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MINI-REVIEW

Non-genomic regulation and disruption of spermatozoal in vitro hyperactivation by oviductal hormones

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Abstract During capacitation, motility of mammalian spermatozoon is changed from a state of "activation" to "hyperactivation." Recently, it has been suggested that some hormones present in the oviduct are involved in the regulation of this hyperactivation in vitro. Progesterone, melatonin, and serotonin enhance hyperactivation through specific membrane receptors, and 17β-estradiol suppresses this enhancement by progesterone and melatonin via a membrane estrogen receptor. Moreover, γ-aminobutyric acid suppresses progesterone-enhanced hyperactivation through the γ-aminobutyric acid receptor. These hormones dose-dependently affect hyperactivation. Although the complete signaling pathway is not clear, progesterone activates phospholipase C and protein kinases and enhances tyrosine phosphorylation. Moreover, tyrosine phosphorylation is suppressed by 17β-estradiol. This regulation of spermatozoal hyperactivation by steroids is also disrupted by diethylstilbestrol. The in vitro experiments reviewed here suggest that mammalian spermatozoa are able to respond to effects of oviductal hormones. We therefore assume that the enhancement of spermatozoal hyperactivation is also regulated by oviductal hormones in vivo.

Keywords Amine · Amino acid · Hyperactivation · Non-genomic regulation · Spermatozoa · Steroid

Introduction

In the oviduct, mammalian spermatozoa fertilize the oocyte. Before fertilization, however, spermatozoa must be capacitated [1–4]. Capacitation is a qualitative change in the spermatozoa that is needed for fertilization of the oocyte. Capacitated spermatozoa exhibit two reactions associated with capacitation. One is an acrosome reaction that occurs at the head of a spermatozoon. This reaction is a specialized exocytosis that is required for penetration of the zona pellucida (ZP) and for binding to the oocyte [1, 2, 4, 5]. The other is hyperactivation that occurs at the flagellum. Hyperactivation induces a specialized flagellar movement that creates the driving force for swimming in the oviduct and for penetrating the ZP [1–4]. Moreover, it has been shown that the ability of spermatozoon to be hyperactivated correlates with the success of in vitro fertilization [6].

By use of a specific culture medium, capacitation is also made to occur in vitro. During in vitro capacitation, spermatozoa show motility change, such as from "activation" to "hyperactivation." Just after swim up in a specific culture medium, spermatozoa are activated (movies 1, 3, and 5). In many animals, activated spermatozoa show a small bend amplitude in flagellar movement and swim linearly. After incubation for some hours (for example, 3–4 h in hamster and mouse spermatozoa and 4–5 h in rat spermatozoa), most spermatozoa show hyperactivated motility (movies 2, 4, and 6). However, the movement pattern of hyperactivated spermatozoon basically depends on animals [1]. In hamster

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and mouse (movies 2 and 4), hyperactivated spermatozoa show a large amplitude and a large asymmetric beating pattern in flagellar movement. Sometimes, their spermatozoa writhe and swim in the form of eight characters. In rat spermatozoa (movie 6), many hyperactivated spermatozoa show the large amplitude of head, the arched movement of middle peace of flagellum, and the decrease of progressive movement although identification of their movement pattern is difficult.

During spermatozoal capacitation, hyperactivation occurs spontaneously and time-dependently [1–3, 7–9]. The first stimulation for capacitation/hyperactivation is the removal of cholesterol from a spermatozoal plasma membrane by albumin [10–13]. The next step is Ca^{2+} influx and cAMP production stimulated by HCO_3^-. Stimulation by Ca^{2+} and HCO_3^- activates certain protein kinases and phosphorylates proteins [14–19]. Additionally, the suppression of protein phosphatases induces hyperactivation and protein phosphorylation [20]. Tyrosine phosphorylation is well known as a capacitation-associated intracellular signal [14, 15, 19]. The most popular tyrosine phosphorylation molecule is an 80-kDa protein, which was identified as an A-kinase anchoring protein (AKAP) [21]. These stimulations and signal transductions are associated with regulation of capacitation, and induce the acrosome reaction and hyperactivation.

After the 1980s, it has been reported that several molecules that are found from the oviductal and follicular fluids affect spermatozoal acrosome reaction and hyperactivation [11–13, 22–39]. Progesterone and serotonin are well-known classical effectors of the acrosome reaction [22, 40]. Recently, it has been suggested that progesterone, melatonin, and serotonin act as inducers or enhancers of the acrosome reaction and hyperactivation [11–13, 29–31, 33], and that 17β-estradiol acts as a suppressor for these processes [32, 34]. Moreover, it has been reported that γ-aminobutyric acid (GABA) acts as an inducer of the acrosome reaction and hyperactivation in humans, rams, and rats [35–38], although it acts as a suppressor of hyperactivation in hamsters [39]. In the present article, we review effects of the above molecules on hyperactivation.

