Expression of the cystic fibrosis transmembrane conductance regulator in rat spermatids: implication for the site of action of antispermatogenic agents


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To establish whether cystic fibrosis transmembrane conductance regulator (CFTR) is functionally expressed in the testis, we subjected spermatogenic cells from rat testes to analysis of CFTR mRNA, protein and channel activity. CFTR mRNA was detected in the testes of mature but not immature rats using reverse transcription–polymerase chain reaction analysis. Western blot analysis performed with a CFTR specific antibody revealed immunoreactivity in the membrane extract of spermatogenic cells. Immunohistochemical studies localized CFTR in round and elongated spermatids, but not in the fully developed spermatozoa. Using a whole-cell patch clamp technique, we recorded an inward current activated by intracellular cAMP (100 µmol/l) in round spermatids. The current displayed a linear I/V relationship and was inhibited by diphenylamine-2-carboxylate (DPC), a chloride channel blocker. Transfection of the rat germ cell CFTR cDNA into human embryonic kidney (HEK) 293 cells caused the expression of a cAMP-activated chloride current with CFTR characteristics. The current was completely blocked by the antispermatogenic agents 1-(2,4-dichlorobenzyl)-indazole-3-carboxylic acid, lonidamine (500 µmol/l) and 1-(2,4-dichlorobenzyl)-indazole-3-acrylic acid, AF2785 (250 µmol/l). These results taken together provide evidence that CFTR is differentially expressed in spermatids during spermiogenesis. We speculate that CFTR may interact with aquaporin to bring about cytoplasmic volume contraction which is an essential feature of spermiogenesis.

Key words: antispermatogenic agents/CFTR/ion channels/spermatogenic cells

Introduction

Spermatogenesis is a complex cellular event during which stem cells in the seminiferous tubules divide, differentiate and remodel into the haploid spermatozoa, the male gametes (Austin and Short, 1972; Steinberger and Steinberger, 1975). In order for this complex cellular event to occur in an orderly fashion during the spermatogenic cycle, cell division and differentiation must be precisely controlled by signals arising from within and outside the seminiferous tubules. Changes in membrane potential of germ cells are likely to be an important part of the signalling mechanism. There is a large body of evidence that ion channels are essential elements in cell signalling for cell division, differentiation, and maturation of somatic cells (Hilde, 1992). Ion channels therefore may also play an important role in the control of gamete functions (Hagiwara and Kawa, 1984; Darszon et al., 1999).

Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) gene which encodes a membrane protein located primarily in the apical membrane of polarized epithelial cells. CFTR is now shown to act in two major ways. (i) It acts as a cAMP-activated chloride channel, and (ii) it is a regulator of other membrane conductances. CFTR mutations result in a variety of abnormal functions associated with epithelia and exocrine glands. A growing body of evidence suggests that mutation of the CFTR gene is also associated with a severe reduction in fertility, especially in male CF patients. Much of this infertile state is related to developmental abnormalities that result in blockage or absence of the vas deferens (Oates and Amos, 1994; Chillion et al., 1995). Testicular biopsy of some CF men have revealed a decreased number of mature spermatids in the seminiferous epithelium and many of the testicular spermatozoa are malformed. Van der ven et al. have reported that 17.5% of infertile men with poor sperm quality and who are apparently healthy have at least one mutation in the CFTR gene (Van der Ven et al., 1996). These observations pointed to the involvement of CFTR in spermatogenesis although Mak et al. have not detected CFTR mutations in infertile men with primary testicular failure (Mak et al., 1999). There have been reports that CFTR is
expressed in germ cells at specific stages of spermatogenesis (Trezise et al., 1993) and that functional CFTR protein is expressed in the Sertoli cells (Boockorf et al., 1998). Our previous studies also revealed that functional CFTR protein is present in the cells of epididymis (Wong et al., 1992; Leung et al., 1996; Wong, 1998; Gong et al., 2000). The goal of this study was to determine whether CFTR protein is expressed in spermatogenic cells in adult rats and to investigate its role, if any, in spermatogenesis.

