Molecular Aspects of Medicine 55 (2017) 102–109

Contents lists available at ScienceDirect

Molecular Aspects of Medicine

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

Adenosine in sperm physiology

Ilaria Bellezza, Alba Minelli*

Dipartimento di Medicina Sperimentale, Università di Perugia, Polo Unico Sant'Andrea delle Fratte, Piazzale Gambuli, 06132 Perugia, Italy

ARTICLE INFO

Article history:
Received 13 September 2016
Received in revised form
23 November 2016
Accepted 23 November 2016
Available online 24 November 2016

Keywords:
Spermatogenesis
Epididymal maturation
Capacitation
Acrosome reaction
Adenosine receptors

ABSTRACT

It has long been known that ATP and cAMP are deeply involved in sperm function whereas the role of adenosine and adenosine receptors is still far from being totally construed. The presence of adenosine in male reproductive tract and adenosine receptors on spermatozoa is strongly suggestive of a functional role of these receptors in sperm physiology and function. Spermatozoa are highly differentiated cells with fertility as the only goal. This paper, by an extensive review of the literature, outlines our current understanding of the role and effects of adenosine and adenosine receptors in spermatogenesis and in the acquisition of sperm fertilizing capacity which occurs in the femal genital tract, where the motile sperm fertilize an egg to form a zygote.

1. Background

Organs of the male reproductive system are primarly controlled by the autonomical nervous system (ANS), with the contribution of ATP released by the nerves that control the reproductive system in a process defined as purinergic co-transmission (Burnstock, 2014a). Thus, nucleotides and their by-products, i.e. nucleosides, are also involved in the control of the reproductive system (Andersson and Wagner, 1995; Burnstock, 2014b; Gorodeski, 2015). Data in the literature suggest a role for extracellular nucleotides and nucleosides in modulating the functions of male genital tract and controlling fertility/reproduction by acting on cell-surface receptors, termed purine receptors. Firstly described by Burnstock (1978), these receptors have been further unravelled by Fredholm et al. (1994, 1996). On the basis of their natural ligands, purine receptors are grouped in two subclasses, P1 and P2, the former binding adenosine, the latter adenine nucleotides. P2 purine receptors exist in various subtypes, while P1/adenosine receptors are classified as A1, A2a, A2b, A3. All adenosine receptors (AdoR) are G-protein- coupled proteins, and, on the basis of their effects on cyclic adenosine 3′,5′-monophosphate (cAMP) levels, further subdivided in stimulatory (A2a and A2b) and inhibitory (A1 and A3). It is therefore clear that the activation of AdoR can increase or reduce the second messenger levels thereby intervening in the regulation of cAMP levels. Besides purine nucleotides, sperm cAMP, whose synthesis uses a unique molecular machinery not found in somatic cells (Buck et al., 1999; Chen et al., 2000; Esposito et al., 2004), is deeply involved in the control of sperm function, i.e. the regulation of motility (Hoskins et al., 1975; Garbers and Kopf., 1980; Tash and Means., 1988) and the acquisition of the fertilizing capacity (Harrison, 2003). Indeed, phosphodiesterase inhibitors, which increase cAMP levels, enhance sperm motility as well as the fertilizing potential. Caffeine, as a phosphodiesterase inhibitor, produces beneficial effects in human spermatozoa from patients suffering from astheno- or oligozoospermia (Aitken et al., 1983). These results prompted clinical interest in the use of reagents that increase intracellular cAMP levels in the treatment of infertility associated with the impairment of sperm motility and/or fertilizing potential (Barkay et al., 1984). Because of the modulation of cAMP levels by AdoR, adenosine has long been suspected to play an important role in regulating male reproduction. The testicles generate morphologically mature spermatozoa which are poorly motile and incapable of fertilizing the ovum. Spermatozoa become motile after their deposition into the vagina and acquire the ability to undergo the acrosomal reaction and fertilize the ovum in the fallopian tube. The present paper summarizes data about the expression of P1/AdoR receptors and their functions during the most salient processes in the life of a sperm cell, i.e. spermatogenesis in the testis, sperm maturation in the epididymis, and finally, acquisition of the fertilizing capacity in the female internal genitalia.

