

Receptors in Spermatozoa: Are They Real?

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Sperm develop in the seminiferous tubules of testes following a complex developmental process called spermatogenesis. A series of morphologic remodeling occurs during spermiogenesis before the sperm are released into the lumen of the tubules by spermiation (Eddy and O'Brien, 1994). Mammalian testicular spermatozoa are relatively nonmotile and incapable of fertilizing an ovum. In most species, sperm attain progressive motility and fertilizing capacity during their transit through various regions of the epididymis. Changes in the sperm membrane and cytoplasm, by addition and deletion of several proteins and molecules, occur during the epididymal transit (Moore, 1990). During ejaculation, sperm are exposed to various components of the seminal plasma that adhere to the sperm membrane. The ejaculated sperm are incapable of fertilizing an oocyte. They must undergo physiologic changes that are collectively called capacitation, leading to acrosomal exocytosis during the transit through the female genital tract (Yanagimachi, 1994). One of the major steps besides tyrosine phosphorylation during capacitation is the removal of seminal plasma-adsorbed proteins from the sperm surface (Naz and Rajesh, 2004). These changes are a prerequisite for successful fertilization. It is generally believed that these processes are regulated by several hormones and cytokines/growth

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factors present in seminal plasma and the female genital tract. The receptors for these hormones and cytokines/growth factors have been reported to be present on the sperm membrane, and the list is ever growing. The sperm are transcriptionally and translationally quiescent, and they can be successfully capacitated in vitro without any hormones and cytokines/growth factors. In lieu of these findings, the physiologic significance of several of these receptors seems enigmatic. The present article will review the receptors for well-known hormones, growth factors, and neurotransmitters that have been reported in the literature, and their authenticity and physiologic relevance will be discussed. The cognate proteins on the sperm surface that have been delineated by monoclonal and polyclonal antibodies (at least more than 100) and have been implicated in various sperm functions, especially oocyte zona pellucida binding, have been excluded from this review (Anderson et al, 1987; Eddy and O'Brien, 1994; Naz et al, 2005). Only receptors that are well known and have a definite role in other cell types are included.

Results and Discussion

Hormone Receptors—Various hormone receptors which have been delineated in sperm are summarized in Table 1.

Gonadotropin-Releasing Hormone Receptor. Gonadotropin-releasing hormone (GnRH) is a decapeptide released from the hypothalamus which binds to membrane-bound receptors in the anterior pituitary for secretion of gonadotropins (follicle-stimulating hormone [FSH] and luteinizing hormone [LH]) (Clayton, 1989). GnRH-R cDNA has been cloned from mouse, rat, and human tissues (Tsutsumi et al, 1992; Chi et al, 1993; Perrin et al, 1993). The cognate GnRH-R has a molecular mass of approximately 50 to 60 kd. Besides anterior pituitary, GnRH-R is present in other tissues. GnRH-R of 45 kd has also been reported in ejaculated human sperm (Lee et al, 2000). Incubation of human sperm with GnRH increases sperm binding with human oocyte zona pellucida in the hemizona assay (Morales, 1998). GnRH antagonist completely inhibits the enhancing effect. There is no effect of GnRH on sperm capacitation and/or acrosomal exocytosis. It is hypothesized that increased sperm binding to oocytes is due to exposure and/or change in affinity of zona receptors on sperm membranes.

Table 1. *Hormone receptors present in sperm*

Receptor	Size (kd)		Sperm subcellular localization	Localized by	Function	Reference
	Other cells	Sperm				
GnRH*	38	45	Acrosome	Immunohistochemistry, Western blot	Sperm-zona binding	Lee et al, 2000
LH/hCG*	78	80	Head, midpiece, tail	Immunocytochemistry	Sperm motility	Eblen et al, 2001
Estrogen α	66	45, 66	Equatorial segment	Immunofluorescence labeling, confocal laser scanning, Western blot	Capacitation, acrosomal exocytosis, fertilizing ability	Solakidi et al, 2005
Estrogen β	60	64	Midpiece, mitochondria	Immunofluorescence labeling, confocal laser scanning, Western blot	...	Solakidi et al, 2005
Androgen	98	110, 90	Midpiece	Immunofluorescence labeling, confocal laser scanning, Western blot	Sperm motility, fertilizing ability	Solakidi et al, 2005
Progesterone	99	54, 65; 28, 57	Acrosome	Western blot	Capacitation, acrosomal exocytosis, chemoattractant	Jacob et al, 1998; Luconi et al, 1998; Wu et al, 2005
Leptin	131	145	Tail	Western blot	...	Jope, 2003
Calcitonin	60	...	Acrosomal cap, midpiece, tail	Fluorescence microscopy, immunofluorescence	Capacitation	Adeoya-Osiguwa and Fraser, 2003

* GnRH indicates gonadotropin-releasing hormone; LH/hCG, luteinizing hormone/human chorionic gonadotropin.