Non-genomic regulation of hyperactivation by progesterone

Progesterone was found to be an inducer of the acrosome reaction in human follicular fluid [22]. Additionally, in hamsters, 20 ng/ml of progesterone increased ZP penetration and enhanced hyperactivation [11, 25]. In hamsters, moreover, the concentration of progesterone was 4.2–7.4 μg/ml in the follicular fluid, and was 44.04–175.06 ng/ml in the oviductal fluid [41]. Therefore, it seems that the progesterone present in the follicular fluid induces the acrosome reaction and that the progesterone present in the oviductal fluid increases ZP penetration and enhances hyperactivation. Although progesterone regulates cell functions through genomic signals in somatic cells, it regulates the acrosome reaction, ZP penetration, and hyperactivation through non-genomic regulation in mammalian spermatozoa [23, 24, 30, 42]. In human spermatozoa, progesterone stimulates an influx of Ca^{2+} associated with CatSper activation, tyrosine phosphorylation, chloride efflux, and cAMP increase, and subsequently induces the acrosome reaction and hyperactivation [24, 30, 42–45]. In hamster spermatozoa, progesterone enhances hyperactivation together with tyrosine phosphorylation [11]. Although the traditional genomic progesterone receptor (PR) does not exist in spermatozoa, a novel non-genomic PR exists at the plasma membrane of spermatozoa [23, 24, 27, 28, 42]. Moreover, it has been suggested that progesterone binds to the acrosome region where PR are localized in human and hamster spermatozoa [11, 46]. Downstream of the spermatozoal PR, phospholipase C (PLC) [47] and/or protein kinase A (PKA) [48] are involved in the progesterone-induced acrosome reaction in mouse and human spermatozoa. In hamster spermatozoa, PLC, PKA, and protein kinase C (PKC) are involved in the progesterone-enhanced hyperactivation downstream of the PR [11, 49].

It is well known that tyrosine phosphorylation sites, including AKAP, are associated with the regulation of spermatozoal capacitation/hyperactivation [1, 2, 14, 15, 19]. In several cases [11, 20], tyrosine phosphorylation is enhanced when spermatozoal hyperactivation is induced by molecules present in the oviduct. Although it is not clear which kinases cause tyrosine phosphorylation of spermatozoal proteins, it has been reported that tyrosine phosphorylation is regulated through Ca^{2+} signals associated with an inositol 1,4,5-tris–phosphate (IP_3) receptor-gated Ca^{2+} store located at the base of the flagellum and calmodulin-dependent protein kinase [7, 9, 16, 17, 50]. Moreover, it has also been reported that tyrosine phosphorylation is regulated through cAMP-PKA signals [1, 14, 15, 19]. Because PLC, IP_3 receptor, PKA, and PKC are involved in enhancement of hyperactivation in hamster spermatozoa [11, 49], it seems that progesterone enhances spermatozoal hyperactivation through binding to PR and activation of PLC. This binding results in the production of IP_3 and diacylglycerol, release of intracellular Ca^{2+} from an IP_3 receptor-gated Ca^{2+} store, activation of PKC, activation of adenylate cyclase, production of cAMP, activation of PKA, and enhancement of tyrosine phosphorylation (Fig. 1).

