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Alteration of Acrosome Reacting Ability of Mouse Spermatozoa during Epididymal Transit

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생쥐 정자의 부정소 통과 과정 중 첨체반응 능력 변화

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요 약

생쥐의 부정소에서 진행되는 정자 성숙과정 동안의 첨체반응 능력의 변화를 조사하였다. 자발적 첨체반응 및 난포액, 프로게스테론, 또는 A23187에 의해 유발되는 첨체반응은 모두 정자의 성숙에 의존적으로 일어났다. 두부 부정소의 매우 적은 정자만이 난포액 및 프로게스테론에 반응하여 첨체반응을 일으켰으며 체부 및 미부 부정소간에 첨체반응율에 차이가 없었다. 반면 A23187 처리시 상당수의 두부 부정소 정자가 첨체반응을 진행하였다. 이러한 결과에서 두부 부정소를 거쳐 체부 부정소에 도달한 생쥐 정자는 첨체반응 능력을 획득하며 이 과정에서 정자의 원형질막 표면에서는 첨체반응을 유발하는 물질과 상호작용에 필요한 변화가 진행되는 것으로 사료된다. 반면 정자내로의 Ca^{2+} 유입 후 진행되는 막융합과 첨체내용물의 분비에 필요한 능력은 두부 부정소 정자도 일부 갖고 있는 것으로 사료된다.

Key words: Sperm, Acrosome reaction, Epididymis, Mouse.

INTRODUCTION

Mammalian testicular sperm is unable to fertilize the oocyte and it must undergo "maturation" during the epididymal transit (Pavlok, 1974; Cooper, 1986). Ejaculated sperm, however, still needs a final maturation process in the female genital tract, collectively described as sperm capacitation, to obtain the ability to react acrosome reaction (AR) (Austin, 1951; Chang, 1951). In mouse, capacitated sperm binds to zona pellucida, undergoes AR in a Ca^{2+}-dependent manner, penetrates the investments of oocyte, and eventually fuses with oolemma (Yanagimachi, 1994).

Extensive changes in physiological and biochemical properties of sperm occur during sperm maturation in epididymis. Sperm acquires foward motility in epididymis (Hoskins & Vijayarhaghavan, 1990). Antigen redistribution (Gye *et al.*, 1994), appearence of new antigens originated from epididymal fluid (Suzuki & Yanagimachi, 1986),

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modification of fertilization antigen (Lakoski *et al.*, 1988), and increase in protein mobility (Hammerstedt *et al.*, 1979) in plasma membrane are evident during this period. As a result, sperm surface antigens involved in the recognition and binding to egg investments matured (Saling, 1982; Olson & Winfrey, 1991) and sperm became able to react physiological acrosome reaction (Lakoski *et al.*, 1988; Klemm & Engel, 1991; Biegler *et al.*, 1994).

Acrosome reaction, Ca^{2+}-dependent exocytotic event is a final differentiation of spermatozoa and is regulated by unique cellular and environmental factors associated with either gametes or the reproductive tracts. A numbers of biomolecules present in female genital tracts act on sperm cell surface molecules which are coupled to effector and modify signaling pathways leading to fusion between plasma membrane and outer acrosomal membrane(OAM) and exocytosis of acrosomal content (Ward & Kopf, 1993). Undoubtedly, acrosome reaction is essential for fertilization and it is important to elucidate precise nature of development of acrosome reacting ability of epididymal sperm to understand fertilization in mammals. Acrosome reaction of sperm consists of two phases. The early events include interactions between sperm surface receptor(s) and extracellular ligands and subsequent cell signaling leading to Ca^{2+} influx. Although many of biological molecules were known to induce AR in mammalian sperm (reviewed in Meizel, 1985), it has not yet been elucidated whether the properties of sperm surface to interact with AR inducers were altered during sperm maturation. The late events of AR are Ca^{2+}-dependent intracellular signaling culminating to fusion between the plasma membrane and outer acrosomal membrane and release of acrosomal contents. However, it has not yet been elucidated whether immature sperm lacks an ability to undergo an membrane fusion after the influx of extracellular Ca^{2+}. Therefore development of acrosome reacting ability of sperm during the epididymal transit must be descriminated according to different phases of AR mentioned above. Mouse sperm is good model for study of AR because AR can be easily measured by staining with Coomassie dye (Moller *et al.*, 1990) after exposure of sperm to various biological molecules or chemicals *in vitro*.

