Biochemical and Biophysical Research Communications 446 (2014) 1017–1021

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Aquaporin-4 as a molecular partner of cystic fibrosis transmembrane conductance regulator in rat Sertoli cells

Tito T. Jesus [a], Raquel L. Bernardino [a], Ana D. Martins [a], Rosália Sá [b], Mário Sousa [b,c], Marco G. Alves [a], Pedro F. Oliveira [a,*]

[a] CICS – UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal
[b] Department of Microscopy, Laboratory of Cell Biology & Multidisciplinary Unit for Biomedical Research, UMIB-FCT, Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Portugal
[c] Centre for Reproductive Genetics Alberto Barros, Porto, Portugal

ARTICLE INFO

Article history:
Received 7 March 2014
Available online 19 March 2014

Keywords:
CFTR
Aquaporin-4
Sertoli cells
Water transport

ABSTRACT

Sertoli cells (SCs) form the blood–testis barrier (BTB) that controls the microenvironment where the germ cells develop. The cystic fibrosis transmembrane conductance regulator (CFTR) plays an essential role to male fertility and it was recently suggested that it may promote water transport. Interestingly, Aquaporin-4 (AQP4) is widely expressed in blood barriers, but was never identified in SCs. Herein we hypothesized that SCs express CFTR and AQP4 and that they can physically interact.

Primary SCs cultures from 20-day-old rats were maintained and CFTR and AQP4 mRNA and protein expression was assessed by RT-PCR and Western blot, respectively. The possible physical interaction between CFTR and AQP4 was studied by co-immunoprecipitation.

We were able to confirm the presence of CFTR at mRNA and protein level in cultured rat SCs. AQP4 mRNA analysis showed that cultured rat SCs express the transcript variant c of AQP4, which was followed by immunodetection of the correspondent protein. The co-immunoprecipitation experiments showed a direct interaction between AQP4 and CFTR in cultured rat SCs.

Our results suggest that CFTR physically interacts with AQP4 in rat SCs evidencing a possible mechanism by which CFTR can control water transport through BTB. The full enlightenment of this particular relation between CFTR and AQP4 may point towards possible therapeutic targets to counteract male subfertility/infertility in men with Cystic Fibrosis and mutations in CFTR gene, which are known to impair spermatogenesis due to defective water transport.

1. Introduction

Spermatogenesis takes place within the seminiferous tubules, where Sertoli cells (SCs) directly interact with the developing germ cells [1]. Adjacent SCs connect through specialized junctions, establishing the Sertoli/blood–testis barrier (BTB) and controlling the passage of substances [1,2]. Hence, SCs control the seminiferous tubular fluid (STF) composition and the physiochemical milieu where spermatogenesis occurs [1,3]. The STF composition is dependent of ions and water movements [4]. Thus, distinct types of ion and water transport proteins have been identified in the plasmatic membrane of these cells (for review [3]). Nevertheless, their involvement on STF establishment remains unknown.

The cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-regulated membrane transporter of the ATP-binding cassette superfamily [5], widely expressed in cells of the reproductive tract [6]. Noteworthy, mutations in the gene that encodes the CFTR protein have been associated with several abnormalities on the male reproductive system and with abnormal production, quality and number of sperm cells [7,8]. CFTR presence was reported in the cytoplasm and plasma membrane of SCs [9], where it plays a role in HCO_3^- transport [10]. Moreover, CFTR plays an important role in epithelial cells fluid volume regulation [5], enhancing osmotic water permeability via direct molecular interactions with water channels (Aquaporins). This additional role of CFTR in controlling water permeability may have an impact on the development of the Cystic Fibrosis (CF).

Aquaporins (AQPs) are essential for water homeostasis regulation and for providing a continuous and rapid water movement across epithelia [11–13]. During spermatogenesis, there is a striking reduction of germ cells volume, largely due to the osmotically

* Corresponding author. Address: Health Sciences Research Centre, University of Beira Interior, Av. Infante D. Henrique, 6201-506 Covilhã, Portugal. Fax: +351 275 329099.
E-mail address: pfobox@gmail.com (P.F. Oliveira).

http://dx.doi.org/10.1016/j.bbrc.2014.03.046
0006-291X/

driven fluid efflux [14], being expectable that AQPs are key players in this process. Aquaporin-4 (AQP4) has been briefly identified by immunocytochemistry in the seminiferous tubules [15]. Interestingly, AQP4 is densely expressed in the blood–brain barrier (BBB) [16], which exhibits remarkable similarities with BTB [17]. Several cellular functions have been attributed to AQP4, such as regulation of extracellular space volume, potassium buffering, fluid circulation and resorption, waste clearance, neuroinflammation, osmosensation, cell migration, and Ca^{2+} signaling (for review see [16]).