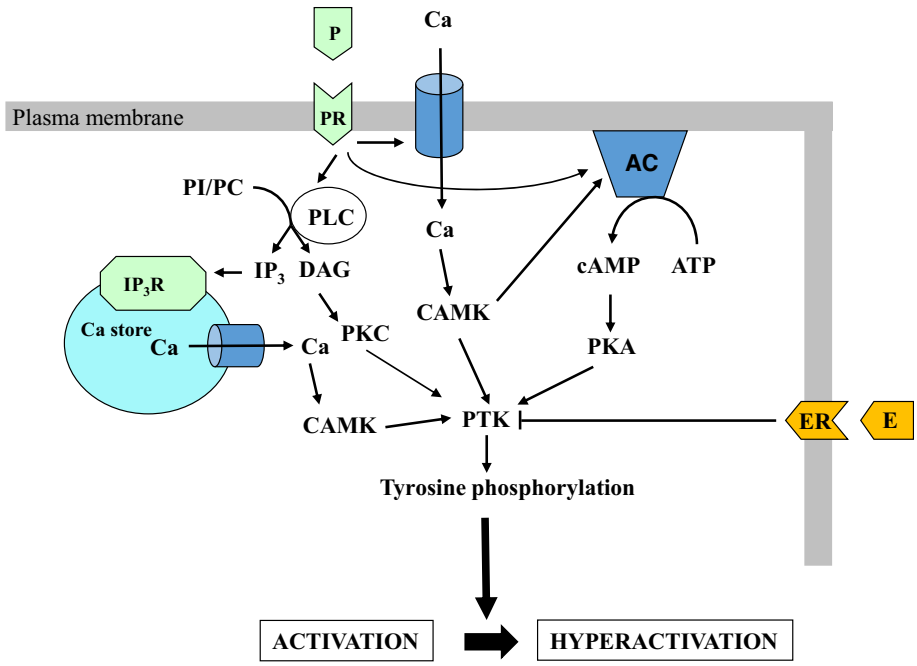

Fig. 1 Hypothesized mechanism for the regulation of hyperactivation enhancement by progesterone and estradiol. *AC* adenylate cyclase, *ATP* adenosine triphosphate, *CAMK* calmodulin-dependent protein kinase, *cAMP* cyclic adenosine monophosphate, *DAG* diacylglycerol, *E* estradiol, *ER* estrogen receptor, IP_3 inositol 1,4,5-tris–phosphate, IP_3R inositol 1,4,5-tris–phosphate receptor, *P* progesterone, *PC* phosphatidylcholine, *PI* phosphatidylinositol, *PKA* protein kinase A, *PKC* protein kinase C, *PLC* phospholipase C, *PR* progesterone receptor, *PTK* protein tyrosine kinase

Suppression of progesterone-enhanced hyperactivation by 17β-estradiol

In human spermatozoa, it has been reported that 17β-estradiol suppresses the progesterone-induced acrosome reaction through non-genomic regulation associated with membrane estrogen receptor (ER) [24, 29–31]. Although the detailed suppressive mechanism of 17β-estradiol is not clear, differences in Ca^{2+} influx due to progesterone and 17β-estradiol are considered important [29, 31]. The progesterone spike follows that of Ca^{2+} in spermatozoa, whereas 17β-estradiol gradually increases the intracellular Ca^{2+} concentration in spermatozoa [29, 31].

In hamster spermatozoa [32, 51], 17β-estradiol has been shown to suppress progesterone-enhanced hyperactivation through ER-inhibiting tyrosine phosphorylation (Fig. 1). Because the ER is present in the plasma membrane at the head of hamster spermatozoa [32], it seems that 17β-estradiol suppresses progesterone-enhanced hyperactivation through non-genomic regulation (Fig. 1). Suppression of progesterone-enhanced hyperactivation by 17β-estradiol occurs in a dose-dependent manner [32, 51]. The effect of 20 ng/ml of progesterone is suppressed by >20 pg/ml of 17β-estradiol. It seems that spermatozoal hyperactivation is regulated by the balance of progesterone and 17β-estradiol concentrations. Because the concentrations of progesterone and 17β-estradiol vary during the female estrous cycle [4], it seems that mammalian spermatozoa (at least hamster spermatozoa) are hyperactivated in response to progesterone and 17β-estradiol changes in the oviduct [8, 32, 51].

Disruption of the effects of steroids on hyperactivation by diethylstilbestrol (DES)

Diethylstilbestrol (DES) is an endocrine-disrupting chemical that affects some reproductive systems [52, 53]. Although it had not previously been known whether DES affects gametic function, a recent study [51] has suggested that DES affects the non-genomic regulation of hyperactivation by progesterone and 17β-estradiol. The effect of DES alone on progesterone-enhanced hyperactivation is very weak [51]. However, when spermatozoa are exposed to DES together with 17β-estradiol, DES suppresses progesterone-enhanced hyperactivation by accelerating the effect of 17β-estradiol [51]. Specifically, 20 pg/ml of 17β-estradiol with 20 pg/ml of DES was found to significantly suppress enhancement of hyperactivation by 20 ng/ml of progesterone, while 20 pg/ml of 17β-estradiol alone did not significantly suppress enhancement by 20 ng/ml of progesterone [51]. It seems that the effects of DES described above disrupt hyperactivation of hamster spermatozoa through non-genomic regulation associated with progesterone and 17β-estradiol.

Interaction between steroids and other molecules

Melatonin is an enhancer of spermatozoal hyperactivation [12, 33]. In hamsters, it has been shown that melatonin enhances spermatozoal hyperactivation via melatonin receptor type 1 [12]. In rams and humans, it has been shown that melatonin increases some spermatozoal

functions (e.g., motility, capacitation, fertility rate, antioxidant enzyme activity) through decreasing nitric oxide (NO) [33, 54–57]. Although low concentrations of NO induce capacitation through a mitogen-activated protein kinase cascade [58–63], high concentrations of NO suppress spermatozoal functions [59, 61]. Generally, melatonin indirectly suppresses the reproductive system (e.g., steroidogenesis and spermatogenesis) through the central nervous system in seasonal breeding animals [4, 62, 63]. In contrast, melatonin directly affects spermatozoal functions of seasonal breeding animals and human [12, 33, 54–57]. Moreover, a very recent study [34] has shown that 17β-estradiol suppresses melatonin-enhanced hyperactivation in hamster spermatozoa. The effect of melatonin and 17β-estradiol interaction on hyperactivation of hamster spermatozoa is direct, although the mechanisms behind this interaction are not at all clear.

