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Modulation of spermatozoon acrosome reaction

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ABSTRACT

Spermatozoon acrosome reaction is an exocytotic event of the utmost importance for the development of mammalian fertilisation. Current evidence shows that the triggering of the acrosome reaction (AR) could be regulated by the action of diverse compounds, namely, metabolites, neurotransmitters and hormones. The aim of the present review is to describe the modulating effects of several compounds that have been classified as inductors or inhibitors of acrosome reaction. Among AR inductors, it is necessary to mention progesterone, angiotensin II, atrial natriuretic peptide, cathecolamines, insulin, leptin, relaxin and other hormones. Regarding the inhibitors, oestradiol and epidermal growth factor are among the substances that retard AR. It is worth mentioning that gamma-aminobutyric acid, a neurotransmitter known to be an inhibitor in the central nervous system, has been shown to induce AR. The multiple hormones located in the fluids of the female reproductive tract are also likely to act as subtle regulators of AR, constituting a fundamental aspect for the development of successful fertilisation. Finally, it is necessary to emphasise that the study of regulation exerted by hormones and other compounds on AR is essential for further understanding of mammalian reproductive biology, especially spermatozoon physiology.

Key words: capacitation, gamma-aminobutyric acid, hormone modulation, sex steroids, spermatozoon acrosome reaction.

INTRODUCTION

The complex process of mammalian reproduction is based on a series of highly regulated and synchronised physiological events (Colombo, 2006; Familiari et al., 2006). The development of successful fertilisation depends on several aspects, among which it is worth mentioning physical (i.e., mechanical), biochemical, endocrine, behavioural and environmental factors. In mammals in which semen is deposited mainly in the vagina, e.g. humans, the existing spermatozoa have to ascend through the female reproductive tract (Vigil et al., 1994; Vigil et al., 1995). The latter can be considered a microenvironment that supplies the conditions needed to guarantee survival, capacitation and migration of spermatozoa required for subsequent fusion with the oocyte (Vigil et al., 1995). Fertilisation also depends on the morphological characteristics of the spermatozoon and the oocyte (Vigil, 1987; Familiari et al., 2006) and it is known that alterations in either gamete can have an impact on its attainment (Vigil et al., 1985; Vigil, 1987; Bustos-Obregón et al., 1995).

The spermatozoon is a haploid cell (n) consisting of a head, neck, mid-piece and flagellum (Fawcett, 1975). The nucleus and the genetic material are located in the head, along with the acrosome (discussed in detail below). The flagellum is responsible for the motility of the spermatozoon due to the presence of structures such as the axoneme and a set of mitochondria that supply the energy required by the flagellar beating (Fawcett, 1975). Spermatozoa are produced in the testicles in a well-regulated process of differentiation known as spermatogenesis (von Kölliker, 1841), which involves all the phenomena through which a group of diploid cells (2n) become haploid spermatozoa.

As regards the acrosome –from Greek ακρος (acros) meaning "highest", and σωμα (soma) meaning "body"– this is an organelle, which by localisation and shape may resemble a hood, found in the apical region of the spermatozoon covering the anterior extremity of the nucleus. The acrosome has been described as a secretory vesicle, specifically as a modified lysosome (Moreno and Alvarado, 2006), and comprises varied enzymatic content including acrosin, acrogranin, hyaluronidase and other enzymes present in classic organelles, such as peroxisome, lysosome, and even in cytoplasm (McRorie and Williams, 1974; Moreno and Alvarado, 2006; Zhao et al., 2007). Each spermatozoon is endowed with an acrosome varying across species in shape and size, and its formation is a complex, highly regulated phenomenon as compared to the biogenesis of other organelles and secretory vesicles. In fact, many of its proteic components are synthesized in stages prior to the development of the male gamete (Moreno and Alvarado, 2006).

The objective of the present review is to describe the regulation exerted on spermatozoon acrosome reaction (AR), with special interest in the hormone modulation this process is subject to.

WHAT IS AR?