Since AQP4 mediates water flux through BBB, we aimed to full disclose its expression in isolated SCs. Moreover, CFTR has been reported to interact with AQPs to regulate osmotic water permeability, thus, we hypothesized that CFTR can interact with AQP4 providing new insight on how CFTR can regulate water membrane movements.

2. Materials and methods

2.1. Chemicals

NZY M-MuLV Reverse Transcriptase (M-MLV RT), random hexamer primers, dNTPs and NZTaq 2× Green Master Mix, agarose and DNA ladder were obtained from NZYTech (Lisboa, Portugal). Primers were obtained from STABVIDA (Oeiras, Portugal). All other chemicals were purchased from Sigma–Aldrich (St. Louis, USA), unless stated otherwise.

2.2. Animals

Male Wistar rats (Rattus norvegicus) were housed under a 12 h light–12 h darkness cycle and constant room temperature (20 ± 2 °C) in our accredited animal facilities, with food and water ad libitum. Accommodation, maintenance and animal handling were performed in accordance with national and international guidelines, particularly with the 'Guide for the Care and Use of Laboratory Animals', available in the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), and the EU rules for the care and handling of laboratory animals (Directive 2010/63/EU).

2.3. Primary cultures of rat Sertoli cells

Male Wistar rats (20-day-old) were sacrificed by cervical dislocation. The testes were excised and washed. SCs were isolated as reported by Oliveira and collaborators [18]. Specific protein markers, anti-mullerian hormone (AMH) and vimentin, were used to assess cultures purity [19]. After 96 h, cultures were examined by phase contrast microscopy and only the cultures with cell contaminants below 5% were used.

2.4. RT-PCR

The extraction of total RNA (RNAt) from brain, lung and SCs was performed using the E.Z.N.A. Total RNA Kit (Omega Bio-Tek, Norcross, USA) as indicated by the manufacturer. RNA concentration and absorbance ratios (A260/A280) were determined by spectrophotometry (Nanophotometer™, Implen, Germany). The RNAt was reversely transcribed as reported by Alves and collaborators [20]. The resulting complementary deoxyribonucleic acid (cDNA) was used with exon–exon spanning primer sets designed to amplify CFTR and AQP4 cDNA fragments (Table 1). Polymerase chain reactions (PCR) were carried out as described by Alves and collaborators [20]. Primers' sequences, optimal annealing temperature, number of cycles required for exponential amplification phase of fragments, fragments size and positive control are indicated (Table 1). cDNA-free sample was used as negative control. Samples were run in 1% agarose gel electrophoresis and visualized using software Molecular Imager FX Pro Plus Multilmager (Biorad, Hercules, USA) coupled to an image acquisition system (Vilber Lourmat, Marne-la-Vallée, France). The size of the expected products was compared to a DNA ladder.

2.5. Western blot

Western blot was performed as previously described by Alves and collaborators [21]. In brief, proteins samples (50 μg) were fractionated on a 12% SDS–PAGE and transferred to polyvinylidene difluoride membranes. The membranes were blocked and incubated overnight at 4 °C with rabbit anti-AQP4 (1:500, AQP41-A, Gentaur, Gdansk, Poland) or goat anti-CFTR (1:500; SC-8909, Santa Cruz Biotechnology, Heidelberg, Germany). The immune-reactive proteins were detected separately with goat anti-rabbit IgG-AP (1:5000, Sc 2007, Santa Cruz Biotechnology, Heidelberg, Germany) or donkey anti-goat IgG-AP (1:5000, Sc 2020, Santa Cruz Biotechnology, Heidelberg, Germany). Membranes were reacted with ECF detection system (GE, Healthcare, Weßling, Germany) and read with the BioRad FX-Pro-plus (Bio-Rad, Hemel Hempstead, UK). The densities from each band were obtained using the Quantity One Software (Bio-Rad, Hemel Hempstead, UK).