Materials and methods

Preparation of rat germ cells

All experiments on animals were performed in accordance with the guidelines on the use of laboratory animals laid down by the Animal Ethics Committee of the Chinese University of Hong Kong. Eighty-day old (~300 g body weight) male Sprague–Dawley rats were used as a source of spermatocytes and spermatids. Nine-day old (~20 g body weight) male rats were used as a source of spermatogonia. Rats were killed by CO₂ inhalation. The testes were dissected out and immersed in sterile phosphate-buffered saline (PBS): 140 mmol/l NaCl, 10 mmol/l Na₂HPO₄, 2.7 mmol/l KCl, 1.8 mmol/l KH₂PO₄, pH 7.4. The testes were decapsulated and the blood vessels were removed. Dispersed seminiferous tubules were then transferred to 50 ml tubes with 50 ml PBS added. After the tubules had settled down to the bottom of the tube, the supernatants containing Leydig cells and red blood cells were discarded. Seminiferous tubules were minced in Petri dishes with sterile blades and scalpels for 10 min. The minced tubules were washed with PBS and transferred to 50 ml centrifuge tubes and centrifuged lightly at 200 r.p.m. for 2 min. The supernatant containing germ cells was filtered consecutively through mira cloth, nylon mesh (100 µm), glass wool, and nylon mesh (20 µm) to remove tissue debris, cell clumps, elongated spermatids and spermatozoa. The filtrates were centrifuged at 105 g for 4 min. The pellets were used for isolation and purification of different cell types.

Fractionation of different spermatogenic cells by discontinuous Percoll gradient

Spermatogenic cells were collected and fractionated on a discontinuous Percoll gradient of 20–65%. After centrifugation at 1164 g for 20 min at room temperature, cell fractions were recovered and washed with PBS. Total RNA and protein extracts were analysed by reverse transcription–polymerase chain reaction (RT–PCR) and Western blotting respectively. Some of the pellets were gently reconstituted in 2 ml of culture medium for patch clamp recording.

RT–PCR

Total RNA was isolated from the rat germ cells by TRIzol reagent (Gibco BRL, Gaithersburg, MD, USA). Approximately 5 µg total RNA was reverse-transcribed by SuperScript™ premptilization system (Gibco BRL) according to the manufacturer’s protocol. This was the cDNA template for use in the following PCR experiments.

Primers used in cloning and PCR of rat CFTR cDNA are summarized in Table I. The PCR were carried out by combining the following reagents in a final volume of 50 µl: 1× PCR buffer, 1.5 mmol/l MgCl₂, 5 mmol/l dNTP, 10 µmol/l primer pair, 1 unit Taq DNA polymerase and 2 µl cDNA template. These PCR mixtures were subjected to 25–28 cycles at 95°C for 1 min, 55°C for 1 min and 72°C for 3 min. The PCR products were resolved on a 1.2% agarose gel and visualized under UV followed by ethidium bromide staining.

In selected experiments, PCR co-amplification was performed to yield semi-quantitative results. Briefly, an internal control primer pair of rat ribosomal S16 (Table I) was co-amplified with either CFTR-Nhel and CFTR-Xhol primer pair (specifically amplifying the P3 portion of the CFTR cDNA) or aquaporin 7 (AQP7) primer pair (Table I). These reactions were carried out in a similar condition as described above. Under such conditions, the production of S16 and P3 or AQP7 amplicons were all in the linear phase when aliquots of PCR products were withdrawn in cycles 22, 25 and 28 for gel analysis. To confirm the authenticity of these amplicons, PCR products of expected sizes (S16, 385 bp; P3 of CFTR, 1.4 kb; AQP7, 824 bp) were gel-purified and subjected to partial DNA sequencing (Perkin Elmer/Applied Bioscience 310 Automated DNA sequencer).

Cloning of CFTR cDNA into pcDNA vector for transfection into HEK293 cells

The full-length rat spermatogenic CFTR cDNA was firstly cloned into the pGEM-T vector (Promega Corporation, Madison, WI, USA) and its authenticity was confirmed by PCR and partial DNA sequencing. The cDNA fragment encoding rat germ cell CFTR was excised from pGEM-T/cftr using the restriction enzyme NotI. The insert was then ligated into a NotI-restricted pcDNA3 vector (Invitrogen, Carlsbad, CA, USA) by rapid DNA Ligation kit (Gibco BRL). The orientation of pcDNA/cFTR was checked by PCR. For the expression studies, human embryonic kidney 293 (HEK 293) cells (American Type Culture Collection, Rockville, MD, USA) were grown in minimal essential medium (MEM) (Gibco BRL), supplemented with 10% fetal bovine serum, 100 units/ml penicillin (Gibco BRL), and 100 units/ml streptomycin (Gibco BRL), in a 5% CO₂ incubator at 37°C. Exponentially growing cells were rinsed twice with PBS (100 mmol/l sodium phosphate and 150 mmol/l sodium chloride, pH 7.4), dispered with fresh medium, seeded on a 20 µl poly-D-lysine coated dish. HEK 293 cells were transfected with rat CFTR cDNA using LipofectAMINE® solution (Gibco BRL) according to manufacturer’s protocol. Cells were used for patch clamp recording 40–50 h after the transfection.

