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ROLE OF FERTILIZATION PROMOTING PEPTIDE (FPP) IN MODULATING MAMMALIAN SPERM FUNCTION

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1. ABSTRACT

Fertilization promoting peptide (FPP), structurally similar to thyrotrophin releasing hormone, is produced by the prostate gland and secreted into seminal plasma. Recent *in vitro* studies have provided evidence that FPP elicits biologically relevant responses in mouse, human and boar spermatozoa. In the presence of nanomolar concentrations of FPP, spermatozoa become fertilizing more quickly and then are inhibited from undergoing spontaneous acrosome loss, an event that would make them non-fertilizing. *In vivo*, these responses would be very important in maximizing the availability of potentially fertilizing spermatozoa. Adenosine, which can elicit the same responses as FPP, is known to modulate the adenylyl cyclase(AC)/cAMP signal transduction pathway; current evidence indicates that FPP and adenosine act via separate receptors on the same signal transduction pathway. Mouse spermatozoa are known to have adenosine receptors and a putative receptor for FPP (TCP-11) has been identified. Unlike many surface receptors, TCP-11 has no obvious transmembrane regions whereby modulation of AC could occur. Recent evidence suggests that FPP receptors may dimerize with adenosine receptors to activate the signaling pathway, with stimulatory adenosine receptors involved in the stimulation of capacitation, but inhibitory receptors involved in inhibition of spontaneous acrosome loss. These results indicate that FPP plays an important role in normal sperm function and that it might be used in new therapeutic strategies designed to alleviate some causes of sperm dysfunction.

2. INTRODUCTION

Fertilization promoting peptide (FPP; pGlu-Glu-ProNH_2) is structurally similar to thyrotrophin releasing hormone (TRH; pGlu-His-ProNH_2) and so can cross-react with TRH antibodies. Evidence for TRH-like material in mammalian prostate glands and semen was reported in the early 1980s (1,2). However, it was not until 1989 that Cockle and her colleagues (3) were able to isolate FPP from the rabbit prostate complex, then characterize it and demonstrate that FPP was indeed distinct from TRH. Later studies identified FPP in the prostate glands and/or semen of several other mammals (e.g., man, several rodents; 4) as well as the pituitary glands of birds and mammals (5,6,7). Although no specific function has yet been demonstrated for FPP of pituitary origin, considerable evidence indicates that FPP of prostatic origin may play an important role in modulating mammalian sperm function *in vivo*.

FPP production appears to be under androgen control since it cannot be detected in the prepubertal rabbit prostate but is present following the androgen surge at the onset of puberty (8). The presence of FPP in semen at quite high concentrations (mean of ~50 nmol l^{-1} in humans; 9) suggested that the tripeptide might have a biological role relating to spermatozoa and recent studies have provided evidence for this, hence the name 'fertilization promoting peptide'.

In order to understand the importance of the responses elicited by FPP, it is necessary to have some knowledge of what is required to turn a non-fertilizing spermatozoon into a potentially fertilizing one. At the time of release from the male reproductive tract, mammalian spermatozoa are morphologically complete and capable of independent motility, but they are unable to fertilize oocytes, even if mixed directly with them *in vitro*. Spermatozoa require a species-specific length of time in a permissive environment to 'switch on' in a functional sense. Normally, this 'switching on', which is referred to as capacitation, occurs in the female reproductive tract, but it is also possible to provide suitable conditions *in vitro* that will support capacitation. This means that conditions can be manipulated so as to gain insight into mechanisms of action involved in specific responses. Although our current knowledge is still fragmentary, a considerable body of evidence indicates that the adenylyl cyclase(AC)/cAMP signal transduction pathway plays an important role in capacitation (10,11). Once capacitated, a spermatozoon has the potential to interact successfully with an oocyte, undergoing an acrosome reaction in response to oocyte-associated molecules, penetrating the zona pellucida, fusing with the oocyte plasma membrane and then depositing its

genetic information in the oocyte cytoplasm. Although capacitated spermatozoa can undergo the acrosome reaction spontaneously, this is a dead-end step since acrosome-reacted cells are non-fertilizing, even if they retain motility (12).