2.6. Co-immunoprecipitation

Immunoprecipitation of CFTR was performed using the Dynabeads® Co-Immunoprecipitation Kit (Life Technologies, Calsbad, USA). Anti-CFTR goat antibody was conjugated to magnetic beads according to the manufacturer's protocol. In brief, harvested SCs (50 mg) were lysed in extraction buffer (1× IP Buffer, NaCl 100 mM, Protease Inhibitors 1:400) and centrifuged (2600*g* for 5 min). The final supernatant contained the cell lysate ready to be used immediately for co-immunoprecipitation. For co-immunoprecipitation, CFTR antibody-coupled beads were washed in extraction buffer. The supernatant was discarded and the pelleted beads were resuspended in the cell lysate. This suspension was incubated at 4 °C for 30 min. The supernatant was then removed and the beads washed three times in the extraction buffer. The beads were then washed in the Last Wash Buffer (LWB) (1× LWB, Tween 20 0.02%). Finally, the beads were resuspended in the Elution Buffer and gently mixed. Protein expression was evaluated by slot blot after confirmation of antibodies specificity by Western blot.

2.7. Slot-blot

Protein samples (5 μg) derived from co-immunoprecipitation were diluted in phosphate buffer saline (PBS) and transferred to activated polyvinylidene difluoride membranes using a Hybri-slot manifold system (Biometra, Göttingen, Germany). The membranes were then blocked and incubated overnight at 4 °C with rabbit anti-AQP4 antibody (1:500) or goat anti-CFTR antibody (1:500). The immune-reactive proteins were detected separately with goat anti-rabbit IgG-AP (1:5000) or donkey anti-goat IgG-AP (1:5000). Membranes were then reacted with ECF and read using a BioRad FX-Pro-plus.

3. Results

3.1. Aquaporin-4 is expressed in rat Sertoli cells

Several types of AQPs have been identified throughout the male reproductive tract. However, the expression of AQP4, which is

Table 1
Oligonucleotides and cycling conditions for PCR amplification of cystic fibrosis transmembrane conductance regulator (CFTR), Aquaporin-4 transcript variant a (AQP4a or M1) and Aquaporin-4 transcript variant c (AQP4c or M23).

Gene	Sequence (5′–3′)	AT (°C)	Amplificon size (bp)	C	PtC
CFTR	Forward: GTTGGGAATCAGCGATGGAG	65	156	40c.	Lung
AN: NM_031506.1	Reverse: TGACTGTGTAGGGAAGCACA				
AQP4a	Sense: CAGGGAAGGCATGAGTGACG	56	198	40c.	Brain
AN: NM_012825.3	Antisense: TCTGAGCCACCCCAGTTAAT				
AQP4c	Sense: GAAGACAGCACCTGTGATAGC	58	234	40c.	Brain
AN: NM_001142366.1	Antisense: CACAGGTAGGGGGTTCTCTG				

Abbreviations: AN, GenBank accession number; AT, annealing temperature; C, number of cycles during exponential phase of amplification; PtC, positive control.

known to play a crucial role in water membrane transport through blood barriers, remained to be elucidated in SCs. Thus, we evaluated the expression of AQP4 isoforms in cultured rat SCs. It has been described that AQP4 exists in several isoforms. Besides the first two classical AQP4 isoforms described in rat brain, M1 (or AQP4a) and M23 (or AQP4c) [22], other new AQP4 isoforms were recently identified by screening the cDNA rat brain library [23]. They were named AQP4b, AQP4d, AQP4e (or Mz) and AQP4f but their role and tissue localization remains to be unraveled, being that each isoform corresponds to an alternative transcript variant (variants a–f, respectively) [23]. Therefore, we investigated the presence of the two classical and functional isoforms in isolated SCs. When we analyzed the expression of the mRNA transcript variant a of AQP4 in rat SCs, we were unable to detect any expression of this variant, as the 198 bp product was only detected in the positive control (Fig. 1, Panel A). However, we were able to detect a 234 bp product corresponding to the mRNA expression of the AQP4 transcript variant c (Fig. 1, Panel B). Furthermore, using a specific AQP4 antibody we were able to further confirm the protein expression of this AQP in SCs by detecting a single band with an apparent molecular weight of approximately 32 kDa in the immunoblot analysis. (Fig. 1, Panel C and D).

3.2. Rat SCs express CFTR mRNA and protein

Boockfort and collaborators [7] have previously reported the CFTR presence in cultured SCs from Sprague–Dawley rats and it has been proposed that CFTR plays a crucial role in seminiferous fluid secretion and ionic composition [10,24]. As could be expected, we were also able to detect the presence of the 156 bp product of CFTR mRNA in cultured rat SCs (Fig. 2, Panel A). Moreover, we used a specific anti-CFTR antibody and we were able to detect a single specific staining of approximately 165 kDa by Western blot analysis, which corresponds to the intact CFTR protein (Fig. 2, Panel B and C).