Analysis of germ cell cytosol and membrane extracts by Western blot

Germ cell cytosols were prepared from their corresponding primary cultures with minor modifications (Li et al., 2000). Round spermatids were briefly rinsed with lysis buffer: 20 mmol/l Tris, pH 7.4 containing 1 mmol/l EDTA, 2 mmol/l PMSF. Thereafter, cells were resuspended in lysis buffer at three times their packed cell volume and incubated at 4°C for 1 h. The cell sample was then centrifuged at 15 000 g for 30 min at 4°C and the supernatant was used as cytosol. Cell pellets were resuspended in solubilization buffer: lysis buffer containing 0.1% SDS (w/v) and 0.1% Triton X-100 (v/v), incubated at 4°C for 1 h and centrifuged at 40 000 g for 90 min at 4°C. The supernatant was designated as membrane extract. The protein content was estimated by the Coomassie blue dye-binding assay with bovine serum albumin (BSA) as a standard (Bradford, 1976). Approximately 75 µg of cytosol and membrane extracts were resolved on a 7% T polyacrylamide gel under denaturing and reducing conditions as described previously (Laemmli, 1970). The immunoreactive CFTR band was visualized by staining the corresponding blot with the monoclonal anti-CFTR antibody directed against the first extracellular loop (MATG 1016 at 1/5000 dilution; Transgene SA, Strasbourg, France).
CFTR expression in spermatogenic cells

Figure 1. Activation of CFTR current by cAMP in a round spermatid. (A) Time course of cAMP-evoked whole-cell current. Membrane potential was held at -70 mV. 100 µmol/l cAMP was applied to the pipette (internal) solution containing 120 mmol/l CsCl and 20 mmol/l TEA-Cl. The external solution was Krebs–Henseleit (K–H) solution. (B and C) cAMP-activated current at different voltages in the absence and presence of cAMP (100 µmol/l). The voltage protocol used consisted of a stepwise depolarization, in steps of 20 mV, from -100 mV to +100 mV with a holding potential of -70 mV. (D) I/V plot obtained by changing the membrane potential from -100 to +100 mV within 180 ms using the ramp test protocol.

Table I. Primers used in cloning and reverse transcription–polymerase chain reaction of CFTR and AQP7

<table>
<thead>
<tr>
<th>Primer Pair</th>
<th>Primer sequence</th>
<th>Corresponding region</th>
<th>Expected size</th>
<th>Reference</th>
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<tbody>
<tr>
<td>CFTR-KpnI</td>
<td>5'-CACGGTACCATCATGCAGAAGTCGCCTCTG-3'</td>
<td>12 to 4448 bp</td>
<td>4.4 kb (F)</td>
<td>Fiedler et al. (1992)</td>
</tr>
<tr>
<td>CFTR-XhoI</td>
<td>5'-AGCCTCGAGCACTAGAGCCGGGTCTCTTGC-3'</td>
<td>1725 to 3062 bp</td>
<td>1.3 kb (P1)</td>
<td>Fiedler et al. (1992)</td>
</tr>
<tr>
<td>CFTR-KpnI</td>
<td>5'-CACGGTACCATCATGCAGAAGTCGCCTCTG-3'</td>
<td>1725 to 3062 bp</td>
<td>1.3 kb (P2)</td>
<td>Fiedler et al. (1992)</td>
</tr>
<tr>
<td>CFTR-XhoI</td>
<td>5'-AGCCTCGAGCACTAGAGCCGGGTCTCTTGC-3'</td>
<td>3045 to 4448 bp</td>
<td>1.4 kb (P3)</td>
<td>Fiedler et al. (1992)</td>
</tr>
<tr>
<td>CFTR-XhoI</td>
<td>5'-AGCCTCGAGCACTAGAGCCGGGTCTCTTGC-3'</td>
<td>7 to 817 bp</td>
<td>824 bp</td>
<td>Suzuki-Toyota et al. (1999)</td>
</tr>
<tr>
<td>S16-F</td>
<td>5'-TCCGCTGCACTCCGTTCAAGTCTT-3'</td>
<td>15 to 400 bp</td>
<td>385 bp</td>
<td>Chan et al. (1990)</td>
</tr>
<tr>
<td>S16-R</td>
<td>5'-GCCAAACTTCTTCTTGGATTCGCAGCG-3'</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

F = full-length sequence of rat CFTR cDNA; P1, P2 and P3 = first, middle and last portions of rat CFTR cDNA respectively.

