Abstract. Hamster sperm hyperactivation is enhanced by progesterone, and this progesterone-enhanced hyperactivation is suppressed by 17β-estradiol (17βE2) and γ-aminobutyric acid (GABA). Although it has been indicated that melatonin also enhances hyperactivation, it is unknown whether melatonin-enhanced hyperactivation is also suppressed by 17βE2 and GABA. In the present study, melatonin-enhanced hyperactivation was significantly suppressed by 17βE2 but not by GABA. Moreover, suppression of melatonin-enhanced hyperactivation by 17βE2 occurred through non-genomic regulation via the estrogen receptor (ER). These results suggest that enhancement of hyperactivation is regulated by melatonin and 17βE2 through non-genomic regulation.

Key words: Estradiol, Hyperactivation, Melatonin, Non-genomic regulation, Spermatozoa

In mammals, only capacitated spermatozoa can fertilize an egg. Capacitated spermatozoa generally show two major responses, known as “acrosome reaction” and “hyperactivation” [1–3]. The acrosome reaction is an exocytosis occurring at the sperm head, and is required for penetration of the zona pellucida (ZP) [1]. Hyperactivation is a modification of flagellar movement to create the driving force for penetrating the ZP [1–3]. Moreover, the ability of spermatozoa to be hyperactivated correlates with the success of in vitro fertilization [4].

It has been recently reported that hyperactivation is regulated by several hormones and transmitters (e.g., progesterone [5–10], 17β-estradiol (17βE2) [8, 10], melatonin [11, 12], serotonin (5-HT) [13] and γ-aminobutyric acid (GABA) [14–18]). In the hamster, progesterone, melatonin and serotonin enhance hyperactivation in a dose-dependent manner [7, 10, 11, 13]. Moreover, progesterone enhances hyperactivation through non-genomic regulation associated with a progesterone receptor (PR), phospholipase C (PLC), inositol 1,4,5-tris-phosphate receptor (IP3R), protein kinases and tyrosine phosphorylations [7, 9]. Melatonin enhances hyperactivation via melatonin receptor type 1 (MT1) [11]. Serotonin enhances hyperactivation via the 5-HT2 and 5-HT4 receptors [13]. In humans, progesterone and melatonin change motility parameters and enhance hyperactivation [5, 6, 12]. It has also been shown that 17βE2 and GABA dose-dependently suppress progesterone-enhanced hyperactivation in the hamster [8, 9, 18]. Furthermore, 17βE2 suppresses progesterone-enhanced hyperactivation through non-genomic regulation associated with the estrogen receptor (ER) and tyrosine dephosphorylations [8]. GABA suppresses progesterone-enhanced hyperactivation via the GABAA receptor [18]. Interestingly, in humans, rams and rats, GABA increases hyperactivation via the GABAA receptor [14–17].

In hamster spermatozoa, there are three enhancers of hyperactivation: progesterone [7, 9, 10], melatonin [11] and serotonin [13]. Moreover, there are two suppressors of progesterone-enhanced hyperactivation: 17βE2 [8, 10] and GABA [18]. In order to understand the regulatory mechanisms of sperm hyperactivation caused by enhancers and suppressors, interactions among them need to be examined. Therefore, in the present study, we examined whether melatonin-enhanced hyperactivation of hamster spermatozoa is suppressed by 17βE2 and GABA.

Materials and Methods

Chemicals

Hypotaurine, (–)epinephrine, 17α-estradiol (17αE2), 17βE2, fluroscein isothiocyanate and bovine serum albumin (BSA)-conjugated 17βE2 (BSA-17βE2), GABA, melatonin, sodium taurocholate, sodium metabisulphite, and tamoxifen were purchased from Sigma-Aldrich (St Louis, MO, USA). BSA fraction V was purchased from Merck KGaA (Darmstadt, Germany). Other reagent-grade chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Animals and preparation of hyperactivated spermatozoa

Spermatozoa were obtained from the posterior epididymis of sexually mature male golden hamsters (Mesocricetus auratus). The experimental plan was approved by the Animal Care and Use Committee of the Dokkyo Medical University (Experimental permission number: 0107), and the experiment was carried out according to the Guidelines for Animal Experimentation of the University.