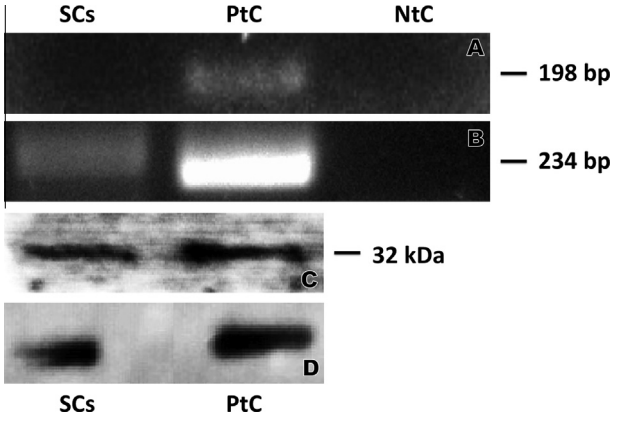

Fig. 1. Expression of Aquaporin-4 (AQP4) in cultured rat Sertoli cells (SCs) showing representative image of RT-PCR (Panel A and B), Western Blot (Panel C) and slot blot (Panel D). (NtC, no-template control; PtC, positive control brain cDNA).

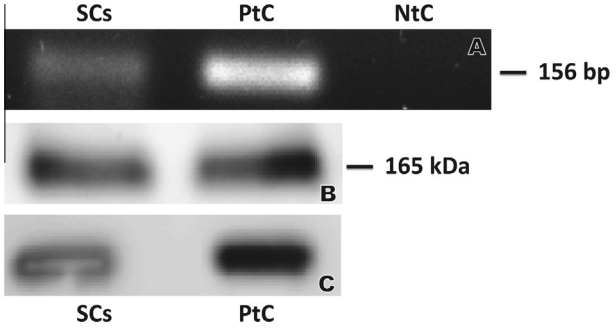

Fig. 2. Expression of CFTR in cultured rat Sertoli cells (SCs) showing representative image of RT-PCR (Panel A), Western Blot (Panel B) and slot blot (Panel C). (NtC, no-template control; PtC, positive control lung cDNA).

3.3. CFTR interacts with Aquaporin 4 in rat Sertoli cells

CFTR expression has been reported in germ cells of various developmental stages evidencing its role during spermatogenesis [25–27]. In that process, CFTR has been suggested to be involved in water fluxes regulation [25]. Since adjacent SCs form the BTB and are responsible for the establishment of the intratubular fluid microenvironment, being known to highly express CFTR, we hypothesized that this HCO_3^- transporter may modulate water fluxes in SCs by molecular interactions with AQPs. Since it has been reported that AQP4 plays a key role in water transport through BBB, we hypothesized that it could be present not only in SCs (which form the BTB), but also interact with CFTR in these cells. To test our hypotheses, we used a co-immunoprecipitation protocol and were able to efficiently conjugate the anti-CFTR antibody to magnetic beads and to precipitate the CFTR protein from cultured rat SCs (Fig. 3, Panel A) by the specific staining detected using the same anti-CFTR antibody and the Slot-blot technique (Fig. 3, Panel B). Interestingly, using the same sample (obtained after conjugating the anti-CFTR goat antibody to magnetic beads and after the co-immunoprecipitation protocol) we were also able to detect the presence of a specific staining correspondent to the AQP4 (Fig. 3, Panel C). These results obtained using a specific anti-AQP4 antibody, clearly indicate that AQP4 derived from cultured rat SCs co-immunoprecipitated with CFTR.

4. Discussion

The SCs are epithelial somatic cells that transport electrolytes and water to create a proper intraluminal environment for the occurrence of spermatogenesis. This process is highly regulated and depends of several membrane transporters [3]. Among those, the expression of functional CFTR has been described in SCs [7].