Immunohistochemistry

Paraffin sections (3 µm) of mature rat testes were dewaxed and hydrated. Antigens were retrieved by treatment in 10 mmol/l citrate buffer (pH 6.0) for 5 min in a microwave oven. They were then rinsed twice with pure water and incubated in methanol containing 3% H2O2 for 15 min. After rinsing with pure water and PBS, sections were incubated in normal blocking serum (Vectastain Elite ABC kit, Vector Labs, Burlingame, CA, USA) for 30 min and then with the monoclonal anti-CFTR antibody directed against the first nucleotide binding fold (MATG 1061; Transgene SA, France), diluted 1:100 with diluting buffer (PBS with 0.01% Triton X-100, 0.01% Tween 20, and 0.1% BSA) at 4°C overnight. Sections were washed three times with PBS and incubated with biotinylated secondary antibody (ABC kit) for 30 min. After washing three times with PBS, the sections were incubated with Vectastain Elite ABC reagent (ABC kit) for 30 min, and finally washed three times with PBS again. Visualization was achieved by immersing sections in a peroxidase substrate solution (Vector DAB substrate kit) until the desired stain intensity developed. Slides were rinsed with pure water for 5 min, counterstained with Lillie–Mayer’s haematoxylin (Merck, Darmstadt, Germany), dehydrated, and mounted for observation. Negative controls were obtained by omission of primary antibodies.

Whole-cell patch clamp recordings

After about 30 min incubation at 32°C, germ cells adhered to the bottom of the dish. Recordings were made from solitary cells which
Figure 2. Inhibition of CFTR current by DPC in a round spermatid. (A) Effect of extracellular DPC (500 µmol/l) on the cAMP-activated current. Membrane potential was held at −70 mV. 100 µmol/l cAMP was applied to the pipette (internal) solution containing 120 mmol/l CsCl and 20 mmol/l TEA-Cl. The external solution was Krebs–Henseleit (K–H) solution. (B and C) cAMP-activated current at different voltages before and after addition of DPC (500 µmol/l). The voltage protocol used consisted of a stepwise depolarization, in steps of 20 mV, from −100 mV to +100 mV with a holding potential of −70 mV. (D) I/V plot obtained from changing the membrane potential from −100 to +100 mV within 180 ms using the ramp test protocol.

Results

Separation of germ cells using discontinuous Percoll gradient

In the testes from 9-day old rats, the spermatogonia represent the most abundant cells present in the seminiferous epithelium. In the testes from 80-day old rats, two classes of cells were in the majority (≈80%). The first class of cells had large diameters (>18 µm), a large nucleus to cytoplasm ratio and condensed chromatin visible inside the nucleus. They were identified as pachytene spermatocytes. The second class of cells was characterized by their smaller round shape (11–14 µm in diameter), short and thin flagellum and developing acrosome on the nucleus. These cells were identified as early or round spermatids (Austin and Short, 1972). Spermatogonia and the pre-leptotene or leptotene spermatocytes were in the minority. These cells were separated by the discontinuous Percoll gradient. The spermatocytes could be recovered from the 30% Percoll layer, the spermatids from the 37 and 40% layers, and the spermatogonia from the 45% layer. The cell debris and clumped cells were separated out in the 20 and 25% layers.
CFTR expression in spermatogenic cells

Figure 3. (A) Detection of CFTR mRNA from rat round spermatids. Reverse transcription–polymerase chain reaction products amplifying different regions of CFTR cDNA. F represents the 4.4 kb full-length CFTR cDNA. P1, P2 and P3 represent the 1.7, 1.3 and 1.4 kb segments of the CFTR cDNA respectively. M is the molecular weight marker. (B) Detection of CFTR protein in the rat round spermatids. Approximately 75 µg of cytosol and membrane extracts were resolved by polyacrylamide gel electrophoresis under denaturing and reducing conditions. The presence of immunoreactive CFTR protein was visualized by staining with anti-CFTR antibody MATG 1016. M = molecular weight marker: 205 kDa (myosin), 120 kDa (β-galactosidase).