Hyperactivated spermatozoa were prepared using the modified Tyrode’s albumate pyruvate (mTALP) medium [20] containing 101.02 mM NaCl, 2.68 mM KCl, 2 mM CaCl2-2H2O, 1.5 mM MgCl2-6H2O, 360 μM NaH2PO4-2H2O, 35.70 mM NaHCO3, 4.5
mM D-glucose, 90 μM sodium pyruvate, 9 mM sodium lactate, 500 μM hypotaurine, 50 μM (-)-epinephrine, 200 μM sodium taurocholate, 5.26 μM sodium metabisulfitite, 0.05% (w/v) streptomycin sulfate, 0.05% (w/v) potassium penicillin G and 15 mg/ml BSA (pH 7.4 at 37°C under 5% (v/v) CO₂ in air) according to the method described previously [19]. A drop (~5 μl) of posterior epididymal spermatozoa was placed in a culture dish (diameter, 35 mm) with 3 ml of the mTALP medium, followed by incubation at 37°C for 5 min to allow spermatozoa to swim up. All of the mTALP medium containing motile spermatozoa was placed in a new culture dish and incubated for 4 h at 37°C under 5% (v/v) CO₂ in air to allow hyperactivation to occur. As stock solutions, melatonin (1 μM), 17αE₂ (20 μg/ml) and 17βE₂ (20 μg/ml) were dissolved in ethanol; tamoxifen was dissolved at 1 mM in dimethyl sulfoxide; and GABA (5 mM) and BSA-E₂ (7.4 μM) were dissolved in pure water. GABA, 17αE₂, 17βE₂, tamoxifen or vehicle was added to the mTALP medium after swim up, and after 5 min of incubation, 17βE₂ or vehicle was added to the mTALP medium (Figs. 1, 2, 3A, 3B, 5A, 5B). Tamoxifen or vehicle was added to the mTALP medium after swim up, and after 5 min incubation, 17βE₂, BSA-17E₂ or vehicle was added to the mTALP medium again. After additional incubation for 5 min, melatonin or vehicle was added to the mTALP medium (Figs. 3C, 3D, 4). Mixtures of 17βE₂ and melatonin or vehicle were added to the mTALP medium after swim up (Figs. 5C, 5D). Melatonin or vehicle was added to the mTALP medium after swim up, and after 5 min of incubation, 17βE₂ or vehicle was added to the mTALP medium (Figs. 5E, 5F). In all experiments, the maximal concentration of vehicle was 0.3% by volume.

Measurement of the percentage of hyperactivated spermatozoa

The percentage of hyperactivated spermatozoa was measured according to the method described previously [19]. Motile spermatozoa were recorded on videotape using a phase-contrast illumination unit attached to a microscope (IX70, Olympus, Tokyo, Japan) with a CCD camera (Progressive 3CCD, Sony, Tokyo, Japan) and a small CO₂ incubator (MI-IBC, Olympus). Each observation was performed
at 37 C, recorded for 2 min, and analyzed by slow-motion playback and manual counting of the numbers of total spermatozoa and hyperactivated spermatozoa in four different fields per point of analysis. The analysis was done in a blinded manner. Motile spermatozoa that exhibited asymmetric and whiplash flagellar movement and a circular or octagonal swimming locus were defined as hyperactivated spermatozoa in four different fields per point of analysis.

Statistical analysis

Data are expressed as the mean ± standard deviation (SD). Statistical analysis was carried out using Tukey’s test of analysis of variance. P < 0.05 was considered significantly different.