* Corresponding author.
E-mail address: alba.minelli@unipg.it (A. Minelli).

http://dx.doi.org/10.1016/j.mam.2016.11.009
0098-2997/

2. Adenosine receptors in testes and spermatogenesis

The testes contain, in the seminiferous tubules, sperm cells at different stages of maturation and Sertoli cells, which support germ cell development into spermatozoa. They also contain Leydig cells, which produce and secrete testosterone and, surrounding the seminiferous tubules, peritubular myoid cells which, with their regular contractions, drive sperm cells into the testis efferent tubules and epididymis. Autoradiographic analysis in the rat testes showed a localization of AdoR within seminiferous tubules suggesting an association with spermatocytes (Murphy et al., 1983). Later it was shown that a large portion of agonist binding sites, found in the testis, are localized on the Sertoli cell plasma membrane and that these are functional receptors associated with a biological response (Monaco and Conti, 1986). The same Authors also reported that AdoR are present in the immature seminiferous tubules of 10-day-old rats, when spermatogenesis has proceeded to the earliest stages of meiosis, and seminiferous tubules are mainly composed of spermatogonia, a few spermatocytes, and Sertoli cells (Clermont and Perey, 1957). Nevertheless, the Authors did not rule out the possibility that AdoR could also be localized on the germ cells and suggested that AdoR and adenosine are involved in regulating the energetic equilibrium of the seminiferous tubule cells. Sertoli cells, which provide the metabolic substrates and maintain the levels of ATP in germ cells, migth use the AdoR as sensors of the energetic balance of the germ cells. Indeed, changes in germ cells ATP levels might result in a release of adenosine that can function as a signal for the Sertoli cells. Northern blot analysis and in situ hybridization studies by Rivkees (1994) revealed high levels of testicular A3 AdoR messenger RNA, confirmed by Mazzoni et al. (1995) and lower levels of A1 AdoR messenger RNA. Neither A2a nor A2b AdoR gene expression could be detected. Moreover, in situ hybridization and comparative polymerase chain reaction studies showed high level A3 AdoR gene expression in germ cells (spermatocytes and spermatids), whereas high levels of A1 AdoR gene expression were found in Sertoli cells. These inhibitory receptors were shown to be functional, with the A1AdoR expression undergoing an age-dependent reduction (Bhat et al., 1998). A1 AdoR were also found in bovine and rat testicular tissue (Murphy et al., 1983; Stiles et al., 1986; Cushing et al., 1988) whereas A1 and A2 AdoR were found in trout testicular cells (Loir, 2001), suggesting that all the events, either cAMP-dependent or independent, induced by the activation of testicular AdoRs, may participate in the regulation of trout male germ cell proliferation. Indeed, in fish, extracellular adenosine and ATP migth influence the *in vitro* proliferation of spermatogonia, thus potentially mediating the influence of the nervous system on the control of spermatogenesis. Study of the pharmacological profile of responses to adenosine analogs strongly suggested involvement of a cell surface A3AdoR in mice sperm (Burnett et al., 2010), supported by the blockade of A3AdoR agonist action by pertussistoxin (PTX). Sensitivity to PTX is consistent with the known coupling of A3R and A1R to Gαi in sperm (Kopf et al., 1986; Minelli et al., 2008). More direct informations on the effects of adenosine and AdoR on spermiogenesis appeared in 2012 when Minutoli and co-workers (Minutoli et al., 2012) showed that polydeoxyribonucleotide (PDRN), an agonist of A2AdoR (Thellung et al., 1999), up-regulated the expression of vascular endothelial growth factor (VEGF) in an experimental model of testicular torsion. VEGF is present in the epithelium of the prostate, seminal vesicle, and in semen (Brown et al., 1995) and has a well-known protective role on spermatogenic activity (Tunçkiran et al., 2005). The administration of PDRN dramatically improved spermatogenic activity in rats while the co-administration of the A2AdoR antagonist abrogated the effects of PDRN on spermatogenesis activity. These results point to a functional role of sperm A2AdoR in supporting spermatogenic activity in rats.

