chain called the R-domain which contains numerous phosphorylation sites. There are also two nucleotide binding folds (NBF). Since the gene was discovered in 1989, more than 500 mutations have been identified. This large number of mutations are responsible for a wide spectrum of disease expressions in CF.

CFTR is a membrane protein. When targeted to the apical membrane of epithelial cells, it functions as a small conductance Cl⁻ channel with a regulatory domain. When the cell is at rest, the Cl⁻ channels are closed and the cells do not secrete. When the cells are stimulated by cAMP-elevating agents, the cAMP generated phosphorylate the CFTR through protein kinase A. When the protein is phosphorylated, the Cl⁻ channel opens allowing Cl⁻ to diffuse out of the cell and secretion ensues. Before the channel is activated, binding and hydrolysis of ATP at the two NBFs are necessary. In CF, CFTR proteins are either absent from the apical membrane, or if present, they are abnormal and cannot be regulated by cAMP.

The work described above provides functional evidence for the existence of CFTR protein in the epididymis. More direct evidence comes from determination of the CFTR gene or the gene product. Using in-situ hybridization, Tizzano et al. (1994) were able to detect CFTR mRNA in the post-natal human epididymis. They found that expression is strongest in the caput but declines along the duct. In the cauda, expression was found to be less consistent, in that some segments stained positively while others did not. However, no CFTR mRNA was detected in the human testis. In our laboratory, using a monoclonal antibody against the R domain of the human CFTR, we have localized the CFTR protein to be in the luminal border of the human cauda epididymal epithelium, consistent with its role as an apical chloride channel (Wong et al., 1992).

In ~97% of the men with clinical CF, the vas deferens on both sides are missing and these men are therefore infertile. This anomaly may be traced back to the embryonic stage where the Wolffian duct is differentiated into the scrotal vas and distal epididymis. It is conceivable that fluid secretion by the Wolffian duct is necessary for normal development. When secretion is blocked in CF, normal development is interrupted hence leading to vas agenesis. It has been shown that the expression of the CFTR gene is developmentally regulated. Cultured epithelial cells from the human fetal vas deferens have been shown to express the CFTR gene (Harris et al., 1991). Its product, a small conductance cAMP-activated Cl⁻ channel, has been identified in the human fetal vas deferens cells (Pollard et al., 1991).

**CF mouse model**

To study how secretion by the epididymis is impaired in CF, we took advantage of the CF mouse model of CF generated
by the North Carolina Group by gene targeting (Snouwaert et al., 1992). The CF mice can survive up to 40 days after which they usually die of intestinal obstruction. Interestingly, their epididymides look apparently normal and their fertility is little affected. We cultured the epididymal epithelia from the CF mouse and studied their responses to secretory agonists (Leung et al., 1996). The epididymal epithelia from normal animals can respond to Ca\(^{2+}\) elevating agonist such as ionomycin and apically applied ATP, and also to cAMP, indicating that both the Ca\(^{2+}\) and the cAMP pathways are in place and working to stimulate secretion. However, in the CF mouse which is homozygous for the disrupted gene, the response to cAMP is abolished while the responses to Ca\(^{2+}\) agonists are preserved. These results confirm that the mouse epididymis expresses the CFTR gene and its disruption leads to a loss of cAMP-driven secretion. However, in the CF mouse, a significant level of secretion can still be obtained with the Ca\(^{2+}\) agonists. The Ca\(^{2+}\) pathway can therefore function as an alternative pathway which can compensate for the loss of the CFTR proteins. This may explain why the mouse epididymis is apparently normal in CF. In man, it is conceivable that the Ca\(^{2+}\) pathway is less prominent so that a defect in the CFTR protein cannot be compensated for by the alternative pathway. This may explain why the human epididymis is very vulnerable to CFTR gene mutation. However, this point needs to be verified in future work.