Results

Effects of 17βE₂ and GABA on melatonin-enhanced hyperactivation

We examined whether melatonin-enhanced hyperactivation is suppressed by 17βE₂ and GABA in a manner similar to progesterone-enhanced hyperactivation [8, 18]. After swim up, 20 ng/ml 17βE₂ or 5 μM GABA was added to the mTALP medium containing motile spermatozoa, and after additional incubation for 5 min, 1 nM melatonin was added to the same medium. After supplementation with 1 nM melatonin, spermatozoa were incubated for 4 h in order to induce hyperactivation. As shown in Fig. 1A, the percentage of motile spermatozoa was not affected by addition of 1 nM melatonin and 20 ng/ml 17βE₂ when spermatozoa were exposed to 1 nM melatonin after exposure to 17βE₂. In contrast, as shown in Fig. 1B, melatonin-enhanced hyperactivation was significantly suppressed by 20 ng/ml 17βE₂. After incubation for 1 or 1.5 h, melatonin-enhanced hyperactivation was significantly suppressed by 17βE₂ (1 h, Melatonin: 17.4% ± 3.17, 17βE₂ → Melatonin: 10.67% ± 2.45; 1.5 h, Melatonin: 47.89% ± 6.86, 17βE₂ → Melatonin: 26.98% ± 11.72) but not significantly suppressed by 17βE₂ after incubation for 2 h. GABA did not affect the percentages of motile spermatozoa, hyperactivated spermatozoa and melatonin-enhanced hyperactivation (Figs. 1C, 1D).

Effect of tamoxifen on suppression of melatonin-enhanced hyperactivation by 17βE₂

Because melatonin-enhanced hyperactivation was suppressed by 17βE₂ (Fig. 1), we examined whether the mechanism acted via the ER. In order to examine whether the ER was associated with suppression of melatonin-enhanced hyperactivation caused by 17βE₂, we used tamoxifen, which is a popular ER antagonist [24–26]. It was reported that several micromoles of tamoxifen acted as an ER antagonist [27–29], although a high concentration of tamoxifen inhibited protein kinase C (PKC) in some cases [30, 31]. In a previous study [8], 1 μM tamoxifen inhibited only suppression of progesterone-enhanced hyperactivation caused by 17βE₂. After swim up in the present study, 1 μM tamoxifen was added to the mTALP medium containing motile spermatozoa, and after additional incubation for 5 min, 1 nM melatonin was added to the same medium. After supplementation with 1 nM melatonin, spermatozoa were incubated for 4 h. As shown in Figs. 3A and 3B, 1 μM tamoxifen did not affect the percentages of motile spermatozoa, hyperactivated spermatozoa and melatonin-enhanced hyperactivated spermatozoa.
After swim up, 1 μM tamoxifen was added to the mTALP medium containing motile spermatozoa, and after additional incubation for 5 min, 20 ng/ml 17βE₂ was added to the same medium. After additional incubation for 5 min again, 1 nM melatonin was added to the same medium. Data are expressed as the mean ± SD. (A, B) (Control): mTALP medium with 0.1% (v/v) EtOH and 0.1% (v/v) DMSO; (Melatonin): mTALP medium with addition of 1 nM melatonin, 0.1% (v/v) EtOH and 0.1% (v/v) DMSO; (Tamoxifen → Melatonin): mTALP medium with addition of 1 nM melatonin, 1 μM tamoxifen, 0.1% (v/v) EtOH and 0.1% (v/v) DMSO; (17βE₂ → Melatonin): mTALP medium with addition of 1 nM melatonin, 20 ng/ml 17βE₂, 0.2% (v/v) EtOH and 0.1% (v/v) DMSO; (Tamoxifen → 17βE₂ → Melatonin): mTALP medium with addition of 1 nM melatonin, 20 ng/ml 17βE₂, 1 μM tamoxifen, 0.2% (v/v) EtOH and 0.1% (v/v) DMSO. * Significant difference compared with “Control” (P < 0.05). ** Significant difference compared with “Control” and “17βE₂ → Melatonin” (P < 0.05). DMSO, dimethyl sulfoxide; 17βE₂, 17β-estradiol; EtOH, ethanol; mTALP medium, modified Tyrode’s albumin lactate pyruvate medium.

Non-genomic suppression of melatonin-enhanced hyperactivation by 17βE₂

Because 17βE₂ suppresses progesterone-enhanced hyperactivation through non-genomic regulation via the membrane ER [8], we examined whether melatonin-enhanced hyperactivation was also suppressed by 17βE₂ through non-genomic regulation using BSA-17βE₂, which binds only to the membrane ER and not to the intracellular ER, thereby blocking entry of BSA-17βE₂ into the cell [32, 33].