3. Adenosine receptors in the epididymal maturation

Testicular mature spermatozoa acquire their fertilization capacity after completion of two post-testicular maturational processes, the former occurring in the epididymis, the latter in the female tract. Epididymal maturation involves the incorporation of proteins secreted by the epididymal epithelium in the male reproductive tract and the acquisition of progressive motility. Nevertheless, after epididymal maturation, the stored sperm are still incapable of fertilizing an egg (Yeung et al., 1993; Hinton et al., 1996; Jones and Murdoch., 1996; Orgebin-Crist and Davies., 2003; Robaire et al., 2006; Cornwall, 2009**).** The epididymis is formed by a convoluted tubule characterized by morphologically and functionally distinct regions (the initial segment, caput, corpus, and cauda). In the epithelium, lining the epididymal lumen, there are four cell types (narrow, clear, principal, and basal cells) with specific localization and all contributing to the establishment and regulation of a unique luminal environment for the concentration, maturation, storage, and viability of spermatozoa (Wong and Uchendu, 1990; Robaire et al., 2006; Da Silva et al., 2007; Cornwall., 2009; Shum et al., 2008, 2011). Clear cells, present in the caput, corpus, and cauda epididymidis, express the proton pumping ATPase (V-ATPase) in the apical membrane and secrete protons while principal cells, present in all epididymal regions, secrete bicarbonate ions, which, in turn, stimulate cAMP production via a bicarbonate −sensitive adenylyl cyclase and prime spermatozoa before ejaculation. The apical V-ATPase accumulation in clear cells is regulated by ATP acting on P2 receptors and by adenosine acting on P1 receptors. ATP, released into the lumen from sperm and principal cells, is metabolized into adenosine by local nucleotidases. ATP and adenosine trigger the apical accumulation of V-ATPase that leads to the luminal acidification which is essential for sperm maturation and storage. Purinergic receptors are expressed in primary cultures of rat epididymal cells and epithelial cells of mouse epididymis (Wong, 1988; Shariatmadari et al., 2003). In particular, messenger RNA transcripts specific for three AdoR genes (A1, A2b, and A3) and seven P2/ATP receptor genes (P2X1, P2X2, P2X3, P2X4, P2X6, P2Y2, P2Y5) were detected in epididymal epithelial cells (Belleannee et al., 2010). ATP, whose release from cells still remains for the most part unknown, is rapidly degraded by ectonucleotidases located in the epididymal luminal fluid and epithelial cell apical membranes. Activation of P1 receptors by adenosine results in either a decrease (A1 and A3 receptors) or increase (A2b receptor) in intracellular cAMP. Interestingly, since myristoylated protein kinase (mPKI), a PKA inhibitor, abolishes the response elicited by adenosine, the participation of the A2bAdoR in the activation of clear cells has been postulated. Moreover, the V-ATPase is regulated by luminal angiotensin II through the activation of basal cells, which are provided of body projections that cross the tight junction barrier. Basal cells then secrete nitric oxide, which then diffuses and, by activating the cGMP pathway, stimulates proton secretion in clear cells (Belleannee et al., 2010). In conclusion, there is an elaborate and still elusive communication network between all the epididymal cell types to control luminal acidification and subsequent fertility, but AdoRs seem to be minimally involved in this process.

4. Adenosine receptors in the acquisition of the fertilizing capacity

4.1. First step: capacitation

In the '50s, Chang (1951) and Austin (1952) reported that