AR consists of the exocytosis of acrosomal content. This generally involves the fusion and fenestration of the spermatozoon plasma membrane with the outer acrosomal membrane. As a consequence of this process, small lipid cumuli are generated from both membranes, stabilising one another until they become independent units. The membranes that contain the enzymes of the acrosome lose continuity

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and stability leading to release of acrosome content to the external medium (Barros et al., 1967; Nagae et al., 1986; Llanos, 1989; Moreno and Alvarado, 2006). These series of events of membrane fusion have been studied mainly in the principal segment of the acrosome. However, AR has also been described at the equatorial segment level, in stages following exocytosis in the apical region (Nagae et al., 1986; Vigil, 1987; Vigil, 1989).

When AR has concluded, the spermatozoon has suffered various physiological changes that will later determine fertilisation, namely: a) acrosome enzyme release, which favours the passing of the spermatozoon through the zona pellucida; b) exposition of the inner acrosome membrane as a new cell surface domain (Nolan and Hammerstedt, 1997; Jungnickel et al., 2001); and c) in the case of the principal acrosome segment, acquisition of the fusogenic ability of the plasma membrane in the spermatozoon equatorial segment. These three events are important and necessary for fertilisation to occur. It has been described that the post-equatorial segment can also acquire this fusogenic capacity (Vigil, 1989; Jungnickel et al., 2001). Although the AR takes place first in the principal segment of the acrosome and later in the equatorial segment, our research establishes that the AR can occur asynchronically in the spermatozoon head, i.e., the fusion of the plasma membrane and the outer acrosome membrane can take place at different times and at different sites (Vigil, 1987; Vigil, 1989). In fact, it is possible to observe spermatozoa with a partial AR at the principal segment and at the equatorial segment of the acrosome. It has also been observed that, given a partial AR of the equatorial segment, both the remaining plasma membrane of the unreacted part of the equatorial segment and the plasma membrane of the post-equatorial segment can acquire fusogenic capacity (Vigil, 1987; Vigil, 1989). This is supported by ultrastructural morphological evidence obtained after observing a spermatozoon in the oocyte cytoplasm with one side of the equatorial segment intact and the other side of the equatorial segment reacted (Figure 1). The ultrastructural evidence obtained by using an immature zona-free hamster oocyte shows that fusion between the oocyte plasma membrane and the spermatozoon plasma membrane across the equatorial and post-equatorial can occur (Vigil, 1987; Vigil, 1989).

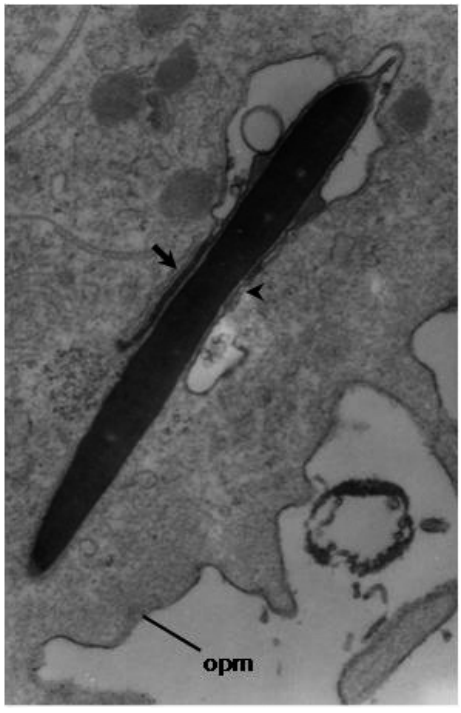

Figure 1: Transmission electron micrograph showing a hamster spermatozoon inside an immature hamster oocyte cytoplasm. The spermatozoon shows one side in which the acrosome equatorial segment is intact (arrow) and the other side in which the acrosome equatorial segment has reacted (arrow head). The oocyte plasma membrane is identified as opm (23000X).

