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Article of the Month

The endocannabinoid 2-arachidonoylglycerol promotes sperm developement through activation of cannabinoid-2 receptors

Mauro Maccarrone

Department of Biomedical Sciences, University of Teramo, 64100 Teramo, Italy, & European Center for Brain Research/Santa Lucia Foundation, 00143 Rome, Italy

Abstract

The exact role of the endocannabinoid system (ECS) during spermatogenesis has not been clarified. Scientists at the University of Rome "Tor Vergata" (Italy) and at the Endocannabinoid Research Group – Consiglio Nazionale delle Ricerche (Pozzuoli, Italy) have used purified germ cell fractions representative of all phases of spermatogenesis and primary cultures of spermatogonia, to demonstrate that male mouse germ cells possess an active and complete ECS [Grimaldi et al. Proc Natl Acad Sci USA 2009;106:11131-6]. They found that an autocrine endocannabinoid signalling might be operational during spermatogenesis, and that the ECS is modulated during meiosis, with the levels of the endocannabinoid 2-arachidonoylglycerol peaking before meiosis. In addition, activation of type-2 cannabinoid (CB_2) receptors was found to promote meiotic progression of germ cells, by increasing the expression of early meiotic prophase genes.

Keywords: endocannabinoids; human reproduction; male fertility; meiosis; spermatogenesis.

Author's address: Mauro Maccarrone, mmaccarrone@unite.it

Background information

Over the past half century, fertility rates have declined at an unprecedented speed, so that infertility has become an urgent public health issue especially in developed countries, affecting one out of six couples [2]. Although assisted reproduction technologies have overcome many infertility causes, pregnancy rates are still relatively low, and have not increased in the last years [1]. Against this background, it is all the more important that our understanding of the molecular details of reproduction, both by the male and female sides, is improved, and that reliable diagnostic markers of infertility and novel therapeutic targets to fight the reproductive diseases are identified.

In the last decade, a new group of lipid mediators, collectively termed "endocannabinoids" (eCBs), have emerged as critical signals in various aspects of human reproduction (for a recent and extensive review see [7]). Remarkably eCBs, like their Cannabis-derived counterpart Δ^9-tetrahydrocannabinol [11], regulate embryo oviductal transport, implantation and fetal development (in females), and sperm survival, motility, capacitation and acrosome reaction (in males) [7]. The eCBs and the array of proteins that bind, transport and metabolize them (collectively known as "endocannabinoid system", ECS) appear to be highly conserved in evolution, emerging from the divergence of protostomian/deuterostomian and regulating reproductive processes already in invertebrates and lower vertebrates [10]. Most of these actions can be attributed to the eCB anandamide (*N*-arachidonoylethanolamine, AEA). In line with this, a reduced level of the AEA-degrading enzyme fatty acid amide hydrolase (FAAH) in circulating maternal lymphocytes has been recognized as a marker of human infertility [5], and conversely a blood level of AEA above a certain threshold is nearly 100% predictive of miscarriage in women at risk [4]. All in

Explanations

Acrosome reaction	release of an enzymatic arsenal from the head of the spermatozoa, needed to penetrate a female germ cell (oocyte or egg).
Autocrine	relative to a form of signaling in which a cell secretes a chemical messenger (in this case an endocannabinoid) that binds to receptors on the same type of cell.
Capacitation	a complex modification of spermatozoa, that enables them to reach and fertilize an oocyte.
Epigenetic	a type of regulation of gene expression that does not affect the nucleotide sequence, but chemically modifies DNA (e.g., through methylation or acetylation).
Haploid	the number of chromosomes in a germ cell (oocyte or sperm), which is 23 in humans.
In vitro	based on cellular systems kept in culture in the laboratory.
In vivo	based on animal models housed in the laboratory.
Meiosis	cell division process that leads one diploid cell (i.e., a cell with a double set of chromosomes) to generate four aploid cells (each containing a single set of chromosomes).
Mitosis	cell division process that leads one cell (mother) to generate two cells with the same set of chromosomes as the mother.
Oocyte	female germ cell (oocyte or egg).
Oviductal	relative to the tract that connects the ovary to the uterus.
Pachytene	relative to a certain stage of meiosis (pachytene stage).
Placentation	a process refering to the formation, type and structure, or arrangement of placentas.
Spermatogenesis	a highly coordinated process, that leads to the maturation of male germ cells (spermatozoa).