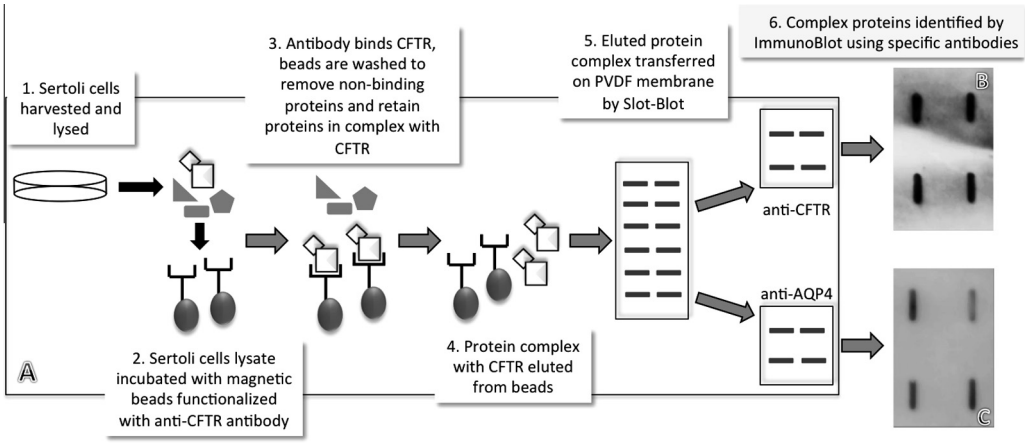

Fig. 3. Molecular interaction of Aquaporin-4 (AQP4) and CFTR in cultured rat Sertoli cells as determined by co-immunoprecipitation (Panel A). Immunoprecipitated CFTR protein was detected (Panel B) and co-immunoprecipitated AQP4 protein derived from the Sertoli cells eluted (Panel C). (PVDF, polyvinylidene difluoride).

In several conditions associated with CFTR mutation, an abnormal germ cell development has been reported [9], suggesting that normal CFTR expression is required for a successful spermatogenesis. Noteworthy, nearly 20% of men with infertility due to sperm abnormalities have a mutation in the CFTR gene [28]. Thus, we tested if CFTR was expressed in cultured rat SCs. We identified a RT-PCR product of predicted size consistent with the CFTR sequence, when RNA from cultured rat SCs was used as a template. These results show that cultured rat SCs express CFTR mRNA. To determine whether CFTR messenger RNA was translated into a functional protein, we used an immunoblot analysis. With this approach, we were able to detect immunoreactive bands in rat SCs samples using a specific antibody against CFTR. These immunoblot studies were conclusive and confirm the presence of CFTR protein in cultured rat SCs.

CFTR enhances osmotic water permeability in various cells or tissues [29–31] and it interacts with AQPs in rat epididymis, establishing a synergistic interaction [32]. Water movements are essential for the luminal fluid environment [33] however the presence of specific water transporters (AQPs) in SCs has been discussed. Some AQP isoforms have been consistently described in SCs [34–36]. Those isoforms are expected to be involved in STF secretion [3,33]. Herein we chose to investigate AQP4 expression in cultured SCs since: (1) the presence of AQP4 was only reported in SCs using immunohistochemical analysis of testicular tissue sections [15] and thus lacked confirmation; (2) AQP4 is the predominant water channel in mammalian brain and is abundantly expressed in astrocytes that support BBB [37], a structure similar to the BTB. AQP4 may be transcribed as six distinct mRNAs (transcript variants a–f) [37], where AQP4a (also M1) and AQP4c (also M23) are the classical and functional water transport channels, with brain expressing both isoforms and tissues outside of the central nervous system expressing predominantly AQP4c [38]. These two AQP4 isoforms differ in their sizes and water transport rates (when expressed in epithelial cells), being that AQP4c results in a shorter variant with increased single-channel osmotic water permeability [38,39]. Hence, we evaluated the presence of transcripts a and c in cultured rat SCs and we only detected mRNA transcript variant c (AQP4 M23). The presence of AQP4 protein was also confirmed by immunoblot. To the best of our knowledge, our results are the first to unequivocally demonstrate the presence of this aquaporin isoform in rat SCs.

CFTR, besides functioning as an ion channel, also acts as a regulator of several other membrane transport proteins, among which are epithelial Na^+ channels [40] and outwardly rectifying Cl^- channels [41]. Besides, AQPs are also essential in the establishment of intratubular fluid composition. Recent data showed that CFTR interacts with several AQPs in various cellular systems [29,30], including in the epididymis [32]. It has been suggested that these interactions may be of clinical outcome to CF, in which mutation of the CFTR gene leads to fluid accumulation [42,43]. Thus, since it has been suggested that CFTR can interact with AQPs, we further investigated the possibility of a physical interaction between CFTR and AQP4 in cultured rat SCs. The direct interaction between AQP4 and CFTR observed in the present study, using the co-immunoprecipitation technique, strongly suggests that CFTR might serve as a regulator of AQPs and water homeodynamics in SCs, as it was previously shown to occur in other cells of the male reproductive tract [31,32]. To the best of our knowledge, our results are the first to unequivocally demonstrate a molecular interaction between CFTR and AQP4 in cultured rat SCs.