Activation of CFTR Cl– current by cAMP in rat spermatogenic cells

Germ cells recovered from the Percoll gradient were seeded onto 35 mm dishes pre-coated with poly-D-lysine for ionic current measurement using a whole-cell patch clamp technique. 120 mmol/l CsCl and 20 mmol/l tetraethylammonium chloride (TEA-Cl) were used in the internal solution to block potassium channels. Efforts in detecting the cAMP-activated current had not met with success initially as no cAMP-activated inward current could be detected in spermatogonia and spermatocytes. However, when the round spermatids were studied, inward currents with CFTR characteristics were recorded in 20% of the cells studied. Figure 1A shows the time course of current activation in round spermatids when cAMP (100 µmol/l) was included in the pipette. The activated current had a linear I/V relationship (Figure 1D), was independent of time and voltage (Figure 1B and C), and was inhibited by DPC (500 µmol/l) (Figure 2).

Detection of CFTR mRNA and protein in rat spermatogenic cells

The presence of CFTR mRNA in rat round spermatids was revealed by RT–PCR. Different sets of primer pairs were used to amplify different regions of the CFTR gene as described in the Materials and methods (Figure 3A). In selected experiments, the PCR products (P1, P2 and P3) were gel-purified and subcloned into the pGEM®-T vector for partial DNA sequencing. Identical sequence homologies were yielded when comparing the partial sequences with the CFTR entries in GenBank database (data not shown). To determine whether the CFTR mRNA detected by RT–PCR may indeed be translated into protein, cytosol and membrane extracts of round spermatids were prepared and analysed by Western blotting using anti-CFTR antibody. After polyacrylamide gel electrophoresis, a complex protein pattern was revealed by Coomassie blue staining (data not shown). When a similar gel was electroblotted onto a nitrocellulose membrane and stained with an anti-CFTR antibody MATG 1016 (Transgene SA, 1/5000 dilution), a single immunoreactive band at ~145 kDa was detected in the membrane extract (Figure 3B). The size was consistent with the calculated molecular weight of rat CFTR (140 kDa).

The expression of CFTR in the testes from rats at different ages was also studied and compared with that of AQP7 which has also been found to be expressed by rat spermatids (Ishibashi et al., 1997; Suzuki-Toyota et al., 1999). Semi-quantitative RT–PCR revealed that CFTR mRNA was present in the testes of adult rats (20 day old and above) but not of immature rats (10 days old). This pattern of expression was also observed for AQP7 (Figure 4).

Immunolocalization of CFTR protein in the seminiferous tubule

The localization of CFTR protein in the rat testis was revealed by an immunohistochemical study. Stage-specific expression of CFTR protein was observed in the tubules (Figure 5). CFTR immunoreactivity could be seen in the luminal aspect of the seminiferous epithelium (Figure 5B). High power magnification revealed CFTR in the membrane of the round spermatids (stage 8 of the spermatogenic cycle) (Figure 5C), in the elongated spermatids (step 14, and step 16 spermatids) (Figure 5D and E), and in the residual bodies (Figure 5C and F). No immunoreactivity could be detected in the fully grown spermatozoa. These results suggest that the CFTR is mainly expressed by the spermatids.
Reverse transcription–polymerase chain reaction analysis (RT–PCR) of CFTR mRNA (A) and AQP7 mRNA (B) in rat germ cells. Total RNA was isolated from germ cells of rats at different ages (10–90 days old). Approximately 2–5 µg of total RNA was reverse-transcribed and subjected to semi-quantitative RT–PCR as described in Materials and methods. Similar expression patterns were noted for CFTR mRNA and AQP7 mRNA in testes from rats at different ages. M = molecular weight markers; E = rat epididymis (positive control for CFTR); K = rat kidney (positive control for AQP7).

Immunolocalization of CFTR can be protein in rat testis. CFTR seen as brownish peroxidase reaction products. (A) No immunoreactivity was seen when the anti-CFTR antibody was omitted. (B) Immunoreactivity seen in the luminal aspect of the seminiferous epithelium was stage-specific. (C) Staining seen in the membrane of round spermatids at stage 8 of the spermatogenic cycle. (D and E) Staining seen in the step 14 and 16 spermatids (elongated). (F) Staining seen in cytoplasmic lobes and residual bodies. Fully grown spermatozoa were not stained.