The aforementioned physiological and morphological changes are fundamental during oocyte–spermatozoon interaction in the stages following the passage through the zona pellucida. The principal segment of the reacted spermatozoon has been found to possess domains able to interact with proteic components in the oocyte membrane that determine an initial anchoring between the two cells. These interactions later degrade, giving way to the fusion at the equatorial segment (Takano et al., 1993; Primakoff and Myles, 2002). The role of cyritestin, a protein found in the spermatozoon inner acrosome membrane (Linder et al., 1995) in the initial interaction between the oocyte and the principal spermatozoon segment is worth noting. It has also been described that the blocking of cyritestin receptors on the oocyte membrane interferes with spermatozoon binding (Yuan et al., 1997). As to the equatorial segment of the reacted spermatozoon, the existence of a group of lipids called seminolipids has been suggested (Gadella et al., 1995), which seem to be able to interact with SLIP, a protein of the oocyte membrane (Gadella et al., 1995). This could trigger a plasma membrane rearrangement favouring the fusion of both gametes. AR generates a remodelling of the spermatozoon cellular structure, enabling the fusion of its plasma membrane –over the equatorial or post-equatorial segment– with the oocyte plasma membrane (Vigil, 1987; Vigil, 1989; Familiari et al., 2006).

REQUIREMENTS FOR THE OCURRENCE OF AR

The timely occurrence and development of AR involves some prerequisites that depend mainly on changes at molecular level. Among these is capacitation, which is described below:

Capacitation

The physiological aspects of the reproductive process depend on a sequence of episodes that generate both the physical and chemical conditions that enable oocyte fertilisation (Barros et al., 1996; Colombo, 2006; Familiari et al., 2006). One of these events is spermatozoon capacitation, which encompasses a number of modifications that take place as sperm travel along the female reproductive tract and that involve a number of structural and biochemical changes in the spermatozoon (Barros, 1974; Go and Wolf, 1985; Hyne et al., 1985; Llanos, 1989; Fraser, 1995). These changes include an increase in plasma membrane fluidity, a decrease in the level of plasma cholesterol content (Go and Wolf, 1985; Cross, 1998), an increase in intracellular concentrations of calcium and cAMP (Yanagimachi and Usui, 1974; Yanagimachi, 1982; Visconti

et al., 1990; Visconti et al., 1995; Visconti and Kopf, 1998), phosphorylation of tyrosine residues in proteins (Visconti et al., 1995; Leclerc et al., 1996), and a shift in the patterns of spermatozoon movement and motility (Yanagimachi, 1970). These are critical for AR since, as has been described, only capacitated spermatozoa can experience acrosome exocytosis (Bedford, 1983; Llanos, 1989; Yanagimachi, 1995; DeLamirande et al., 1997). The spermatozoon membrane possesses a variety of lipids whose localisation pattern allows for the occurrence of multiple cellular processes, such as protein location and changes in membrane fluidity. During capacitation, certain proteins in the uterine fluid, such as high density lipoprotein and albumin (Langlais and Roberts, 1985) enable extraction of cholesterol from the plasma membrane, leading to the distribution and re-localisation of these molecules in the spermatozoon (Fleming and Yanagimachi, 1981; Bearer and Friend, 1990). Capacitation affects the sensitivity of the male gamete to the diversity of ligands present in the female reproductive tract, especially in the uterus, oviduct and the oocyte, which trigger certain physiological changes in the spermatozoon, hence increasing the probability of oocyte fertilisation (see below). The relationship between gametes during fertilisation is also evidenced by the role exerted by ZP1, ZP2 and ZP3 (proteins present in the zona pellucida), which are capable of inducing AR through their interaction with a receptor located in the spermatozoon plasma membrane (Harkema et al., 1998; O'Toole et al., 2000).

MODULATORS OF AR

A variety of ligands have been reported to modulate AR exerting their effects by means of receptors at the level of the spermatozoon plasma membrane (Ohzu and Yanagimachi, 1982; Meizel, 1985; Hoshi et al., 1988; Morales et al., 1992; Llanos et al., 1993; Llanos et al., 1995; DelRío et al., 2007; Vigil et al., 2008). Some of these compounds are distributed in specific sectors of the female reproductive tract, probably exerting an *in situ* AR control (DelRío et al., 2007).