In conclusion, our results show that CFTR and AQP4 are expressed in rat SCs. Although we did not perform functional studies regarding the role of AQP4 in these cells, their role in water balance and ion homeostasis in the several tissues where they are expressed is well known. We were able to demonstrate that CFTR and AQP4 physically interact in cultured rat SCs. This interaction most likely occurs in *in vivo* conditions when a fully intact BTB is established. However, this needs further investigation beyond this short report. The finding that CFTR regulates water permeability in various tissues, in addition to its role as an ionic channel, raises the possibility that abnormal SCs functioning in men with CF may involve altered water transport, plus to defective HCO_3^- transport, resulting in severe alterations in the intratubular fluid composition and impairment of spermatogenesis. The results presented here raise new questions and point new directions towards the understanding of male subfertility/infertility in men with mutations in CFTR gene. *In vivo* experiments are the next step to clarify the clinical significance of this interaction in SCs and in a full functional BTB. The full enlightenment of these molecular interactions and mechanisms may point towards possible therapeutic targets to counteract male subfertility/infertility in men with CF and mutations in CFTR gene.

Declaration of interests

The authors confirm that this article has no conflict of interests.

Acknowledgments

Supported by FCT, Portugal (PTDC/QUI-BIQ/121446/2010 and PEst-C/SAU/UI0709/2011) co-funded by Fundo Europeu de

Desenvolvimento Regional – FEDER via Programa Operacional Factores de Competitividade – COMPETE/QREN. M.G. Alves (SFRH/BPD/80451/2011) was funded by FCT, Portugal. P.F. Oliveira was funded by FCT (Portugal) through FSE and POPH funds (Programa Ciência 2008). T.T. Jesus and R.L. Bernardino were funded by Santander/Totta – UBI protocol (Portugal). The UMIB was funded by national funds through FCT - Foundation for Science and Technology (Portugal) under the Fcomp-01-0124-FEDER-015893 project.