Functional expression of rat spermatogenic CFTR cDNA in HEK 293 cells

Rat germ cell CFTR cDNA was cloned and expressed in the HEK 293 cells as described in Materials and methods. Cells transfected with the vector only did not exhibit any cAMP-activated current. However, in cells transfected with the vector subcloned with rat germ cell CFTR cDNA, an inward current with CFTR characteristics (results not shown) could be evoked by cAMP (100 µmol/l) (Figure 6A). When the current had
reached a steady state level, addition of the antispermatogenic agents, lonidamine (500 µmol/l) and AF2785 (250 µmol/l), completely blocked the cAMP-activated current in the HEK 293 cells transfected with rat germ cell CFTR cDNA (Figure 6B and C). A lower concentration of lonidamine (50 µmol/l) and AF2785 (50 µmol/l) inhibited the chloride currents by 42.9 and 70.8% respectively (Figure 6D; n = 3).

Discussion

The CFTR encoded by the CFTR gene is a member of the ATP binding cassette (ABC) superfamily. It is present predominantly in the apical membrane of epithelial cells that transport electrolytes and fluid. However, recent work has shown that CFTR expression is not entirely restricted to epithelial cells. CFTR mRNA can be detected in cells of non-epithelial origin, such as the T-lymphocytes, neutrophils, monocytes and alveolar macrophages (Quinton, 1999). CFTR is known to function in two major ways: (i) it acts as a cAMP-activated chloride channel, and (ii) it is a regulator of other membrane conductances, such as the epithelial sodium channels (ENaC) (Ismailov et al., 1996; Chabot et al., 1999), other chloride conductances (Gabriel et al., 1993; Jovov et al., 1995; Kunzelmann and Schreiber, 1999) and aquaporin (Schreiber et al., 1997, 1999).

The epididymis has been shown to express the CFTR gene (Wong et al., 1992; Leung et al., 1996; Wong, 1998). Mutations of the gene are thought to be responsible for male infertility caused by blockage or absence of the vas deferens and the distal half of the epididymis. These structural anomalies are thought to be caused by abnormal fluid transport in the fetal Wolffian duct which fails to differentiate into the epididymis and vas deferens in post-natal life (Wong, 1998). Recently, the CFTR gene is thought to have a more widespread impact on human reproduction following the discovery that a significant proportion of healthy men with poor sperm quality have mutations of the CFTR gene (Van der Ven et al., 1996). While it is possible that this effect may be secondary to partial obstruction of the epididymis/vas deferens causing derangement in spermato genesis (Mak et al., 1999), the possibility exists that the spermatogenic process itself is primarily disrupted by CFTR mutation. Study of CFTR expression and functions in the spermatogenic cells would help to clarify these issues.

Trezise et al. (Trezise and Buchwald, 1991; Trezise et al., 1993) have examined the cell-specific expression of CFTR in the rat testis using in-situ hybridization and found differential expression of CFTR in the germ cells at different stages of the spermatogenic cycle. They found CFTR mRNA is mostly detected in round spermatids. In the present work, RT–PCR analysis using primers designed to amplify the protein-coding regions of the rat CFTR cDNA confirmed the presence of CFTR transcripts in the testes of mature rats. Western blot analysis also detected CFTR protein in the membrane fraction of the germ cells isolated from mature rat testes. In addition,
experiments with rats of different ages revealed the first expression of CFTR in the testes of 20–30 day old rats. This developmental stage correlates well with the emergence of the round spermatids (Russell et al., 1987). Northern blot analysis also showed the first appearance of CFTR mRNA in testes of rats aged between 20–40 days (Trezise et al., 1993).

Evidence for the functional expression of CFTR in germ cells has been provided by the whole-cell patch clamp recordings. Although an inward current was detected in only ~20% of the round spermatids (recovered from 35–40% Percoll gradient layer) patched, this current, if present, displayed a linear I/V relationship, was independent of voltage and time, and was inhibited by diphenylamine-2-carboxylate (DPC), a chloride channel blocker. These properties are consistent with a cAMP-activated current known to be associated with CFTR (Huang et al., 1993).