Aim of this study was to examine the development of AR ability of sperm during epididymal transit. We examined acrosome reacting ability of sperm from different region of epididymis after treatment with ligands or drug acting at two different phases of AR. Follicular fluid was konwn to be a rich source of AR inducers (Meizel, 1985) including acrosome reaction inducing substance (ARIS) (Miska *et al.*, 1994), progesterone (Foresta *et al.*, 1993), lipid transfer protein (Ravnik *et al.*, 1992), atrial natriuretic peptide (Zamir *et al.*, 1995), and glycosaminoglycans (Lenz *et al.*, 1983) which commonly exerted their effects through cell surface receptors (Shi & Roldan, 1995). Therefore follicular fluid and progesterone were employed to induce AR and evaluate ability of epididymal sperm to interact with AR inducing ligands. In contrast, Ca^{2+} ionophore A23187 which mobilizes intracellular Ca^{2+} and induces Ca^{2+} influx in mammalian sperm (Babcock *et al.*, 1976) was used to evaluate sperm ability to undergo AR after Ca^{2+} influx.

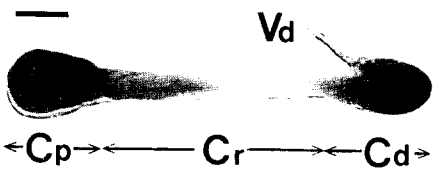

Fig. 1. Microphotograph of mouse epididymis.
Cp, caput epididymis; Cr, corpus epididymis; Cd, cauda epididymis; Vd, vas deferens. Bar = 3 mm.

MATERIALS AND METHODS

1. Sperm preparation

Epididymis was removed from 3 month-old male mice (ICR strain). Blood was cleared from tissues by blotting to filter paper and organ was further dissected into three parts as shown in Fig. 1. Caput, corpus, and cauda region of epididymis were placed in the modified Tyrode solution (Parrish *et al.*, 1988), and tissues were squeezed with forcep to expell their luminal contents. Sperm were collected after 10 min and sperm concentration was adjusted to 2×10^6 sperm/ml with fresh medium. One ml of aliqouts of sperm suspensions were placed in the 4-well dish (Nunc) and preincubated in 5 % CO_2 and 95 % air at 37℃. After 120 min, Ca^{2+}-ionophore A23187 (10 μM in 0.1 % DMSO), progesterone (10 μM in 0.1 % DMSO), or human follicular fluid (10 %, v/v) were added and incubated for further 60 min to induce AR. Follicular fluid was collected from human preovulatory follicles during *in vitro* fertilization program and prescreened their ability to induce AR (Gye & Kim, 1996).

2. Evaluation of the acrosome reaction

Method described in Moller *et al.* (1990) was employed to evaluate AR. After incubation, sperm suspension was fixed with 5 % formaldehyde in phosphate buffered saline (PBS) for 30 min and centrifuged at 5,000 × g for 5 min. Sperm pellet was washed with PBS (pH 7.4) twice and 50 μl of sperm suspension was dropped to slide and air dried. After staining with 0.05 % Coomassie brilliant blue R250 (Bio-rad) for 2 min, slides were washed twice with PBS, mounted with mixture of PBS and glycerol (1:1), and observed under phase contrast microscope. Sperm without blue staining or punctuate pattern of staining on the convex ridge of sperm head was regarded as acrosome-reacted. At least 200 sperm were evaluated per slide and proportions of AR sperm from 4 batches. Differences in the percentage of acrosome-reacted sperm among different treatment groups were statistically analyzed using the PC STAT software. Maturation dependency of AR was expressed as maturation (MA) index (net increase in % AR of cauda sperm/net increase in % AR of caput sperm).

RESULTS

1. Two types of AR patterns were observed

Acrosomal cap of sperm incubated in the presence or absence of various AR inducing substances was different in the Coomassie staining. Acrosome-intact sperm is characterized by smooth surface and intense staining of acrosomal cap. On the other hand acrosome-reacted sperm showed no staining in the acrosomal cap (Fig. 2) due to an complete loss of an acrosomal matrix.

Fig. 2. Microphotograph of mouse sperm stained with Coomassie brilliant blue R250.

Cauda epididymal sperm was stained after incubation in modified Tyrode solution. Sperm with intact acrosome (arrowheads) and acrosome-reacted sperm free from stain in the convex ridge of sperm head (arrows) are seen. Bar = 5 μm.

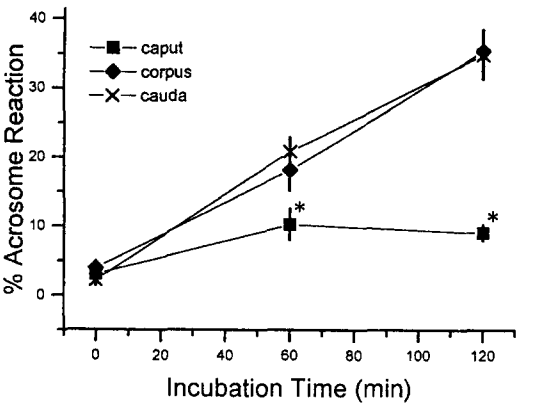

Fig. 3. Spontaneous acrosome reaction of sperm from different regions of epididymis.