After swim up, 1 μM tamoxifen was added to the mTALP medium containing motile spermatozoa, and after incubation for 5 min, 7.4 nM BSA-17βE₂, which is converted into approximately 20 ng/ml 17βE₂, was added to the medium. After additional incubation for 5 min again, 1 nM melatonin was added to the medium. After supplementation with 1 nM melatonin, spermatozoa were incubated for 4 h. As shown in Fig.
INTERACTION OF MELATONIN AND 17βE₂

Dose-dependent effect of 17βE₂ on melatonin-enhanced hyperactivation

In the next step, we examined whether 17βE₂ suppressed melatonin-enhanced hyperactivation in a dose-dependent manner (Fig. 5) because our previous studies [8, 10] reported that progesterone-enhanced hyperactivation was dose dependently suppressed by 17βE₂.

Firstly, we observed sperm movement which were beforehand exposed to estrogen (Figs. 5A and 5B). After swim up, 2 pg/ml to 20 ng/ml 17βE₂ were added to the mTALP medium containing motile spermatozoa, and after additional incubation for 5 min, 1 nM melatonin was added to the medium. After supplementation with 1 nM melatonin, spermatozoa were incubated for 4 h. As shown in Fig. 5A, 17βE₂ did not affect the percentage of motile spermatozoa at the concentration tested. By contrast, 17βE₂ significantly suppressed melatonin-enhanced hyperactivation in a dose-dependent manner (Fig. 5B). After incubation for 1 h, 200 pg/ml to 20 ng/ml 17βE₂ significantly suppressed melatonin-enhanced hyperactivation (Melatonin: 21.38% ± 3.49, 200 pg/ml 17βE₂ → Melatonin: 48.66% ± 20.79), whereas 2 pg/ml to 20 pg/ml 17βE₂ did not. Moreover, the effect of 20 pg/ml 17βE₂ was also not significantly different compared with the control and melatonin (Control: 4.48% ± 4.08, Melatonin: 21.38% ± 3.49, 20 pg/ml 17βE₂ → Melatonin: 13.85% ± 6.59). After incubation for 1.5 h, 20 ng/ml 17βE₂ significantly suppressed melatonin-enhanced hyperactivation (Melatonin: 83.34% ± 2.74, 20 ng/ml 17βE₂ → Melatonin: 48.66% ± 20.79), whereas 2 pg/ml to 20 pg/ml 17βE₂ did not. The effects of 200 pg/ml and 2 ng/ml 17βE₂ were not significantly different compared with the control and melatonin (Control: 45.95% ± 23.47, Melatonin: 83.34% ± 2.74, 200 pg/ml 17βE₂ → Melatonin: 58.85% ± 14.95, 2 ng/ml 17βE₂ → Melatonin: 52.13% ± 18.52). After incubation for 2 h, 17βE₂ did not suppress melatonin-enhanced hyperactivation.