Abbreviations

AEA *N*-arachidonoylethanolamine
ECS endocannabinoid system
FAAH fatty acid amide hydrolase

all, mounting evidence has pointed out that AEA has the potential to direct human fertility towards a positive or negative end, most often by activating type-1 cannabinoid (CB_1) receptors [7]. In addition, the other major subtype of cannabinoid receptors, i.e. type-2 (CB_2) receptor, has been shown to be expressed in various reproductive cells and tissues, where it exerts some distinct actions on placentation, maternal-fetal signalling and embryo development, or Sertoli cell survival and sperm-oocyte interactions [6]. As yet, it was somewhat surprising that a role for the prominent eCB 2-arachidonoylglycerol (2-AG) in fertility remained uncovered, because 2-AG is widely accepted as the genuine endogenous ligand for CB_2, at which it is a much more efficacious agonist than AEA.
The article of the month commented here sheds light on a pivotal role of 2-AG and CB_2 receptors in male fertility [3].

Summary of original article

The importance of the ECS in male reproductive functions has been pointed out in previous papers (see [7] and references therein). AEA reduces sperm-fertilizing capacity in sea urchin sperm, and inhibits key fertilization fuctions such as sperm motility, capacitation, and acrosome reaction in mammals. Accumulated evidence supports a role for the ECS also in the control of testosterone production and other endocrine reproductive functions, however its involvement in spermatogenesis has been only partially explored. Spermatogenesis is a highly coordinated process, that leads to the maturation of male germ cells (spermatozoa); it is characterized by mitotic (spermatogonia), meiotic (spermatocytes), and differentiative haploid (spermatids) phases. Grimaldi and colleagues at the University of Rome "Tor Vergata" (Rome, Italy) and at the Endocannabinoid Research Group – Consiglio Nazionale delle Ricerche (Pozzuoli, Italy) investigated the presence and functional role of the ECS in male germ cells at different stages of differentiation, using purified germ cell fractions representative of each spermatogenesis phase, as well as primary cultures of spermatogonia.
This approach allowed the precise quantification of the CB receptor ligands, AEA and 2-AG, and of the mRNA and protein levels of their metabolic enzymes and receptors. The data indicated that male mouse germ cells possess an active and complete ECS, which was modulated during meiosis, and suggested the presence of an endocannabinoid signal during spermatogenesis. Mitotic cells were shown to possess higher levels of 2-AG, which decreased in spermatocytes (~2-fold) and spermatids (~20-fold). Accordingly, spermatogonia expressed higher and lower levels of 2-AG biosynthetic and degrading enzymes, respectively, as compared to meiotic and postmeiotic cells. Furthermore, during meiosis an increased mRNA level of both biosynthetic and hydrolytic enzymes of AEA was observed, and possibly as a result the levels of AEA remained constant during spermatogenesis. On this basis, the Authors speculated that the up-regulation of AEA metabolic enzymes could serve to maintain an appropriate ''AEA tone'' during meiosis, like that seen in mouse embryos [7]. In addition, because AEA is an agonist for TRPV1 channels, whereas 2-AG is not, they suggested that an AEA tone would ensure the TRPV1-mediated protective action against temperature increase in male germ cells at the pachytene stage, previously proposed by others [8]. On the other hand, 2-AG was suggested to play a pivotal role in promoting the meiotic progression of germ cells, by activating

CB_2 receptors. In fact, the Authors found that the selective CB_2 receptor agonist, JWH133, induced the Erk 1/2 mitogen-activated protein kinase (MAPK) phosphorylation cascade in spermatogonia, an effect attenuated by the selective CB_2 antagonist AM630. Remarkably, JWH133 also induced the progression of spermatogonia toward meiosis, because it increased the number of cells positive for the synaptonemal complex protein 3 (SCP3), a marker of meiotic prophase, and the expression of early meiotic prophase genes like *c-Kit*, *Dmc1*, *Stra8*, *Lhx8*, and *Spo11*. Taken together, the data demonstrated that mouse germ cells possess an active and complete ECS, which is modulated during spermatogenesis. They also suggested the involvement of an autocrine endocannabinoid signalling sustained by 2-AG in the mitotic and meiotic phases of spermatogenesis, and indicated a pivotal role of CB_2 receptors in this process.

Comment

The role of the AEA – CB_1 "couple" in several reproductive events has found solid experimental support, in both *in vitro* and *in vivo* studies. Meanwhile, also a distinct role of CB_2 has been suggested, whereas spotlights were not yet switched on for 2-AG. The article of the month commented here has filled this gap, documenting a pivotal role of the 2-AG – CB_2 "couple" in spermatogenesis, a major reproductive event by the male side. Overall, the new data add further complexity to the hormone – cytokine networks that regulate mammalian fertility through endocannabinoid signalling. In addition, the observation that the ECS is modulated during meiosis calls for attention on the possible interference of eCBs with chromatin homeostasis. In this context, we have recently demonstrated that AEA can control DNA methylation [9], and future studies will tell whether the control of fertility by eCBs can trigger epigenetic modifications of chromatin, thus altering the expression of key reproductive genes.

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