Luteinizing hormone/human chorionic gonadotropin receptor. LH is a glycoprotein secreted by the anterior pituitary. It has 2 polypeptides, α and β chains. The α chain is common among thyroid-stimulating hormone, FSH, LH, and human chorionic gonadotropin (hCG), and the β chain is specific to each hormone. The β chain of LH has more than 80% sequence homology with the β chain of hCG. hCG is primarily produced by the placenta. Apart from the anterior pituitary and placenta, small amounts of LH/hCG are also produced by other tissues, including the testis and fallopian tube (Chowdhury and Steinberger, 1979; Lei et al, 1993; Berger et al, 1994). Both LH and hCG interact with the same membrane receptors that are present in Leydig cells and other tissues. The functional LH/hCG hormone receptors (both mRNA and protein) are also present in head, midpiece, and tail regions of human ejaculated sperm (Eblen et al, 2001). The mRNA transcript was detected by reverse transcriptase-polymerase chain reaction (RT-PCR), and Western blot and radiolabeled ^{125}I -hCG binding delineated an 80-kd receptor protein. Addition of 100 ng/mL hCG or LH to human sperm activates protein kinase A (not protein kinase C) and increases intracellular cyclic adenosine monophosphate (cAMP) levels (Eblen et al, 2001). Interestingly, both males and females of the gene knockout mice of the LH/hCG receptor are infertile (Lei et al, 2001).

Estrogen Receptor. Estrogen is also produced in males and affects reproduction (Hess et al, 1997). In males, it is present in low concentrations in blood but is present at extraordinarily high levels in semen. Estrogen effects are mediated by 2 distinct nuclear receptors, estrogen receptor α (ER α) and ER β . The receptors are encoded by 2 different genes and are expressed in germ cells (O'Donnell et al, 2001). Besides the genomic effect mediated by the 2 nuclear receptors, estrogen also causes nongenomic effects mediated through membrane receptors. Rapid nongenomic effects of estrogen have been demonstrated in several cells (Ho and Liao, 2002) and are mediated by specific membrane receptors. Transfection studies of ER cDNA indicate that both membrane and nuclear receptors originate from a single transcript (Razandi et al, 1999).

An earlier study demonstrated the presence of estrogen binding sites on human spermatozoa (Cheng et al, 1981). Immunofluorescence labeling, confocal laser scanning microscopy, and Western blot analysis verified that ER α (45, 66 kd) is present in postacrosomal, midpiece and tail regions, and ER β (64 kd) in midpiece and tail regions and mitochondria of ejaculated human sperm (Durke et al, 1998; Aquila et al, 2004; Solakidi et al, 2005). Transcripts of both receptors are also present in ejaculated human sperm. Incubation of sperm with 17- β -estradiol affects capacitation, acrosomal exocytosis, and fertilizing ability (Adeoya-

Osigawa et al, 2003). Estrogen stimulates phosphorylation of proteins involved in the phosphatidylinositol-3-OH kinase/Akt pathway and stimulates ERK1/2, which is involved in sperm function (Luconi et al, 1999; Aquila et al, 2004; Luconi et al, 2004). ER α gene knockout male mice are infertile, have reduced mating behavior, and produce poor-quality sperm (Eddy et al, 1996). However, ER β knockout male mice are fertile and exhibit normal sexual behavior but have fewer and smaller litters (Krege et al, 1998).

Androgen Receptor. Androgens are steroid hormones that are important for development of secondary sex characteristics and initiation and maintenance of spermatogenesis. These are genomic effects, mediated through nuclear receptors (Lindzey et al, 1994). However, nongenomic effects of androgen have been described which are nontranscriptional and mediated through membrane receptors (Walker, 2003).