Among the ligands capable of affecting AR, we find the following hormones:

Progesterone

Progesterone is a steroid hormone that possesses a canonical signalling pathway consisting of the union with its nuclear receptors. This binding activates the transcription of several genes; hence, it is called the genomic signalling pathway. In the case of the human spermatozoon, this hormone participates in a range of processes such as: induction of AR (Roldan et al., 1994; Murase and Roldan, 1996; DeLamirande et al., 1997; Nolan and Hammerstedt, 1997) [in our studies, percentage of AR: 58.2 ± 0.84 in progesterone treatment vs. 29.0 ± 0.71 in control, $p < 0.05$; (Vigil et al., 2008)], hyperactivation and increasing the percentage of spermatozoon penetration into hamster oocytes (Sueldo et al., 1993). Such effects are mediated by a non-genomic signalling pathway that operates through receptors present in the spermatozoon membrane (Shah et al., 2003). The action of progesterone is possible due to the increase in phosphorylation of cytoplasmic proteins, together with a transient rise in intracellular calcium concentration (Tesarik and Mendoza, 1993; Tesarik et al., 1993; Rathi et al., 2002). Cumulus oophorus secretes progesterone, thus there are important levels of this hormone present in the periovulatory follicular fluid (Morales et al., 1992). It has been shown that the decline of plasma membrane cholesterol during capacitation would determine the degree of response the human spermatozoon to progesterone (Cross and Razy-Faulkner, 1997).

Oestradiol

Oestrogens are steroid hormones whose participation is fundamental in the female reproductive events, but they have also been reported to exert an important role in the male reproductive system (Hess et al., 1997). Although they classically act through the union with nuclear/cytoplasmic receptors, recent investigations show they could act in a faster/non-genomic via a variety of cell types, including the spermatozoon (Baldi et al., 1998; Luconi et al., 2004; Baldi et al., 2009). Oestradiol binds two subtypes of membrane receptors, α and β, both described in the human spermatozoon plasma membrane as presenting a different location (Solakidi et al., 2005). This generates an influx of calcium as mediator of the non-genomic effects (Luconi et al., 2004; Aquila et al., 2004). Oestradiol has been described as an AR inhibitor, with a lower observed percentage of human reacted spermatozoa as compared to those obtained in incubations with control (without oestradiol) spermatozoa preparations [percentage of AR: 29.4 ± 0.55 oestradiol treatment vs. 32.6 ± 0.55 in control, $p < 0.05$ (Vigil et al., 2008)]. In the physiological context of fertilisation, the spermatozoa have to migrate through the cervical mucus (Vigil et al., 1995). This biological fluid, found in some mammals, such as rabbits, ruminants and primates, possesses rheological properties subject to endocrine regulation, and these characteristics change in physiological and pathophysiological conditions (Vigil et al., 1991; Morales et al., 1993; Vigil et al., 1995; Ceric et al., 2005; Vigil et al., 2009a). After passing through the cervical mucus and endometrial cavity, the spermatozoa in the Fallopian tube come in contact with the follicular fluid. The high concentrations of oestradiol present in the cervical mucus during the female fertile period could exert an inhibitory role on AR, preventing the premature occurrence of the latter during the passage of spermatozoa along the uterine cervix. Progesterone, which has been found in follicular fluid during the periovulatory period (Morales et al., 1992), could play a stimulating effect on AR when spermatozoa are in the proximity of the oocyte. This suggests that the variable concentrations of steroid hormones during the female reproductive cycle could have a crucial role in spermatozoon physiology (Vigil et al., 2009c).