In the next step, hamster spermatozoa were exposed to the mixture of 1 nM melatonin and 2 pg/ml to 20 ng/ml 17βE₂ after swim up and incubated for 4 h in order to mimic the physiological action of estrogen in the spermatozoa (Figs. 5C and 5D). As shown in Fig. 5C, 17βE₂ did not affect the percentage of motile spermatozoa. By contrast, 17βE₂ suppressed melatonin-enhanced hyperactivation in a dose-dependent manner (Fig. 5D). After incubation for 1 h, melatonin slightly enhanced hyperactivation, but the enhancement of hyperactivation caused by melatonin was not significantly different compared with the control (Control: 9.65% ± 2.84, Melatonin: 25.87% ± 8.86). Moreover, 20 pg/ml to 20 ng/ml 17βE₂ slightly suppressed the effect of melatonin, but the level of hyperactivation was not significantly different compared with that with melatonin only (Melatonin: 25.87% ± 8.86, Melatonin + 20 pg/ml 17βE₂: 18.47% ± 11.8, Melatonin + 200 pg/ml 17βE₂: 13.99% ± 7.39, Melatonin + 2 ng/ml 17βE₂: 12.73% ± 3.57, Melatonin + 20 ng/ml 17βE₂: 10.99% ± 6.28). After incubation for 1.5 h, 2 ng/ml and 20 ng/ml 17βE₂ significantly suppressed melatonin-enhanced hyperactivation (Melatonin: 65.55% ± 2.98, Melatonin + 2 ng/ml 17βE₂: 36.68% ± 9.41, Melatonin + 20 ng/ml 17βE₂: 39.35% ± 7.56), whereas 2 pg/ml to 200 pg/ml 17βE₂ did not. After incubation for 2 h, 17βE₂ did not suppress melatonin-enhanced hyperactivation.
Fig. 5. Dose-dependent effects of 17βE₂ on melatonin-enhanced hyperactivation. The percentages of motile (A, C, E) and hyperactivated (B, D, F) spermatozoa are shown (A, B) for when spermatozoa were exposed to melatonin after exposure to 17βE₂, (C, D) for when spermatozoa were simultaneously exposed to melatonin and 17βE₂, and (E, F) for when spermatozoa were exposed to melatonin before exposure to 17βE₂. In A and B, after swim up, 17βE₂ or vehicle was added to the mTALP medium containing motile spermatozoa. After incubation for 5 min, melatonin or vehicle was added to the mTALP medium containing motile spermatozoa. In C and D, after swim up, mixtures of melatonin and 17βE₂ or vehicle were added to the mTALP medium containing motile spermatozoa. After incubation for 5 min, 17βE₂ or vehicle was added to the mTALP medium containing motile spermatozoa. Data are expressed as the mean ± SD. (Control): mTALP medium with 0.2% (v/v) EtOH; (Melatonin): mTALP medium with added 1 nM melatonin and 0.2% (v/v) EtOH; (20 ng/ml 17βE₂ → Melatonin, Melatonin + 20 ng/ml 17βE₂): mTALP medium with addition of 1 nM melatonin, 20 ng/ml 17βE₂ and 0.2% (v/v) EtOH; (2 ng/ml 17βE₂ → Melatonin, Melatonin + 2 ng/ml 17βE₂): mTALP medium with addition of 1 nM melatonin, 2 ng/ml 17βE₂ and 0.2% (v/v) EtOH; (200 pg/ml 17βE₂ → Melatonin, Melatonin + 200 pg/ml 17βE₂): mTALP medium with addition of 1 nM melatonin, 200 pg/ml 17βE₂ and 0.2% (v/v) EtOH; (20 pg/ml 17βE₂ → Melatonin, Melatonin + 20 pg/ml 17βE₂): mTALP medium with addition of 1 nM melatonin, 20 pg/ml 17βE₂ and 0.2% (v/v) EtOH; (2 pg/ml 17βE₂ → Melatonin, Melatonin + 2 pg/ml 17βE₂): mTALP medium with addition of 1 nM melatonin, 2 pg/ml 17βE₂ and 0.2% (v/v) EtOH. *Significant difference compared with “Control” (P < 0.05). † Significant difference compared with “Melatonin” (P < 0.05). Items with significant differences are indicated by symbols in the same color. 17βE₂, 17β-estradiol; EtOH, ethanol; mTALP medium, modified Tyrode’s albumin lactate pyruvate medium.
Finally, we observed sperm movement which were beforehand exposed to melatonin (Figs. 5E and 5F). Motile spermatozoa were exposed to 2 pg/ml to 20 ng/ml 17βE2 after exposure to 1 nM melatonin for 5 min. After addition of 17βE2 to the mTALP medium, spermatozoa were incubated for 4 h. As shown in Figs. 5E and 5F, 2 pg/ml to 20 ng/ml 17βE2 did not affect the percentages of motile spermatozoa, hyperactivated spermatozoa and melatonin-enhanced hyperactivation.