The presence of testosterone binding sites have been demonstrated on sperm and correlated with motility (Warikoo et al, 1986). Recently immunofluorescence labeling and confocal microscopy localized androgen receptors (ARs) in midpiece and mitochondria of ejaculated human sperm. Western blot analysis using AR antibodies specifically detected 110- and 90-kd protein bands, corresponding to AR (Solakidi et al, 2005). Tissue-specific AR gene knockout in Sertoli cells affects androgen function and causes infertility due to an arrest in spermatogenesis (Chang et al, 2004).

Progesterone Receptor. Progesterone, a female hormone, is also involved in male reproduction. The physiologic responses to progesterone are mediated through 2 types of nuclear receptors, progesterone receptor A (PR-A) and PR-B. These 2 classic nuclear receptors are encoded by a single gene (Gadkar-Sable et al, 2005). Besides nuclear receptor binding, progesterone also binds on sperm surfaces through specific receptors (Ambhaikar and Puri, 1998). These receptors are localized on the membrane and are unrelated to the classic receptors.

Western blot analysis with peroxidase-conjugated progesterone detected 2 progesterone-binding proteins of 54 and 57 kd in lysate of human sperm. These 2 receptors are present on membranes but not in the cytoplasm (Luconi et al, 1998). In boar sperm, a 71-ka PR protein has been delineated (Jang and Yi, 2005); in canine sperm, 2 proteins of 54 and 65 kd have been found that bind to progesterone (Wu et al, 2005). These differences in molecular mass of progesterone-binding proteins among various species may be due to species differences and/or methods of sperm lysate preparation. Progesterone mRNA transcripts have also been detected in sperm (Sachdeva et al, 2000). Progesterone-bovine serum albumin-fluorescein isothiocyanate conjugate

identified PRs in the acrosome (Ambhaikar and Puri, 1998; Sachdeva et al, 2005; Wu et al, 2005), equatorial, and postacrosomal regions of sperm (Jacob et al, 1998; Contreras and Llanos, 2001). Incubation of sperm with progesterone affects sperm motility, capacitation, and acrosomal exocytosis (Baldi et al, 1998, 1999; Contreras and Llanos, 2001; Wu et al, 2005). Experiments have demonstrated that progesterone stimulates sperm motility and acrosome reaction in a dose-dependant manner.

Leptin Receptor. Leptin is a 16-kd protein secreted by adipose tissue (Zhang et al, 1994). Its role has been implicated in reproduction (Ahima and Flier, 2000). Leptin acts on target tissues via membrane-bound receptors (Tartaglia et al, 1995). These receptors are classified into long form and short form (Tartaglia, 1997). Leptin and its receptors are present in testes (Tena-Sempere et al, 2001), and leptin has been detected in seminal plasma (Glander et al, 2002). Leptin levels in seminal plasma are negatively correlated with sperm progressive motility. Leptin receptor (145-kd protein) is present in the tail region of ejaculated spermatozoa (Jope, 2003). The exact isoform has not been delineated.

Calcitonin Receptor. Calcitonin is a 32-amino acid peptide hormone that regulates calcium homeostasis. Calcitonin is present in seminal plasma, and its receptors exist in spermatozoa (Sjoberg et al, 1980; Silvestroni et al, 1987). Calcitonin receptors are present in acrosomal, midpiece, and tail regions of spermatozoa (Adeoya-Osiguwa and Fraser, 2003). Calcitonin stimulates cAMP production in noncapacitated sperm (Adeoya-Osiguwa and Fraser, 2003).

Cytokine Receptors—Various cytokine and growth factor receptors that have been delineated in sperm are summarized in Table 2.

Interferon α and γ Receptors. Interferons (IFNs) are a heterogenous family of proteins that have the ability to protect body cells from viral infection (Friedmann, 1990). IFN α is produced by peripheral blood mononuclear cells in response to viral infection, and IFN γ is produced by T lymphocytes and natural killer cells (Biron et al, 1999). IFN receptors have been cloned and characterized: IFN α receptor is 68.5/95–100 kd and IFN γ receptor is 48.8–52.5/90 kd (Farrar and Shreiber, 1993; Constantinescu et al, 1994). Indirect immunofluorescence, immunoscanning electron microscopic, immunoprecipitation, and Western blot procedures localized IFN α and γ receptors of similar molecular masses in acrosomal, midpiece, and tail regions of spermatozoa of several mammalian species, including humans (Naz et al, 2000). Incubation of spermatozoa with IFNs decreases sperm motility (Naz and Kumar, 1991).