Angiotensin II

This hormone possesses a wide range of physiological functions, among which arterial blood pressure control and plasma volume regulation are worth mentioning since they currently constitute an important therapeutic target in cardiovascular pathologies. These effects are produced by the union of Angiotensin II to surface receptors, among which two types have been described, AT_1 and AT_2 (Griendling et al., 1996). Some studies have shown that, in bovines and humans, this hormone can act as an AR inductor by binding to its AT_1 receptor (Gur et al., 1998; Köhn et al., 1998). This AR modulation is dependent on extracellular calcium

concentration and can be inhibited by the administration of losartan, a selective AT_1 receptor inhibitor (Vinson et al., 1995; Gur et al., 1998). The AT_1 receptor is found mainly on the tail of the spermatozoon. However, in capacitated bovine spermatozoa it is most commonly present on the head (Gur et al., 1998). A study in equines Sabeur et al. (2000) found that the addition of a dose of angiotensin II ranging from 1 to 100 nmol/L to capacitated spermatozoa during 20 min resulted in a significant increase in live reacted acrosome spermatozoa (percentage of AR: 24.9 to 22.6 in treated cells vs. 9.8 in control, $p < 0.05$). Such effect could be mediated by the above mentioned AT_1 receptor (Sabeur et al., 2000). Angiotensin II levels have been reported in follicular fluid (Heimler et al., 1995), a finding that leads to the hypothesis that this hormone can have a physiological role in *in vivo* induction of AR, but the underlying mechanisms of the process have not as yet been clearly explained.

Atrial natriuretic peptide

Atrial natriuretic peptide (ANP), also known as atrial natriuretic hormone, is a powerful vasodilator peptide produced by heart muscle cells (Potter et al., 2009). ANP has been found in mammalian reproductive tissue, *e.g.* oviducts (Zhang et al., 2006), as well as in ovarian follicular fluid (Anderson et al., 1994). Evidence suggests that the spermatozoon possesses ANP receptors (Rotem et al., 1998; Zhang et al., 2006). To date, it has been possible to determine that ANP induces AR in human (Anderson et al., 1994; Anderson et al., 1995; Rotem et al., 1998), bovine (Zamir et al., 1995), giant panda (Zhang et al., 2005) and pig spermatozoa (Zhang et al., 2006). The latter evidence suggests that ANP may be involved in the regulation of the acrosome exocytosis and the fertilising ability of mammalian spermatozoa, and it has been suggested that the cGMP-dependent protein kinase pathway possibly participates in this process (Zhang et al., 2006).

Catecholamines

Adrenalin and noradrenalin are catecholamine hormones widely known for their effect on the autonomic nervous system. In spite of this, high concentration levels of these hormones have been determined in the oviductal fluid of mammals (Way et al., 2001). Noradrenalin is described as an AR inducing hormone in bovine spermatozoa, showing an optimal concentration for maximal response and generating higher rates of capacitation, as compared to control incubations (Way and Killian, 2002). Similar effects have also been reported for the addition of adrenalin. Nevertheless, noradrenalin evidences a more relevant increase, both in the percentage of reacted bull spermatozoa and the observed capacitation rates (Way and Killian, 2002). Finally, the catecholamine hormone dopamine has no effect whatsoever on the characteristics of the bull spermatozoa under study (Way and Killian, 2002).

Epidermal growth factor

Epidermal growth factor (EGF), also known as epidermal growth hormone, is a 53 amino acid polypeptide known to be an inductor of cellular growth, proliferation and differentiation in various tissues. EGF has been reported to exert a role in cellular proliferation in the human testicles, favouring mice spermatozoa production (Tsutsumi et al., 1986). EGF receptors have been found in the spermatozoa of different mammals, including humans (Naz and Ahmed, 1992). These receptors correspond to the classic 170 kDa protein in somatic cells (Lax et al., 1994). EGF has been described as an AR inhibitor in humans, causing a dose-dependent decrease in the percentage of reacted spermatozoa (percentage of AR: 51.62 ± 4.23 EGF treatment vs. 67.02 ± 4.36 in control; $p < 0.05$). This could also lead to a reduction in the rate of penetrated oocytes and a decline in the spermatozoon kinetic variables such as velocity and flagellar beat frequency (Naz and Kaplan, 1993).