Sperm from different regions of epididymis were incubated in modified Tyrode solution for 120 min and acrosome reacted sperm was monitored at 0, 60, and 120 min after incubation.

Error bars are SD (n=4). *, significantly ($p<0.05$) different from % AR of caput sperm.

2. Spontaneous acrosome reaction

Proportion of acrosome reacted sperm in the unincubated sperm suspension was not different among the sperm from different portions of epididymis and was not exceeded 5 %. There was no difference in the AR according the the portions of epididymis. Spontaneous AR of sperm incubated for 120 min was 6.1 % in caput, 23.9 % in corpus, and 28.1 % in cauda epididymal sperm. When the incubation was prolonged for 180 min % AR was 9.1 % in caput, 35.4 % in corpus, and 34.9 % in cauda epididymal sperm (Fig. 3). MA index of spontaneous AR for 3 hr was 3.8.

3. Follicular fluid-induced acrosome reaction

Proportions of acrosome reacted sperm incubated in the presence of hFF for 60 min was 10.4 % in caput, 44.6 % in corpus, and 45.7 % in cauda epididymal sperm (Table 1). Increases of AR due to hFF treatment (% AR of hFF treated groups - % AR of not treated control) were 1.3 % in caput, 9.2 % in corpus, and 10.8 % in cauda epididymal sperm. MA index of follicular fluid-induced AR was 8.3.

4. Progesterone(P_4)-induced acrosome reaction

Proportions of acrosome reacted sperm incubated in the presence of 10 μM P_4 for 60 min were 9.7 % in caput, 46.1 % in corpus, and 48.1 % in cauda epididymal sperm (Tabel 1). Increase of AR due to P_4 treatment (% AR of P_4 treated groups - % AR of 0.1 % DMSO treated group) were 0.5 % in caput, 11.7 % in corpus, and 15.8 % in cauda epididymal sperm. MA index of

Table 1. Follicular fluid-, progesterone-, or A23197-induced acrosome reaction of sperm recovered from different region of epididymis

Region of epididymis	Treatments				
	Non	hFF	DMSO	Progesterone	A23187
Caput	9.1±1.1	10.4±0.8	9.2±1.9	9.7±2.3[c]	29.5±1.1[c]
Corpus	35.4±1.6[a]	44.6±1.9[a, b]	34.4±2.2[a]	46.1±2.2[a, c]	76.1±5.6[a, c]
Cauda	34.9±3.5[a]	45.7±3.8[a, b]	32.3±1.89[a]	48.1±1.3[a, c]	85.1±2.3[a, c]
MA index	3.8	8.3	–	31.6	2.6

Sperm collected from different region of epididymis were preincubated for 120 min. hFF (10 %, v/v), progesterone (10 μM in 0.1% DMSO), or A23187 (10 μM in 0.1% DMSO) was added and incubated for 60 min. Values are % mean ± SD (n=4).

[a] significantly ($p<0.001$) different from % AR of caput sperm.

[b] significantly ($p<0.01$) different from % AR of untreated sperm.

[c] significantly ($p<0.01$) different from % AR of DMSO treated sperm.

MA index = net increase in % AR of cauda sperm/net increase in % AR of caput sperm.

progesterone-induced AR was 31.6.

5. A23187-induced acrosome reaction

Most of A23187-induced acrosome reacted sperm were cleared from staining in the convex ridge of sperm head. Proportion of acrosome reacted sperm incubated in the presence of 10 μM A23187 for 60 min was 29.5 % in caput, 76.1 % in corpus, and 85.1 % in cauda epididymal sperm (Table 1). Increases of AR due to A23187 treatment (% AR of A23187 treated groups - % AR of 0.1 % DMSO treated group) were 20.3% in caput, 41.7 % in corpus, and 53.8 % in cauda epididymal sperm. MA index of A23187-induced AR was 2.6.

DISCUSSION

The ability of sperm to undergo AR was dependent on both epididymal maturation and incubation time. MA index was 3.8 in spontaneous AR, 8.3 in follicular fluid-induced AR, 31.6 in P_4-induced AR, and 2.3 in A23187 induced AR. It suggested that maturation dependency of spontaneous AR and ionophore-induced AR was lower than that of ligand-induced AR. Only a small portion of caput epididymal sperm responded to ligands examined here. However some extent of caput epididymal sperm underwent AR when the sperm was preincubated for 120 min and exposed to A23187. It suggested that development of acrosome reacting ability is highly dependent on sperm's ability to interact with AR inducing ligands. In addition, maturation of intracellular machineries responsible for signaling of Ca^{2+} influx and membrane apparatus for fusion might be implicated in the development of acrosome reacting ability. There was no large difference in AR between corpus and cauda epididymal sperm. Main function of cauda epididymis was referred to store the mature sperm (Waites *et al.*, 1980). It suggested that most of sperm matured during their transit through the proximal portion of epididymis and there was no remarkable change in the AR ability during the sperm residence in the cauda epididymis in compared to that in caput and corpus epididymis. Difference in MA index between ligand- or Ca^{2+} ionophore-induced AR among the sperm from different portions of epididymis reflects differential

maturation of acrosome reacting ability in response to AR inducing substances and rise in $[Ca^{2+}]_i$.