Interleukin 2 α and β Receptors. Flow cytometry and transmission electron microscopy detected interleukin 2

Table 2. Cytokine and growth factor receptors present in sperm

Receptor	Size (kd)		Sperm subcellular localization	Localized by	Function	Reference
	Other cells	Sperm				
Interferon α	100	100	Acrosome, tail	Indirect immunofluorescence, immunoscanning electron microscopy, Western blot	Sperm motility	Naz et al, 2000
Interferon γ	85	85	Posterior head, midpiece, tail	Immunoprecipitation, Western blot	Sperm motility	Naz et al, 2000
Interleukin 2 α	31	...	Head, midpiece	Flow cytometry, transmission electron microscopy	...	Fierro et al, 2002
Interleukin 2 β	61	...	Head, midpiece	Flow cytometry, transmission electron microscopy	...	Fierro et al, 2002
Interleukin 3 α	43	38, 66	Head, midpiece, tail	Western blot	Glucose uptake	Rauch et al, 2004
Interleukin 3 β	99	...	Tail	Western blot	Glucose uptake	Rauch et al, 2004
Interleukin 5 α	46	61	Head, neck	Western blot	...	Rauch et al, 2004
Interleukin 5 β	99	105–110	Tail	Western blot	...	Rauch et al, 2004
Interleukin 6 α	80	...	Tail	Indirect immunofluorescence, Western blot	Capacitation	Laflamme et al, 2005
GM-CSF α^*	...	45, 82	Tail	Immunocytochemistry, Western blot	...	Zambrano et al, 2001
GM-CSF β^*	...	105–110	Tail	Immunocytochemistry, Western blot	...	Zambrano et al, 2001
CCR1	41	...	Head, periacrosomal region	RT-PCR, immunofluorescence, Western blot	Chemotaxis	Isobe et al, 2002; Muciaccia et al, 2005
CCR3	42	...	Head, periacrosomal region	Immunofluorescence, Western blot	Chemotaxis	Muciaccia et al, 2005
CCR5	41	RT-PCR, immunofluorescence, Western blot	Chemotaxis	Isobe et al, 2002; Muciaccia et al, 2005
EGF*	165	165, 170	Postacrosomal region	Indirect immunofluorescence	Capacitation, acrosomal exocytosis	Naz and Ahmad, 1992; Damjanov et al, 1993; Furuya et al, 1993; Lax et al, 1994
IGFI*	151	...	Acrosome	Radio receptor assay, immunocytochemistry	Sperm motility	Henricks et al, 1998

* GM-CSF indicates granulocyte macrophage colony-stimulating factor; EGF, epidermal growth factor; and IGFI, insulin-like growth factor I.

(IL2) α and β receptors in head and midpiece regions of human spermatozoa; these receptors have shown some correlation with sperm concentration in semen (Fierro et al, 2002).

Interleukin 3 and 5 Receptors. IL3 and IL5 belong to the cytokine family that regulates proliferation and differentiation of hematopoietic cells. There are 2 types of receptors for each cytokine that show high or low affinity for the ligand depending on their subunit composition. Immunolocalization study demonstrated the presence of these receptors in ejaculated bovine spermatozoa (Rauch et al, 2004). Functional studies indicate that IL3 but not IL5 signals an increased uptake of hexose in sperm cells at picomolar concentrations compatible with expression of IL3 receptors (Rauch et al, 2004).

Interleukin 6 Receptors. IL6, belonging to the hematopoietic cytokine family, is a mediator of in-

flammatory response and is present in female and male reproductive tract secretions (Kishimoto, 1989; Naz and Kaplan, 1994a, b). Its biologic effects are mediated by binding to specific receptors IL6 R α and β (Kishimoto, 1989). IL6 R α of 80 kD has been localized in tail region of human sperm (Laflamme et al, 2005). IL6 is present in human semen and incubation of sperm with IL6 enhances their fertilizing capacity by increasing capacitation and/or acrosomal exocytosis (Naz and Kaplan 1994a, b). IL6 stimulates capacitation by increasing phosphorylation of tyrosine kinase JAK1 (Laflamme et al, 2005).