Insulin

Insulin, a peptide hormone produced in the pancreas by β cells of the islets of Langerhans, has well-known functions in glycaemic homeostasis, also participating in processes of differentiation, growth, development and cell metabolism (Brüning et al., 2000; Saltiel and Kahn, 2001). Several studies have also linked this hormone, as well as its associated signalling pathways, to the regulation of multiple functions implied in reproduction (Ali et al., 1993; Brüning et al., 2000; Lampiao et al., 2009).

The role exerted by insulin in human spermatozoon biology has been evidenced in research on men suffering from diabetes mellitus type 2, whose spermatozoa possess severe structural and morphological defects (Bacetti et al., 2002), reduced motility and lower ability to penetrate hamster oocytes (Shrivastav et al., 1989). A recent study on the *in vitro* effects of this hormone on diverse human spermatozoon variables has determined that treating spermatozoa with insulin significantly increases spontaneous AR, as compared to control (35.33 ± 1.73 % versus 14.56 ± 0.64 %, respectively, $p < 0.05$), and that this administration also leads to an increase in spermatozoon total and progressive motility (Lampiao and du Plessis, 2008). It was also found that inhibiting phosphatidylinositol 3-kinase –an intracellular insulin effector– by means of wortmannin caused a decrease in the observed percentage of AR in insulin treated spermatozoa (Lampiao and du Plessis, 2008). The aforementioned evidence, and the fact that insulin is present in ejaculated spermatozoa (Aquila et al., 2005a), make it possible to attribute eventual functions on spermatozoon physiology to this hormone.

Leptin

Leptin is a peptide hormone with a molecular mass of approximately 16 kDa produced by the gene *LEP,* which was initially reported as the *ob* gene in mice. It is constituted by 166 amino acids, contains a disulphide bridge needed for its biological activity and is produced by adipocytes. This hormone exerts a crucial function in glucose and lipid homeostasis, especially in body mass control through the regulation of food intake and thermogenesis (Farooqi and O'Rahilly, 2009). Current evidence suggests that leptin is involved in the regulation of several processes related to mammalian reproduction (Moschos et al., 2002; Lampiao et al., 2009). The determination by Aquila et al. (2005b) that human ejaculated spermatozoa contain this hormone has led to the questioning of the possible role of leptin on the spermatozoon (Andò and Aquila, 2005). In a study by Lampiao and du Plessis

(2008) designed to determine the *in vitro* effects of leptin on some variables of the human spermatozoon, this hormone in particular has been shown to affect acrosome exocytosis. In fact, treating spermatozoa with leptin significantly increases spontaneous AR as compared to control (36.56 ± 1.93 % vs. 14.56 ± 0.64 %, respectively, $p < 0.05$), and this administration also leads to an increase in spermatozoa motility (Lampiao and du Plessis, 2008). However, the molecular mechanisms underlying the aforementioned results for this hormone have not as yet been elucidated and remain to be determined.

Prolactin

Prolactin (PRL), a hormone synthesised and released by the adenohypophysis lactotroph cells, is responsible for the stimulation and production of milk in the mammary glands, and for exerting other multiple functions related to mammalian reproduction (Smith, 1980). Considering that PRL is present throughout the female reproductive tract (Mori et al., 1988), that its receptor has been identified in mammalian spermatozoa (Hashimoto et al., 1988), and that this hormone shortens the optimal preincubation period for mice spermatozoa to acquire capacitation (Fukuda et al., 1989), it is likely that PRL could be exerting some effect on AR. Stovall and Shabanowitz (1991) studied the effects of PRL on spermatozoon capacitation and its ability to induce AR, and found that, apparently, this hormone has no significant effect on capacitation and AR of the human spermatozoon (Stovall and Shabanowitz, 1991). However, further studies are required in order to assess the role of PRL in the acrosome exocytosis.