Discussion

Under capacitation conditions, sperm hyperactivation spontaneously occurs in vivo and in vitro [1, 3, 34–36]. Spontaneous hyperactivation time-dependently occurs during capacitation processes [1, 3, 34–36]. Recent studies using human and hamster spermatozoa have shown that hyperactivation is enhanced by progesterone, melatonin and serotonin [5–7, 11–13]. Moreover, it has been also shown that progesterone-enhanced hyperactivation of hamster spermatozoa is suppressed by 17βE2 and GABA [8, 10, 18]. Steroids of these hormones, such as progesterone and 17βE2, regulate sperm hyperactivation via non-genomic regulation [2, 7, 8, 37]. In genomic regulation, generally, steroids bind to an intracellular receptor and induce gene expression, whereas in non-genomic regulation, the steroids bind to a membrane receptor and increase the concentration of a second messenger such as Ca2+ and/or cAMP [7, 9, 37]. In order to examine whether the regulatory effects of steroids are non-genomic, a BSA-conjugated steroid was used in previous studies [7, 8, 18] and in the present study (Fig. 4). Because BSA blocks entry of a BSA-conjugated steroid into the cell, the steroid is unable to bind to the intracellular receptor but can bind to the membrane receptor [32, 33]. Therefore, it follows that the effects of a BSA-conjugated steroid will occur through non-genomic regulation. The results obtained from the present study (Fig. 4) suggest that enhancement of hyperactivation by melatonin was suppressed by 17βE2 through non-genomic regulation via a membrane ER.

Progesterone regulates hyperactivation through non-genomic regulation associated with two types of Ca2+ signaling: Ca2+ influx and release of Ca2+ from the Ca2+ store [2, 7, 36–40]. Ca2+ influx is induced by progesterone through the Catsper, which is a sperm-specific Ca2+ channel located in the principal piece of the flagellum [41, 42]. The release of Ca2+ from the Ca2+ store by progesterone is associated with both the PR and PLC [7]. Activation of PLC produces IP3 and diacylglycerol (DAG) from phosphatidylinoline and/or phosphatidylinositol. IP3 releases Ca2+ from the IP3-R-gated Ca2+ store localized at the base of flagellum [36–40]. Ca2+ and DAG regulate hyperactivation through activation of calmodulin-dependent protein kinase II and PKC [9, 43]. After Ca2+ signaling is stimulated by progesterone, many tyrosine phosphorylations, especially the 80- and 85-kDa tyrosine phosphorylations of the fibrous sheath (FS), are increased and enhanced [7, 8, 36]. The 80- and 85-kDa tyrosine phosphorylated FS proteins were identified as the A-kinase anchoring protein, which is a major component of the FS [44]. In general, tyrosine phosphorylation is a very important event during capacitation/hyperactivation [1, 2, 45–47]. It has been suggested that the 80- and 85-kDa tyrosine phosphorylations of the FS are closely associated with capacitation/hyperactivation [19, 46, 48]; and regulated by Ca2+/calmodulin-dependent signals [49] and protein phosphatase 1 [50]. In non-genomic regulation, progesterone also activates adenylate cyclase to increase the cAMP concentration [37, 51, 52]. CAMP is an essential molecule for hyperactivation; and regulates the tyrosine phosphorylations, especially the 80- and 85-kDa tyrosine phosphorylations of the FS, through protein kinase A (PKA) signals [1, 45, 47]. Moreover, progesterone enhances hyperactivation through cAMP–PKA signals [9]. In contrast, 17βE2 suppresses progesterone-enhanced hyperactivation through non-genomic regulation associated with the ER and tyrosine dephosphorylations except for the 80- and 85-kDa tyrosine phosphorylations of the FS [8]. GABA also suppresses progesterone-enhanced hyperactivation via the GABA_A receptor [18]. However, the regulatory mechanisms associated with suppression of progesterone-enhanced hyperactivation by 17βE2 and GABA are still unclear.

It has been shown that melatonin enhances hyperactivation of hamster spermatozoa via MT1 [11]. Moreover, in ram and human spermatozoa, it has been shown that melatonin increases sperm quality, motility, capacitation, fertility rate and the activities of antioxidant enzymes, and decreases nitric oxide (NO) [12, 53–56]. NO at low concentrations increases capacitation through a mitogen-activated protein kinase cascade and tyrosine phosphorylation, especially the 80- and 85-kDa tyrosine phosphorylations of the FS [57–59], whereas at high concentrations, it negatively affects sperm function [58, 60].