Granulocyte Macrophage Colony-Stimulating Factor Receptor. Immunocytochemical studies localized granulocyte macrophage colony-stimulating factor (GM-CSF) α (45 and 82 kd) and β (105–110 kd) receptors to the tail regions of ejaculated human spermatozoa (Zambrano et al, 2001). Incubation with GM-CSF

Table 3. Neurotransmitter receptors present in sperm

Receptor	Size (kd)		Sperm subcellular localization	Localized by	Function	Reference
	Other cells	Sperm				
GABA A*	Equatorial segment	Immunofluorescence, Western blot	Acrosomal exocytosis, motility	Wistrom and Meizel, 1993; Calogero et al, 1999
Glycine	52	...	Periacrosomal region	Immunofluorescence, Western blot	Acrosomal exocytosis	Melendrez and Meizel, 1996; Bray et al, 2002
nAch*	133	...	Postacrosomal region, midpiece	Radioligand binding	Acrosomal exocytosis	Young and Liang, 1991

* GABA A indicates gamma aminobutyric acid A; nAch, nicotinic acetylcholine.

increases uptake of deoxy glucose and deoxy ascorbic acid (Zambrano et al, 2001).

β Chemokine Receptors. β Chemokine RANTES (regulated on activation, normal T expressed and secreted) is a 68-amino acid polypeptide belonging to the chemokine subgroup. It has chemotactic effect (He et al, 1997) and is present in male and female reproductive tract secretions (Hornung et al, 2001; Naz and Leslie, 2000). It binds to receptors, CCR1, CCR3, CCR4, and CCR5 (Rollins, 1997). CCR5 is localized in the periacrosomal regions, and CCR3 is present in the postacrosomal regions of ejaculated human sperm (Muciaccia et al, 2005). mRNA transcripts for CCR1 and CCR5 have also been detected in human sperm (Isobe et al, 2002).

Epidermal Growth Factor Receptor. Epidermal growth factor (EGF) is a 53-amino acid polypeptide present in many tissues and body fluids (Savage et al, 1972; Hirata and Orth, 1979). Mitogenic activity of EGF is mediated through a membrane-bound glycoprotein receptor of 170 kd. EGF receptor has been localized in acrosomal, postacrosomal, and tail regions of human and bull sperm (Naz and Ahmad, 1992; Damjanov et al, 1993; Lax et al, 1994). EGF affects sperm capacitation and acrosomal exocytosis through activation of tyrosine kinase (Furuya et al, 1993; Naz and Kaplan, 1993; Lax et al, 1994).

Insulin-Like Growth Factor I Receptor. Insulin-like growth factor I (IGFI) is present in seminal plasma of humans and bulls and oviductal fluids of pigs (Glander et al, 1996). The source of IGFI in seminal plasma is not known, but oviductal epithelial cells secrete IGFI. IGFI receptor has been characterized using a radio-receptor assay and localized to the acrosomal region of sperm head by immunohistochemistry (Henricks et al, 1998). The same study demonstrated the interaction of IGFI ligand with its receptor in sperm. Sperm treated with IGFI (100 ng/mL) and IGFII (250 ng/mL) increased motility and straight-line velocity (Henricks et al, 1998). Mice homozygous for a targeted mutation of

the *IgfI* gene are infertile dwarfs with smaller testes (Baker et al, 1996).

Neurotransmitter Receptors—Various neurotransmitter receptors which have been delineated in sperm are summarized in Table 3.

γ Aminobutyric Acid Receptors. γ Aminobutyric acid (GABA) is an inhibitory neurotransmitter present in the central nervous system (Krnjevic et al, 1974). It has also been shown to be present in rat oviduct and human seminal plasma (Martin del Rio, 1981; Ritta et al, 1998). GABA A receptor is present in the equatorial segment of human spermatozoa (Wistrom and Meizel, 1993; Ritta et al, 1998). Incubation of human sperm with GABA at high concentration causes hyperactivated motility.