3. FPP'S EFFECTS ON SPERMATOZOA

FPP's effects have been investigated using first epididymal mouse, then ejaculated human and most recently ejaculated boar spermatozoa. In all instances, initial evaluation has utilized the antibiotic chlortetracycline (CTC) to carry out cytological assessments. CTC is reported to bind to membrane-associated divalent cations (13), with greater affinity for Ca^{2+} than Mg^{2+} (14). In several mammalian species, changes in the proportions of cells expressing various CTC fluorescence patterns have been shown to reflect changes in the functional state of the sperm suspensions (15). While most cytological techniques used to evaluate spermatozoa only identify acrosome-intact and acrosome-reacted cells, CTC allows the acrosome-intact category to be further divided into two on the basis of differences in functional state, namely uncapacitated and capacitated. The capacitated but still acrosome-intact category is the one of interest, since this contains the potentially fertilizing cells.

In the initial experiments, uncapacitated epididymal mouse sperm suspensions, which would never have contacted FPP, were incubated in the presence of nanomolar concentrations of FPP. CTC analysis indicated that capacitation was significantly stimulated but there was no increase in spontaneous acrosome reactions (in many instances, a treatment that stimulates capacitation also causes an increase in acrosome reactions). Subsequent experiments demonstrated that, as predicted from the CTC analysis, FPP-treated suspensions were significantly more fertile than untreated controls and this provided the basis for the name for the peptide (16). The fact that FPP-treated cells acquired fertilizing ability sooner than untreated cells would suggest that FPP stimulated hyperactivated motility and this was confirmed using computer assisted sperm analysis (17). Later experiments provided evidence that FPP had a similar effect on human sperm suspensions, stimulating both capacitation (CTC) and demonstrable penetrating ability (using the zona-free hamster oocyte penetration test; 18). The human spermatozoa used in these experiments were prepared from fresh semen samples and so would have come into contact with FPP. The fact that after preparation (using mini-Percoll gradients and washing) spermatozoa were able to respond to FPP suggests that the centrifugation steps involved in the preparation removed at least some of the FPP bound to cells.

Interestingly, FPP was then shown to have an effect on capacitated cells, significantly inhibiting the spontaneous acrosome reaction but not interfering with an acrosome reaction in response to progesterone (19). *In vivo*, this biphasic response to FPP could be of considerable importance since only capacitated, acrosome-intact cells are able to fertilize oocytes. More recently, boar spermatozoa have been shown to respond to FPP in a monophasic way, with the peptide inhibiting spontaneous acrosome reactions but not stimulating capacitation *per se*, i.e., there is no peptide-related decrease in the proportion of uncapacitated cells (20). However, further studies have revealed that the phosphodiesterase inhibitor caffeine, normally present in the medium used for boar spermatozoa, can mask the early responses to FPP, presumably by increasing cAMP concentrations. In caffeine-free medium, FPP elicits a biphasic response (H Funahashi, personal communication).

4. MECHANISM OF ACTION

The biphasic response of epididymal mouse spermatozoa to FPP recalled earlier studies demonstrating that the responses of mouse sperm AC and its production of cAMP to adenosine were biphasic, enzyme activity being stimulated in uncapacitated cells yet inhibited in capacitated cells (21). When adenosine was evaluated using the same protocols as those used for FPP, the responses elicited were the same as those obtained with FPP: adenosine stimulated capacitation in uncapacitated cells and inhibited spontaneous acrosome reactions in capacitated cells (19). Furthermore, FPP and adenosine used together, whether at low concentrations that evoked no significant response when used individually or at high concentrations that evoked a maximal response, were more effective than either used individually (19). These additive effects suggested that FPP and adenosine acted on the same signal transduction pathway but used different receptors. Because adenosine is known to modulate AC/cAMP in mouse spermatozoa by means of specific adenosine receptors (22), we have proposed that FPP also modulates AC/cAMP. Direct evidence has now been obtained that FPP, like adenosine, can stimulate AC production of cAMP in mouse sperm membranes, permeabilized cells and live, intact cells (23).