How the sperm acquires the acrosome reacting ability during epididymal transit? There are several explanations for changes in AR ability during post-testicular sperm maturation.

First, changes in distribution of sperm surface antigens interacting with AR ligands is possibly responsible for this phenomenon. Distribution of glycoconjugates and appearance of new antigens from epididymal fluid are evident during sperm transit through epididymis (Hall & Killian, 1987; Gye *et al.*, 1994). Fluid mosaic nature of sperm plasma membrane makes lateral movement of membrane proteins possible to occur (Flechon, 1985). Gamete interaction leading to AR is believed to occur with ligand-receptor binding manner, and apposition of sperm's receptor to extracellular AR ligands as well as ripening of complementarity between interacting molecules is important for this event. Changes in distribution of antigen responsible for interaction with AR ligands during epididymal transit possibly make sperm prone to undergo AR.

Second, changes in antigenicity of sperm antigens is possibly responsible for this phenomenon. There are increasing bodies of evidences that activities of glycosidase (Hall & Killian, 1987; Skudlarek *et al.*, 1991; Tulsiani *et al.*, 1995) and glycosyltransferase (Ram *et al.*, 1989; Miller *et al.*, 1992; Cooke & Shur, 1994) in sperm surface or luminal fluid of epididymis are different along the length of epididymis. In addition, partial cleavage of peptide portion of antigens might increase fitness of sperm surface antigen to AR inducing ligands.

Third, masking of sperm surface antigens which block the interaction of sperm antigen with AR inducing ligands is the possible cause of inability of caput sperm to undergo AR (Biegler *et al.*, 1994).

Forth, intracellular machineries for downstream events of increase in $[Ca^{2+}]_i$ which culminating to membrane fusion between PM and OAM might be immature in caput epididymal sperm. Acrosome reaction is Ca^{2+}-dependent exocytotic process and a numbers of biomolecules present in female genital tracts can interact with sperm cell surface molecules which are coupled intracellular signaling leading to Ca^{2+} influx, membrane fusion, and exocytosis of acrosomal content. However it seems minor for development of acrosome reacting ability because Ca^{2+} ionophore-induced AR was less dependent on epididymal maturation than that of ligand-induced AR. Maturational changes in other features such as lipid composition of sperm plasma membrane (Suzuki, 1990), activity of enzymes involved in their turnover (Wolf *et al.*, 1988), and organization of membrane fusion apparatus (Beaver & Friend, 1990) partly explain difference in acrosome reacting ability of sperm in different regions of epididymis.

In summary the ability of sperm to undergo AR develops during sperm transit through caput to corpus epididymis. It accompanied development of ability to interact with AR inducing ligands which is responsible for early event of AR leading to Ca^{2+} influx. On the other hand, it seems likely that intracellular signaling machineries after Ca^{2+} influx culminating to membrane fusion between plasma membrane and outer acrosomal membrane start to develop during sperm residence in proximal portion of epididymis. Further studies on the biochemical

changes of sperm during epididymal sperm maturation are needed.

ABSTRACTS

In order to define the changes in acrosome reacting ability of spermatozoa during epididymal transit, known acrosome reaction inducers were challenged to mouse sperm collected from different regions of epididymis, and acrosome reaction (AR) was monitored. Spontaneous, follicular fluid-induced, progesterone (P_4)-induced and A-23187-induced AR were highly dependent on epididymal maturation. Only a small portion of caput epididymal sperm underwent AR in response to ligands examined. There was no obvious difference in AR between corpus and cauda epididymal sperm. However, some extent of caput epididymal sperm responded to A23187 and underwent AR. These results suggested that the ability of sperm to undergo AR develops during epididymal transit. Being able to interact with AR inducing ligands leading to Ca^{2+} influx was the most obvious feature of changes in acrosome reacting ability of sperm transit from caput to corpus epididymis. On the other hand, it seems likely that intracellular machineries for late events of AR after Ca^{2+} influx, such as fusion between plasma membrane and outer acrosomal membrane, start to develop in sperm residing in testis or proximal region of epididymis.

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