It has been shown that progesterone and 17βE2 bind to the head of hamster spermatozoa [7, 8], but it has not been shown where melatonin binds [11]. Ram spermatozoa have two melatonin receptors (MT1 and MT2), which are localized at various sites [61]. In humans, the MT1 receptor is located in the equatorial region of the sperm head [62]. In the present study, we did not show the regulatory mechanism of sperm hyperactivation caused by melatonin and 17βE2. One of the possibilities is the suppression of binding of melatonin to the melatonin receptor by 17βE2. Another is the suppression of intracellular melatonin signals by 17βE2. In a study investigating the regulation of sperm hyperactivation caused by progesterone and 17βE2, neither steroid interfered with binding to their respective receptors [8]. In contrast, one of the intracellular progesterone signal, such as tyrosine phosphorylation, was suppressed by 17βE2 [8]. We expect that 17βE2 affects intracellular melatonin signals in a manner similar to the regulatory mechanism of progesterone and 17βE2 because we previously noted that melatonin enhanced sperm hyperactivation through enhancement of tyrosine phosphorylations [11]. In future studies, we will show the detailed interaction between melatonin and 17βE2.

Progesterone enhances hamster sperm hyperactivation in a dose-dependent manner [7], and progesterone-enhanced hyperactivation is dose-dependently suppressed by 17βE2 and GABA [8, 10, 18]. The concentrations of progesterone and 17βE2 in blood and tissues fluctuate in association with the estrous or menstrual cycle [63]. The concentration of GABA in the female genital tract also fluctuates in association with the estrous cycle [64]. Therefore, we previously proposed that hamster sperm hyperactivation was regulated by balances among the concentrations of progesterone, 17βE2 and GABA [8, 10, 18]. Additionally, picomole or higher concentrations of melatonin enhance hamster sperm hyperactivation [11]. It was reported that picomole or higher concentrations of melatonin were.
detected from the follicular fluid of humans [65, 66]. Based on these reports, we tried to examine the interactions among melatonin, 17βE2 and GABA; in the present study and found that 17βE2 suppressed melatonin-enhanced hyperactivation in a dose-dependent manner, whereas GABA did not (Figs. 1, 4 and 5). 17βE2 suppressed melatonin-enhanced hyperactivation through non-genomic regulation via the ER (Figs. 3 and 4). In another recent study [13], it was reported that serotonin also enhanced hamster sperm hyperactivation. These results and results of previous studies [7, 8, 10, 11, 13, 18] suggest that in the hamster, progesterone, melatonin and serotonin act as enhancers of hyperactivation; and that 17βE2 and GABA act as suppressors. Although we did not investigate relationships among the regulation by progesterone, 17βE2 and GABA, the regulation by melatonin and 17βE2 and the regulation by serotonin, we propose that proteins in the follicular fluid, such as progesterone, melatonin, serotonin, 17βE2 and GABA, regulate hyperactivation of hamster spermatozoa.

It has been suggested that spermatozoa are capacitated/hyperactivated in response to changes in the environment of the oviduct [7, 8, 10, 11, 13, 18, 35]. During capacitation/hyperactivation of hamster spermatozoa, progesterone, melatonin and serotonin act as enhancers and 17βE2, and GABA acts as a suppressor [7, 8, 10, 11, 13, 18]. Although GABA acts as a suppressor of hamster spermatozoa [18], it is an enhancer of other mammalian spermatozoa such as those of humans, rams and rats [14–17]. Because the concentrations of hormones vary during the female’s estrous cycle [63], it seems that mammalian spermatozoa, at least hamster spermatozoa, are capacitated/hyperactivated according to changing concentrations of melatonin and 17βE2 in the oviduct.

There are two regulatory mechanisms of hyperactivation in mammalian or at least hamster spermatozoa. One is a spontaneous regulatory mechanism associated with progesterone, 17βE2, melatonin, serotonin and GABA [7–11, 13, 18] (Fig. 1). In both mechanisms, Ca2+ signals, cAMP–PKA signals and tyrosine phosphorylations are very important [1–3, 7–9, 19, 45, 47]. However, the ligand-dependent regulatory mechanism differs from the spontaneous regulatory mechanism because hyperactivation itself is not suppressed by inhibition of the ligand-dependent regulatory mechanism [7–9, 11, 13, 18] (Fig. 1).

In conclusion, we propose that spermatozoa start to be capacitated/hyperactivated through the spontaneous regulatory mechanism in the oviduct. After that, it seems that capacitation/hyperactivation of spermatozoa is enhanced through a modulatory mechanism associated with changes in the environment of the oviduct.

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