Serotonin is also an enhancer of hyperactivation of hamster spermatozoa [13]. Low concentrations of serotonin enhance spermatozoal hyperactivation through the 5-HT_2 receptor, whereas high concentrations of serotonin enhance hyperactivation through the 5-HT_4 receptor. Serotonin also induces the acrosome reaction through the 5-HT_2 and 5-HT_4 receptors in hamster spermatozoa [40]. Generally, 5-HT_2 receptor and 5-HT_4 receptors activate PLC-Ca^{2+} signaling and adenylate cyclase-cAMP signaling, respectively [64, 65]. Although serotonin signals are similar to progesterone signals, it is not clear whether estradiol suppresses serotonin-enhanced hyperactivation as it does in the case of progesterone.

GABA induces the acrosome reaction and hyperactivation through GABA receptors in human, ram, and rat spermatozoa [35–38]. In several cases, the $GABA_A$ receptor has been shown to be involved in inducing the acrosome reaction and hyperactivation [37, 38]. Although the $GABA_B$ receptor also exists in rat spermatozoa and is localized in the sperm head [66–68], it is unclear whether the $GABA_B$ receptor is involved in spermatozoal functions. Interestingly, several studies have reported that progesterone induces the acrosome reaction and hyperactivation through the $GABA_A$ receptor in human, ram, and rat spermatozoa [35–38], although many studies have reported that progesterone induces and enhances the acrosome reaction and hyperactivation through PR instead [11, 22–24, 26–28, 46, 48]. In contrast, in hamster spermatozoa, GABA suppresses progesterone-enhanced hyperactivation through the $GABA_A$ receptor [39]. Because the concentration of GABA in the oviduct is more than 2.5-fold that in the brain [69] and the concentration of GABA changes in the female genital tract through the estrous cycle [70], it is likely that GABA is involved in the regulation of capacitation in a similar manner to 17β-estradiol, including regulation of the acrosome reaction and hyperactivation. However, the detailed mechanisms behind GABA actions in spermatozoal capacitation are not yet clarified. Additionally, effects of GABA and $GABA_A$ receptor on spermatozoal functions confuse.

Conclusions

Rodent spermatozoa begin to be capacitated after moving into the oviduct. Other many mammalian (including human) spermatozoa present in the oviduct are capacitated. Capacitation-related events (acrosome reaction and hyperactivation), which occurred in a specialized culture medium in vitro, are regulated by molecules present in the oviduct, including progesterone, 17β-estradiol, melatonin, serotonin, and GABA. These molecules induce the acrosome reaction and hyperactivation of mammalian spermatozoa in a dose-dependent manner.

In vitro effects of these molecules on the acrosome reaction and hyperactivation should be confirmed as in vivo effects by in vivo experiments, although observations of effects of the molecules on spermatozoal acrosome reaction and hyperactivation in vivo are very difficult. At least, previous in vitro experiments suggested that mammalian spermatozoa were able to respond to effects of the molecules. Therefore, we consider that mammalian spermatozoa have abilities to respond to influences of the molecules in vivo. Because the concentrations of the molecules present in the oviduct vary during the estrous cycle [4], it seems that mammalian spermatozoa are acrosome-reacted and hyperactivated in response to the changing environment of the oviduct such as the changing concentration of the oviductal molecules [8, 11, 24, 25, 29–32, 51]. Moreover, it seems that regulation of hyperactivation by molecules present in the oviduct, especially progesterone and 17β-estradiol, is unstable because this regulation is easily disrupted by DES accelerating the effect of 17β-estradiol [11, 25, 32, 51].

After beginning to swim, mammalian spermatozoa spontaneously are capacitated in the oviduct in order to be hyperactivated and finally acrosome-reacted. Based on the in vitro experiments reviewed here, we consider that the enhancement of spermatozoal hyperactivation is regulated through ligand-dependent mechanisms associated with oviductal molecules during capacitation. Moreover, we assume that its regulatory mechanisms are associated with changes in the oviduct environment because changes of concentration of oviductal molecules are involved in estrous cycle.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest that could be perceived as prejudicing the impartiality of the presented research.

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Statement on human rights and the welfare of animals The experiment was approved by the Animal Care and Use Committee of the Dokkyo Medical University (Experimental permission number: 0107), and carried out according to the Guidelines for Animal Experimentation in the university.

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