Glycine Receptor. Glycine is an inhibitory neurotransmitter that acts through membrane-bound glycine receptors. Glycine receptor is comprised of α and β subunits and forms pentameric Cl⁻ channels (Rajendra et al, 1997). Glycine receptors of 49 and 50 kd are present in the periacrosomal region of sperm plasma membrane (Melendrez and Meizel, 1996). Incubation with glycine induces acrosomal exocytosis, which is inhibited by strychnine, a glycine receptor antagonist (Melendrez and Meizel, 1995).

Nicotinic Acetylcholine Receptor. Nicotinic acetylcholine receptor (nAch R) is present in the head and tail regions of spermatozoa (Young and Liang, 1991). Western blot and immunofluorescence studies detected the nAch R subunits α 3, α 5, α 7, α 9, and β 4 in sperm (Kumar and Meizel, 2005). Acetylcholine induces acrosomal exocytosis in capacitated sperm. Acrosomal exocytosis of human sperm induced by human recombinant ZP3 is blocked by nicotinic antagonist (Bray et al, 2002).

Miscellaneous Receptors—Various miscellaneous receptors which have been delineated in sperm are summarized in Table 4.

Platelet-Activating Factor Receptor. Platelet-activating factor (PAF) (1-alkyl-2-acetyl-sn-glycero-3-phos-

Table 4. *Miscellaneous receptors present in sperm*

Receptor	Size (kd)		Sperm subcellular localization	Localized by	Function	Reference
	Other cells	Sperm				
PAF*	39	...	Proximal head, acrosome; midpiece; tail	Immunofluorescence, fluorescent microscopy, quantitative RT-PCR*	Capacitation, acrosomal exocytosis, sperm motility	Minhas, 1993; Reinhardt et al, 1999; Roudeshush et al, 2000
Angiotensin II	41	41, 60	Head, tail	Western blot	Acrosomal exocytosis	Vinson et al, 1995; Gur et al, 1998
Odorant	...	41, 60	Tail, midpiece	Immunocytochemistry	Sperm attraction to egg, motility	Vanderhaeghen et al, 1993
ANF*	119	...	Midpiece	Radioreceptor analysis, autoradiography	Acrosomal exocytosis	Silvestroni et al, 1992
DF*	...	23	Acrosomal cap, postacrosomal region, tail	Immunocytochemistry, Western blot	Capacitation	Gibbons et al, 2005

* PAF indicates platelet-activating factor; RT-PCR, reverse transcriptase polymerase chain reaction; ANF, atrial natriuretic factor; and DF, decapacitation factor.

phocholine) is involved in diverse functions of various tissues (Snyder, 1990). PAF is found in spermatozoa (Minhas et al, 1991). Immunofluorescence demonstrated the presence of PAF receptors, mostly in the midpiece and proximal head regions of ejaculated spermatozoa. (Reinhardt et al 1999; Roudeshush et al 2000). Immunofluorescence microscopy and quantitative RT-PCR detected an altered pattern of PAF receptor distribution and its mRNA in abnormal spermatozoa, compared with normal sperm. PAF stimulates human sperm motility, stimulates sperm penetration of cervical mucus, and increases human sperm penetration rates in zona-free hamster oocytes (Minhas, 1993).

Angiotensin II Receptors. The peptide hormone, angiotensin II, acts as a modulator of many cell physiologic functions, which are mediated through binding to 2 distinct receptors: angiotensin II type 1 (AT1) and type 2 (AT2) receptors (Griendling et al, 1996). Follicular fluid is one of the endogenous sources of angiotensin II (Heimler et al, 1995). Immunocytochemical and immunoblotting techniques identified the AT1 receptor protein of 60 kd in tail regions of ejaculated human sperm (Vinson et al, 1995), and a 41-kd protein in head and tail regions of bovine spermatozoa (Gur et al, 1998). Incubation of sperm with angiotensin II increases sperm motility (Vinson et al, 1995) and induces acrosomal exocytosis (Gur et al, 1998).

Odorant Receptor. Discovery of the odorant receptor (OR) gene family has revolutionized the field of chemosensation (Buck and Axel, 1991) and led to understanding of the olfactory system. Sensory system olfactory receptors have been cloned, and their mRNA transcripts have been detected in several tissues (Spehr et al, 2006). OR of 40 kd is present in tail and midpiece regions of ejaculated dog sperm (Vanderhaeghen et al,

1993). Recently human testicular OR, hOR17-4, was cloned and characterized (Spehr et al, 2003). When it was expressed in human embryonic kidney cells and tested with an odorant, bourgeonal, there was an increase in intracellular calcium level. It has been suggested that ORs play an important chemotactic role in bringing sperm to oocyte.