Additional evidence that FPP and adenosine act via separate, specific receptors has been obtained during investigation of an FPP-related peptide, pGlu-Gln-ProNH_2 (Gln-FPP). Gln-FPP, which has been identified in human seminal plasma (24), has no intrinsic biological activity but, when used in combination with FPP, it competitively inhibited responses to FPP in a concentration-dependent manner (25). It also significantly inhibited both ^{3}H-FPP binding to mouse sperm membranes and FPP-induced stimulation of AC activity (23). In contrast, Gln-FPP was unable to inhibit adenosine's stimulation of capacitation (19) and AC/cAMP (23). These results are consistent with FPP and adenosine acting at separate, specific receptors. During preliminary experiments to determine the optimal conditions for conducting the binding studies, it was observed that binding was higher in Ca^{2+}-deficient medium (23). Seminal plasma has very low concentrations of free Ca^{2+}, suggesting that FPP would bind effectively to spermatozoa. However, extracellular Ca^{2+} is required for FPP to elicit responses from spermatozoa (19) and conditions in the female reproductive tract would provide the environment required.

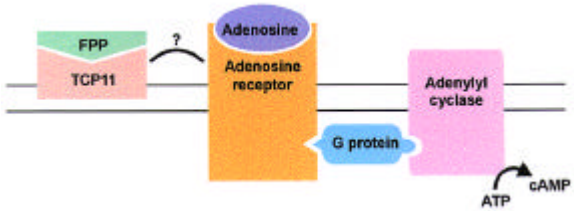

Figure 1. A schematic diagram of how FPP and its putative receptor TCP11 might interact with an adenosine receptor to modulate adenylyl cyclase activity and cAMP production. The biphasic nature of the responses to FPP suggests that two different types of adenosine receptors are involved, initially stimulatory and then inhibitory.

5. PUTATIVE RECEPTOR FOR FPP

Experimental evidence suggested the existence of an FPP-specific receptor and serendipity provided a candidate molecule, namely the protein TCP11. TCP11 is coded for by the mouse *t*-complex gene *Tcp11* (26) which has a human homologue (27). The *t*-complex, located on mouse chromosome 17, is of particular interest because it contains genes known to affect male but not female infertility (28,29). Heterozygous male mice with one wild type chromosome 17 and one *t*-chromosome 17 produce wild type and *t*-bearing spermatozoa in equal quantities, but analysis of their offspring reveals that >90% have been fertilized by *t*-bearing spermatozoa. This transmission ratio distortion (TRD) suggests that *t*-bearing cells have a functional advantage over the wild-type cells *in vivo*. Recent investigations of TCP-11 have revealed a mechanism whereby at least one *t*-complex gene may modulate sperm function to cause TRD.

Tcp11 is expressed only in germ cells in the testis and only during spermatogenesis (26), but it is under translational control since TCP11 protein can only be detected in post-meiotic cells undergoing spermiogenesis (30). The precise localization of TCP11 in maturing spermatozoa has not yet been investigated. However, in mature epididymal cells TCP11 is restricted in its locations on the surface of spermatozoa, namely on the acrosomal cap region of acrosome-intact (but not acrosome-reacted) cells and on the flagellum, fairly consistently on the principal piece but much more variably on the midpiece (31). Since both head and flagellum are involved in successful fertilization (12), this localization would suggest that TCP11 could play an important role in sperm function relating to fertilization. There is no information as to whether TCP11 might have a role prior to leaving the male reproductive tract.