Relaxin

Relaxin, a peptide hormone with a molecular mass of approximately 6000 Da, has been described as having important roles in mammalian pregnancy, being involved in events such as relaxation and softening of the uterus, as well as in pubic symphysis during childbirth. This hormone has been found in human seminal plasma (Essig et al., 1982; Lessing et al., 1986) and it has been found that it exerts physiological effects on spermatozoon motility in certain species of domestic animals, such as bulls (Kohsaka et al., 2003). Miah et al. (2006) showed that boar spermatozoa incubation with relaxin significantly stimulates motility, the percentage of AR and glucose use. A more recent work found that the addition of this hormone leads to an increase of these variables in bovine spermatozoa (Miah et al., 2007). These results suggest the physiological significance of using relaxin on spermatozoon variables in mammals, especially as regards AR induction.

Gamma-aminobutyric acid

Even though gamma-aminobutyric acid (GABA) is not in itself a hormone, it shows interesting effects on AR. It is known that GABA is an inhibitory neurotransmitter in the central nervous system, and participates in most of the inhibitory synapses that enable neuronal activity. In spite of this, this compound has also been also described in a variety of cellular events in non-neuronal peripheral tissues, which has led some authors to suggest it is a trophic factor, or even a hormone (Ong and Kerr, 1990; Gladkevich et al., 2006). GABA is also present in tissues such as the human uterus, oviducts and ovaries (Erdö et al., 1989), as well as in certain fluids, such as human seminal plasma (Leader et al., 1992). Regarding AR, GABA has also been described to be a dose-dependent inductor of this process (Shi et al., 1997). Its effects appear to be mediated by receptors located in the spermatozoon membrane, whose activation implies an increase in cytoplasmic calcium concentration and subsequent acrosome exocytosis (Aanesen et al., 1995). In relation to the latter, two types of GABA receptors have been described, types A and B, both present in the spermatozoon (Aanesen et al., 1995). Studies performed on ligands specific for each receptor have made it possible to conclude that the GABA (A) receptor is the most efficient inductor of AR (Calogero et al., 1999; Hu et al., 2002).

Both GABA and progesterone seem to exert similar effects on processes associated to spermatozoon physiology, such as AR, capacitation and hyperactivation (Calogero et al., 1996; Shi et al., 1997; Calogero et al., 1999). It has also been shown that the GABA receptor in the spermatozoon can be activated by progesterone and some of its metabolites; this interaction results in an increased chloride flux essential to AR initiation (Wistrom and Meizel, 1993). Our research group has recently reported that both GABA and progesterone induced human AR can be regulated by the effects of oestradiol, formerly described as an AR inhibitor [percentage of AR: 58.2 ± 0.84 progesterone treatment vs. 30.0 ± 0.84 progesterone + oestradiol treatment; 33 ± 1.16 GABA vs. 17 ± 1.47 GABA + oestradiol treatment, $p < 0.05$ (Vigil et al., 2008; Vigil et al., 2009b; Vigil et al., 2009c)]. Thus, we have suggested a possible hormone interaction, through non-genomic pathways, among progesterone, GABA and oestradiol in physiological processes such as AR (Vigil et al., 2009c). This interaction could also be present in somatic cells.

The compounds that exert a role on AR modulation are shown in Figure 2.

CONCLUDING REMARKS

Spermatozoon AR is affected by a series of chemical substances and metabolites, as well as by the action of a number of hormones. The meaning and biological relevance of the effects on AR attributed to such hormones are far from being elucidated and thoroughly understood, although this topic is today subject of active research (Meizel, 2004; Andò and Aquila, 2005; Lampiao and du Plessis, 2008; Vigil et al., 2008; Vigil et al., 2009c; Baldi et al., 2009; and other groups). This review was focused upon the hormones that we recognise as the most important modulators of AR. Nevertheless, there may be other hormones acting as regulators of the AR that are yet to be recognised. Considering the current evidence and what has been mentioned, it is probable that the changes in hormonal levels that occur *in vivo* in the reproductive tract of the female of each species during the reproductive cycle subtly regulate the AR, retarding the onset or favouring its timely occurrence. As regards this delicate hormonal modulation that AR could be subject to, it is necessary to mention again the case of steroid hormones, about which more information is available: in the cervix, the oestradiol present in the periovulatory cervical mucus could be exerting the role of AR inhibitor since its onset in such regions of the female reproductive tract would not make fertilisation possible (Ceric et al., 2005; Vigil et al., 2008; Vigil et al., 2009b). On the contrary, in the distal third of the Fallopian tube, there are high levels of progesterone coming