Atrial Natriuretic Factor Receptor. Atrial natriuretic factor (ANF), a 28-amino acid peptide, is secreted by the cardiac atrium and regulates body fluid homeostasis (Brenner et al, 1990). ANF is present in follicular fluid (Steegers et al, 1990). ANF receptor of 135–140 kd has been detected in the midpiece region of spermatozoa (Silvestroni et al, 1992). ANF stimulates acrosomal exocytosis that is inhibited by its antagonist, anantin (Rotem et al, 1998).

Decapacitation Factor Receptor. Decapacitation factor (DF) is a 40-kd protein that prevents capacitation when it binds to sperm (Fraser et al, 1990). DF binds with a specific decapacitation factor receptor (DFR) present on sperm surfaces. Recently it was found that DFR, a 23-kd protein, is a phosphatidylethanolamine-binding protein 1 (PEBP1), localized in acrosomal, postacrosomal, and tail regions of sperm (Gibbons et al, 2005). Incubation of sperm with DF and PEBP1 causes a loss of decapacitating activity.

Conclusions

There are numerous receptors, including 8 hormone receptors, 16 cytokine/growth factor receptors, 3 neurotransmitter receptors, and 5 miscellaneous receptors, that have been reported to be present in mature ejaculated sperm. The list is ever growing. Almost all of these receptors have been shown to be involved, directly or indirectly, in sperm capacitation and/or

acrosomal exocytosis in in vitro studies. Do these receptors have a physiologic significance in vivo? It seems doubtful that all of these receptors are really involved in affecting sperm capacitation/acrosomal exocytosis in vivo due to the following facts:

1. Sperm can be capacitated in vitro, resulting in successful fertilization without any ligand for these receptors and indicating that the receptors are not required for capacitation/acrosomal exocytosis.
2. Many of these receptors, especially the hormone receptors, have been reported to be nongenomic. This is logical because the mature sperm cell is transcriptionally and translationally quiescent. However, there are mRNAs for several proteins present in mature sperm, probably left over after spermatogenesis/spermiogenesis. Our laboratory was first to report the presence of mRNAs in mature sperm (Kumar et al, 1993). Since then, several labs have confirmed these findings on the presence of mRNAs in sperm cells (Ostermeier et al, 2002; Wang et al, 2004; Dadoune et al, 2005; Martins and Krawetz, 2005). Many of the reports on sperm receptors have confirmed their existence by the presence of specific mRNAs in sperm by RT-PCR. What does this really mean? It simply means that there is residual mRNA present in sperm cells for that receptor which was synthesized during sperm development/maturation in the testis/epididymis. It does not confirm the authenticity of the receptor and does not imply that the receptor is being synthesized in mature sperm.
3. Sperm develop in the testis, mature in the epididymis, and then are bathed in secretions of accessory glands in semen. Many proteins in sperm are adsorbed/modified during these processes. Are many of these "receptors" adsorbed or are they real integral membrane proteins? We have reported the presence of c-myc protein (Naz et al, 1991) and c-myc mRNA (Kumar et al, 1993) in ejaculated human sperm cells. c-Myc is an intracellular protein, it can be detected in sperm by immunofluorescence and Western blot, and its antibodies inhibit capacitation/acrosomal exocytosis and human sperm penetration in zona-free hamster oocytes. Many of the receptors may be like the c-myc protein. Our laboratory has examined the immunoreactivity of antibodies of several cytokine/growth factor receptors. It was amazing to find that almost all of them reacted with sperm cells by immunofluorescence, were detected with the relevant protein in sperm lysate in Western blots, and also inhibited sperm capacitation/acrosomal exocytosis in vitro significantly more than the same concentration of control

antibodies (unpublished findings). These data raise doubt on the reality of several of the reported receptors in sperm cells.

In conclusion, receptors for several hormones, cytokines, growth factors, and neurotransmitters have been reported and/or are being reported in mature ejaculated sperm cells. Their authenticity and in vivo physiologic relevance need to be carefully examined before making conclusions that they are real and functionally important.

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