Our immunohistochemical study using an antibody directed against the first nucleotide binding fold of the CFTR protein has identified the protein in the adluminal aspect of the seminiferous epithelium where the post-meiotic germ cells, namely the spermatids and spermatozoa, are located. Higher magnification shows immunoreactivity localized in the membrane of the round spermatids (step 8 spermatids) and elongated spermatids (step 14, 16 and 19 spermatids). However, no immunoreactivity could be detected in the fully grown spermatozoa. The residual bodies, which are cytoplasmic mass sloughed off from the spermatids as they develop into the spermatozoa, also appeared to be stained. The expression of CFTR in post-meiotic germ cells undergoing spermiogenesis (differentiation of spermatids into spermatozoa) may suggest a role for CFTR in the underlying process. During spermiogenesis, major remodelling of the germ cells occurs. Round spermatids change from a spherical into elongated and, finally, flagellar form by loss of cytoplasmic mass. It is likely that CFTR may play a role in volume reduction by causing water efflux from the round spermatids. It is known that the luminal fluid within the seminiferous tubules is hypertonic (Levine and Marsh, 1971) and therefore an osmotic gradient favouring water efflux prevails in the seminiferous tubules. It may be possible that the cAMP-activated chloride channel may play a role in volume regulation per se by causing chloride efflux, or alternatively, CFTR may interact with other membrane proteins to bring about the cell volume change. There is evidence that CFTR activates aquaporin 3 (AQP3) in the airway epithelium (Schreiber et al., 1999), and we propose a similar interaction in the spermatids. AQP7, a water channel with high homology with AQP3, has been found to be expressed in the rat testis (Suzuki-Toyota et al., 1999). Its distribution in the seminiferous tubules is remarkably similar to that of CFTR. As with the latter, AQP7 is localized in the round and elongated spermatids and also in the residual bodies (Suzuki-Toyota et al., 1999). Furthermore, the parallel expression of the mRNA transcripts for the two proteins in the testes of rats of different ages lends support to the contention that CFTR and AQP7 in the spermatids are functionally linked.

One important piece of pharmacological evidence supports a role for CFTR in spermatogenesis. Recently, we discovered that antispermatogenic agents lonidamine and AF2785 (Corsi et al., 1976; Martino et al., 1981; Silvestrini et al., 1984) inhibited chloride secretion stimulated by cAMP and blocked the cAMP-activated chloride current in epididymal epithelia and isolated cells, respectively (Gong et al., 2000; Gong and Wong, 2000). These agents also blocked the cAMP-activated chloride channels in excised membrane patches from rat epididymal cells (X.D.Gong and P.Y.D.Wong, unpublished data). It would be of interest to see if these agents blocked CFTR in the germ cells. Given the infrequent occurrence of the cAMP-activated chloride current in the spermatids, we sought to clone the rat testis CFTR cDNA and express it in human embryonic kidney (HEK 293) cells (Arvispe et al., 1998). We found that transfection of the cells with the rat testis CFTR cDNA elicited a cAMP-activated chloride current which could be blocked by lonidamine and AF2785. No current could be elicited in HEK 293 cells transfected with the vector only. Cheng et al. have studied the effects of lonidamine and AF2785 on the fertility of male rats (Cheng et al., 2000). They have found that when given as a single oral dose, these compounds induced sterility. Infertility was caused by a progressive depletion of elongated spermatids within 2 days of drug treatment, and by 16 days there was loss of virtually all spermatids except that some spermatogonia and a few spermatocytes remained in the deranged tubules. These findings are consistent with an interference of spermiogenesis, most probably as a result of inhibition of germ cell CFTR function.

Our results demonstrate clearly that CFTR is expressed in rat spermatogenic cells, mostly in the spermatids. There is a close parallelism in the differential expression of CFTR and the water channel, AQP7 in the germ cells at different stages of spermatogenesis. We speculate that during spermiogenesis, CFTR may interact with AQP7 to bring about volume reduction in the spermatids (see Figure 7). Contraction of the cytoplasmic volume is an essential event during spermiogenesis (Russell, 1979). The antispermatogenic agents lonidamine and AF2785 suppress spermatogenesis, possibly by inhibiting remodelling.
of the spermatids. On the one hand, this work has provided a biological rationale for poor sperm quality in men harbouring CFTR mutants. On the other hand, the work has led to a new contraceptive strategy of suppressing spermatogenesis by interfering with CFTR in the testis. Experiments with lonidamine and AF2785 in rats have already shown that this approach is feasible.

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References


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