from the follicular fluid, which could promote AR precisely when the spermatozoon and the oocyte are close to encounter, favouring successful fertilisation (Morales et al., 1992; Vigil et al., 2008; Vigil et al., 2009c). The latter constitutes a feasible and coherent explanation in the context of the reproductive process, and possibly other hormones and metabolites of physiological interest could also be participating in this subtle AR regulation, together or simultaneously with steroid hormones.

The variations in the concentrations of cytoplasmic calcium, cAMP and the phosphorylation of proteic residues are currently some of the non-genomic effectors of steroid hormones. Such signalling components have been described both in somatic cells and in spermatozoa (Baldi et al., 2009). The inactivation of the nucleus, and thus the absence of transcription, confers the male gamete an advantage as a study model for the non-genomic action of steroid hormones. For this reason, the knowledge obtained from experiments on spermatozoa could explain the non-genomic response of somatic cells as regards these and other types of hormones.

In keeping with current research on steroid hormones and AR, further studies should focus on determining the hormones that, in fact, exert a significant effect on AR modulation, as well as the molecular mechanisms of the signal transduction pathways that underlie this regulation. This will make it possible to propose a future physiological explanation to integrate the effects that the compounds mentioned above have on AR. This information would certainly help elucidate other even more relevant questions, namely: what is the biological relevance of hormone modulation on AR? And what is the significance of such modulation for the reproductive events posterior to acrosome exocytosis? Our research group is currently aiming to find the answers to these questions.

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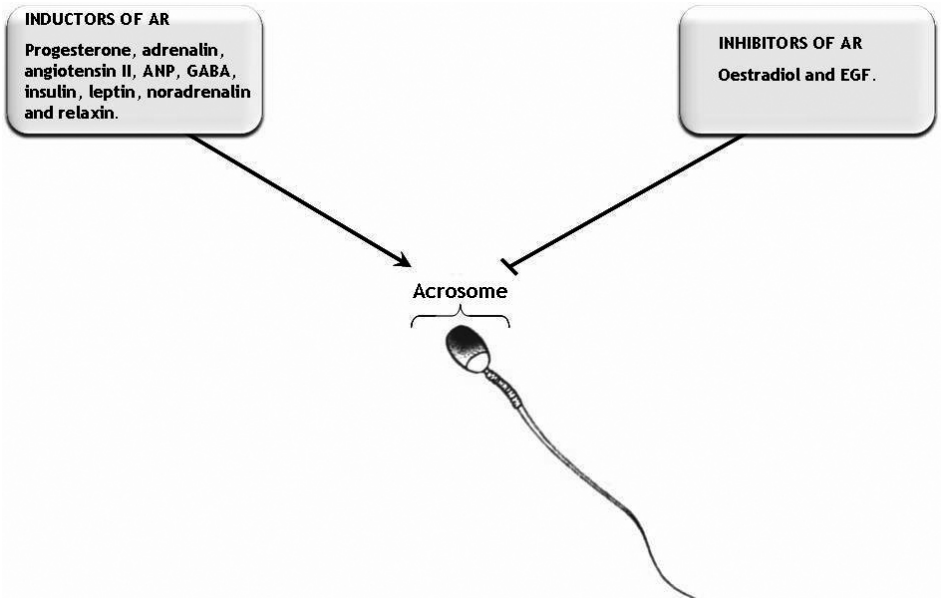

Figure 2: Compounds that exert a role on AR modulation. AR: acrosome reaction; ANP: atrial natriuretic peptide; GABA: gamma-aminobutyric acid; EGF: epidermal growth factor.

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