References

[1] D.D. Mruk, C.Y. Cheng, Sertoli-Sertoli and Sertoli-germ cell interactions and their significance in germ cell movement in the seminiferous epithelium during spermatogenesis, Endocr. Rev. 25 (2004) 747–806.
[2] M.G. Alves, L. Rato, R.A. Carvalho, P.I. Moreira, S. Socorro, P.F. Oliveira, Hormonal control of Sertoli cell metabolism regulates spermatogenesis, Cell. Mol. Life Sci. 70 (2013) 777–793.
[3] L. Rato, S. Socorro, J.E. Cavaco, P.F. Oliveira, Tubular fluid secretion in the seminiferous epithelium: ion transporters and aquaporins in Sertoli cells, J. Membr. Biol. 236 (2010) 215–224.
[4] T.T. Turner, Resorption versus secretion in the rat epididymis, J. Reprod. Fertil. 72 (1984) 509–514.
[5] Y. Liu, D.K. Wang, L.M. Chen, The physiology of bicarbonate transporters in mammalian reproduction, Biol. Reprod. 86 (2012) 99.
[6] P.M. Quinton, Too much salt, too little soda: cystic fibrosis, Sheng Li Xue Bao 59 (2007) 397–415.
[7] F.R. Boockfor, R.A. Morris, D.C. DeSimone, D.M. Hunt, K.B. Walsh, Sertoli cell expression of the cystic fibrosis transmembrane conductance regulator, Am. J. Physiol. 274 (1998) C922–C930.
[8] P.Y. Wong, CFTR gene and male fertility, Mol. Hum. Reprod. 4 (1998) 107–110.
[9] S. Teixeira, R. Sá, A. Grangeia, J. Silva, C. Oliveira, L. Ferráz, A. Alves, S. Paiva, A. Barros, M. Sousa, Immunohystochemical analysis of CFTR in normal and disrupted spermatogenesis, Syst. Biol. Reprod. Med. 59 (2012) 53–59.
[10] W.M. Xu, J. Chen, H. Chen, R.Y. Diao, K.L. Fok, J.D. Dong, T.T. Sun, W.Y. Chen, M.K. Yu, X.H. Zhang, L.L. Tsang, A. Lau, Q.X. Shi, Q.H. Shi, P.B. Huang, H.C. Chan, Defective CFTR-dependent CREB activation results in impaired spermatogenesis and azoospermia, PLoS One 6 (2011) e19120.
[11] P. Agre, L.S. King, M. Yasui, W.B. Guggino, O.P. Ottersen, Y. Fujiyoshi, A. Engel, S. Nielsen, Aquaporin water channels – from atomic structure to clinical medicine, J. Physiol. 542 (2002) 3–16.
[12] D. Brown, J.M. Verbavatz, G. Valenti, B. Lui, I. Sabolic, Localization of the CHIP28 water channel in reabsorptive segments of the rat male reproductive tract, Eur. J. Cell Biol. 61 (1993) 264–273.
[13] E.M. Wintour, Water channels and urea transporters, Clin. Exp. Pharmacol. Physiol. 24 (1997) 1–9.
[14] H.F. Huang, R.H. He, C.C. Sun, Y. Zhang, Q.X. Meng, Y.Y. Ma, Function of aquaporins in female and male reproductive systems, Hum. Reprod. Update 12 (2006) 785–795.
[15] F. Zhuo, S.-Q. Sun, Expression of aquaporin-4 in testis and epididymis of rat, Sichuan J. Anat. 4 (2007) 28–29.
[16] E.A. Nagelhus, O.P. Ottersen, Physiological roles of aquaporin-4 in brain, Physiol. Rev. 93 (2013) 1543–1562.
[17] M.G. Alves, P.F. Oliveira, S. Socorro, P.I. Moreira, Impact of diabetes in blood–testis and blood–brain barriers: resemblances and differences, Curr. Diab. Rev. 8 (2012) 401–412.
[18] P.F. Oliveira, M.G. Alves, L. Rato, S. Laurentino, J. Silva, R. Sa, A. Barros, M. Sousa, R.A. Carvalho, J.E. Cavaco, S. Socorro, Effect of insulin deprivation on metabolism and metabolism-associated gene transcript levels of in vitro cultured human Sertoli cells, Biochim. Biophys. Acta 2012 (1820) 84–89.
[19] K. Steger, R. Rey, S. Kliesch, F. Louis, G. Schleicher, M. Bergmann, Immunohistochemical detection of immature Sertoli cell markers in testicular tissue of infertile adult men: a preliminary study, Int. J. Androl. 19 (1996) 122–128.
[20] M.G. Alves, A. Neuhaus-Oliveira, P.I. Moreira, S. Socorro, P.F. Oliveira, Exposure to 2,4-dichlorophenoxyacetic acid alters glucose metabolism in immature rat Sertoli cells, Reprod. Toxicol. 38 (2013) 81–88.
[21] M.G. Alves, S. Socorro, J. Silva, A. Barros, M. Sousa, J.E. Cavaco, P.F. Oliveira, In vitro cultured human Sertoli cells secrete high amounts of acetate that is stimulated by 17beta-estradiol and suppressed by insulin deprivation, Biochim. Biophys. Acta 2012 (1823) 1389–1394.
[22] J.S. Jung, R.V. Bhat, G.M. Preston, W.B. Guggino, J.M. Baraban, P. Agre, Molecular characterization of an aquaporin cDNA from brain: candidate osmoreceptor and regulator of water balance, Proc. Natl. Acad. Sci. USA 91 (1994) 13052–13056.