**Congenital bilateral absence of the vas deferens (CBAVD)**

As mentioned before, the disease manifestations of CF have many forms owing to a myriad of mutations in the CFTR gene (genotypes). Recently, a lot of attention has been focused on the phenotype, congenital bilateral absence of the vas deferens (CBAVD). Men with CBAVD are apparently healthy with rather normal lung and pancreatic functions. CBAVD appears to be a heterogeneous genetic condition, many cases being rather normal lung and pancreatic functions. CBAV (CBAV) appears to be a heterogeneous genetic condition, many cases being mild forms of CF (De Braekeleer and Férec, 1996). Since the epididymis is most sensitive to CFTR mutation, the only disease expression is absence of the vas deferens. It accounts for ~2% of male infertility.

Chillon et al. (1995) have screened for CFTR gene mutation in 102 men with CBAVD but have no clinical symptoms of CF. They also analysed a DNA variant in the non-coding region of the CFTR gene, the 5T allele. They found that a combination of the 5T allele in one copy of the gene with a CF mutant in the other copy is the most common cause of CBAVD.

How does the 5T variant affect the CFTR protein? The 5T allele is known to give rise to an aberrantly spliced mRNA that lacks exon 9. Such anomalous mRNA is translated into abnormal CFTR which cannot function as a Cl\(^{-}\) channel. Therefore, men with 5T variant in the non-coding region of the gene would produce abnormally low level of CFTR protein in the epididymis. However, there may be sufficient proteins to prevent disease in other organs (such as the lung and the gastrointestinal glands) normally affected by CF. This may explain why the lung and pancreas are normal in CBAVD, but the epididymis is not.

Rave-Harel et al. (1997) have analysed the level of the correctly spliced RNA transcribed from the 5T allele in the nasal and epididymal epithelia in men with CBAVD. They found that these mutations produced very low levels of normal CFTR–mRNA in the epididymis but significantly higher levels in the respiratory epithelial cells sufficient to support normal lung functions. This may explain why the lung function is apparently normal in these men with CBAVD. This highlights that the efficiency of the splicing mechanism differs between different organs of the same individual and among different individuals, which may explain the heterogeneous nature of the disease expression in CF.

**CFTR gene mutations and other causes of male infertility**

To bring this subject to new heights, van der Ven et al. (1996) have tested the possible involvement of CFTR gene in the cause of male infertility other than the CBAVD. They screened a total of 127 unrelated healthy males with various diagnoses of reduced sperm quality for 13 CFTR mutations. They found that the frequencies of mutations in these samples of infertile male were significantly higher than the expected carrier frequencies in the general population. This observation suggests that CFTR mutations not only give rise to clinical CF with debilitating lung and pancreatic problems and CBAVD, they are also seen in a general population of healthy men with reduced sperm quality as the only complaint (Figure 1). This shows that electrolyte and fluid transport in the epididymis involving the CFTR gene may have a broader impact and far-reaching effect on human reproduction. It is believed that further understanding of the gene and its role in the epididymis would provide new therapeutical alternatives for the treatment of male infertility as well as for generating new insights for controlling male fertility.

**Genetic counselling for male infertility caused by CFTR gene mutations**

In the past, CBAVD and CF had been viewed as untreatable causes of male sterility. However, the advent of assisted
reproductive techniques such as microsurgical epididymal sperm aspiration (MESA) in conjunction with in-vitro fertilization (IVF) with or without micromanipulation (ICSI) and embryo transfer (Silber et al., 1990; Asch and Silber, 1992; Silber et al., 1994) has enabled testicular spermatozoa to fertilize eggs without going through the maturation process in the epididymis. This breakthrough in reproductive technology has offered new hope for men with CBAVD and CF to father children. However, before such measures are taken, genetic screening and counselling for the men and their partners are mandatory in safeguarding the offspring from the risk of clinical CF. Detection of a CFTR mutation in the patient’s spouse would indicate a high risk situation whereas the absence of a mutation would indicate a low risk situation. Because the spectrum of CFTR mutations is markedly different among populations, the ethnic background of the patients and their spouses should be taken into consideration to ensure that the most prevalent mutations appropriate to that particular population are included in the screening panel (De Braekeeleer and Férec, 1996). It is believed that genetic screening and counselling for infertile men and their partners requesting MESA/IVF will assume an increasingly important place in reproductive health care as our knowledge of the role of genetic abnormalities in the causation of human infertility (other than CBAVD and CF) is gradually expanded (Meschede and Horst, 1997).

References

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