[23] S.E. Moe, J.G. Sorbo, R. Sogaard, T. Zeuthen, O. Petter Ottersen, T. Holen, New isoforms of rat aquaporin-4, Genomics 91 (2008) 367–377.
[24] W.H. Ko, H.C. Chan, S.B. Chew, P.Y. Wong, Regulated anion secretion in cultured epithelia from Sertoli cells of immature rats, J. Physiol. 512 (Pt 2) (1998) 471–480.
[25] X. Gong, J. Li, K. Cheung, G. Leung, S.B.C. Chew, P. Wong, Expression of the cystic fibrosis transmembrane conductance regulator in rat spermatids: implication for the site of action of antispermatogenic agents, Mol. Hum. Reprod. 7 (2001) 705–713.
[26] S. Hihnala, M. Kujala, J. Toppari, J. Kere, C. Holmberg, P. Höglund, Expression of SLC26A3, CFTR and NHE3 in the human male reproductive tract: role in male subfertility caused by congenital chloride diarrhoea, Mol. Hum. Reprod. 12 (2006) 107–111.
[27] S. Teixeira, R. Sá, A. Grangeia, J. Silva, C. Oliveira, L. Ferráz, Â. Alves, S. Paiva, A. Barros, M. Sousa, Immunohystochemical analysis of CFTR in normal and disrupted spermatogenesis, Syst. Biol. Reprod. Med. (2012) 1–7.
[28] K. van der Ven, L. Messer, H. van der Ven, R.S. Jeyendran, C. Ober, Cystic fibrosis mutation screening in healthy men with reduced sperm quality, Hum. Reprod. 11 (1996) 513–517.
[29] R. Schreiber, R. Nitschke, R. Greger, K. Kunzelmann, The cystic fibrosis transmembrane conductance regulator activates aquaporin 3 in airway epithelial cells, J. Biol. Chem. 274 (1999) 11811–11816.
[30] R. Schreiber, H. Pavenstadt, R. Greger, K. Kunzelmann, Aquaporin 3 cloned from *Xenopus laevis* is regulated by the cystic fibrosis transmembrane conductance regulator, FEBS Lett. 475 (2000) 291–295.
[31] C. Pietrement, N. Da Silva, C. Silberstein, M. James, M. Marsolais, A. Van Hoek, D. Brown, N. Pastor-Soler, N. Ameen, R. Laprade, V. Ramesh, S. Breton, Role of NHERF1, cystic fibrosis transmembrane conductance regulator, and cAMP in the regulation of aquaporin 9, J. Biol. Chem. 283 (2008) 2986–2996.
[32] K.H. Cheung, C.T. Leung, G.P. Leung, P.Y. Wong, Synergistic effects of cystic fibrosis transmembrane conductance regulator and aquaporin-9 in the rat epididymis, Biol. Reprod. 68 (2003) 1505–1510.
[33] Y.S. Cho, M. Svelto, G. Calamita, Possible functional implications of aquaporin water channels in reproductive physiology and medically assisted procreation, Cell. Mol. Biol. 49 (2003) 515–519.
[34] H.H. Badran, L.S. Hermo, Expression and regulation of aquaporins 1, 8, and 9 in the testis, efferent ducts, and epididymis of adult rats and during postnatal development, J. Androl. 23 (2002) 358–373.
[35] L. Hermo, D. Krzeczunowicz, R. Ruz, Cell specificity of aquaporins 0, 3, and 10 expressed in the testis, efferent ducts, and epididymis of adult rats, J. Androl. 25 (2004) 494–505.
[36] T. Tani, Y. Koyama, K. Nihei, S. Hatakeyama, K. Ohshiro, Y. Yoshida, E. Yaoita, Y. Sakai, K. Hatakeyama, T. Yamamoto, Immunolocalization of aquaporin-8 in rat digestive organs and testis, Arch. Histol. Cytol. 64 (2001) 159–168.
[37] C. Iacovetta, E. Rudloff, R. Kirby, The role of aquaporin 4 in the brain, Vet. Clin. Pathol. 41 (2012) 32–44.
[38] G.-Q. Zheng, Y. Li, Y. Gu, X.-M. Chen, Y. Zhou, S.-Z. Zhao, J. Shen, Beyond water channel: aquaporin-4 in adult neurogenesis, Neurochem. Int. 56 (2010) 651–654.
[39] L. Strand, S.E. Moe, T.T. Solbu, M. Vaadal, T. Holen, Roles of aquaporin-4 isoforms and amino acids in square array assembly, Biochemistry (Mosc.) 48 (2009) 5785–5793.
[40] R. Schreiber, A. Hopf, M. Mall, R. Greger, K. Kunzelmann, The first-nucleotide binding domain of the cystic-fibrosis transmembrane conductance regulator is important for inhibition of the epithelial Na^+ channel, Proc. Natl. Acad. Sci. USA 96 (1999) 5310–5315.
[41] E.M. Schwiebert, M.E. Egan, T.-H. Hwang, S.B. Fulmer, S.S. Allen, G.R. Cutting, W.B. Guggino, CFTR regulates outwardly rectifying chloride channels through an autocrine mechanism involving ATP, Cell 81 (1995) 1063–1073.
[42] M. Levin, A. Verkman, Aquaporins and CFTR in ocular epithelial fluid transport, J. Membr. Biol. 210 (2006) 105–115.
[43] J.B. Lyczak, C.L. Cannon, G.B. Pier, Lung infections associated with cystic fibrosis, Clin. Microbiol. Rev. 15 (2002) 194–222.