To undertake physiological investigations, it was necessary to prepare Fab fragments of the purified IgG anti-TCP11 antibodies used for the localization experiments to avoid agglutination of antibody-treated spermatozoa. When sperm suspensions were incubated in the presence of anti-TCP11 Fab fragments and sampled at 30 and 120 minutes, CTC analysis indicated that capacitation was stimulated significantly during the initial incubation and then spontaneous acrosome reactions were inhibited significantly as incubation continued. *In vitro* fertilization experiments confirmed that Fab-treated suspensions became fertilizing more quickly than untreated control suspensions and then maintained high fertilizing ability despite the Fab fragments' inhibition of spontaneous acrosome loss (31). The similarity of these responses to those obtained with FPP and adenosine led to further investigations using FPP, adenosine and Fab fragments in various combinations. Results indicated that FPP and the anti-TCP11 Fab fragments were working at the same FPP-specific site, with responses being inhibited by Gln-FPP, a site distinct from the adenosine-specific site.

The simplest interpretation of these results is that TCP11 is the receptor for FPP. The antibodies, binding to their antigen TCP11, appear to act as agonists and thereby stimulate a signal transduction pathway modulated by TCP11. Given the existing evidence, this would be the AC/cAMP pathway. What evidence is there to support these hypotheses? Very recent experiments have demonstrated that anti-TCP11 Fab fragments significantly inhibited the binding of ^{3}H-FPP to mouse sperm membranes and that significantly less ^{3}H-FPP bound to sperm suspensions treated with ionophore A23187 to induce the acrosome reaction than to untreated suspensions (23). These results are consistent with TCP11, which is restricted to the acrosomal cap region of the sperm head, being the receptor for FPP. The fact that Fab fragments stimulate AC production of cAMP in permeabilized cells (23) is consistent with AC/cAMP being the signal transduction pathway modulated by TCP11.

Thus the current hypothesis is that FPP-TCP11 interaction results in modulation of the AC/cAMP pathway, as does adenosine-adenosine receptor interaction (31). However, although adenosine receptors have seven transmembrane domains typical of external receptors that modulate intracellular enzyme activity (32), TCP11 contains no obvious transmembrane domain. This then raises the question of how FPP-TCP11 might actually interact with AC. Very recent evidence has suggested that responses to FPP may involve dimerization of the FPP receptor and an adenosine receptor (33). In general, adenosine regulation of AC activity involves G proteins (32); consistent with this, GTP analogues have been shown to modulate AC activity in mouse spermatozoa (22) and to modulate responses to FPP (33). Figure 1 indicates schematically how FPP $\leftrightarrow$ TCP11 interactions might modulate AC activity.

If the above hypothesis is correct, then the biphasic nature of the responses to FPP, being first stimulatory and then inhibitory, would suggest involvement initially of stimulatory adenosine receptors and then of inhibitory receptors. The presence of two different adenosine receptors was first hypothesized in 1986 because of the differences in the effects of adenosine on AC activity in uncapacitated (stimulatory) and capacitated (inhibitory) sperm suspensions (21). Very recent investigations of FPP's effect on boar spermatozoa have indicated that both FPP and adenosine can significantly inhibit spontaneous acrosome loss. Considerably lower concentrations ($\leq$ 6.25 nmol l^{-1}) are effective in inhibiting the acrosome reaction

Table 1. Effects of FPP on mammalian spermatozoa

Species	Effect
Mouse	1. FPP has a biphasic effect, stimulating capacitation and fertilizing ability in vitro in uncapacitated cells (16) and then inhibiting spontaneous acrosome loss in capacitated cells (19) 2. adenosine has a similar biphasic effect (19) 3. FPP, like adenosine, modulates AC/cAMP (23) 4. TCP-11, the product of a t-complex gene with a human homologue, may be the receptor for FPP (23, 31) 5. FPP responses may involve dimerization of TCP-11 and adenosine receptors (33) 6. Stimulatory adenosine receptors may be involved in the stimulatory responses to FPP and adeosine, while inhibitory adenosine receptors may be involved in the inhibitory responses (21)
Human	1. FPP stimulates capacitation and penetrating ability in vitro (17)
Boar	1. FPP elicits responses to those observed in mouse, as long as boar spermatozoa are evaluated in caffeine-free medium 2. Lower concentrations of FPP and adenosine are required to obtain a significant inhibition of acrosome loss in boar spermatozoa than are required to stimulate capacitation in mouse spermatozoa (20) 3. This differential sensitivity would be consistent with the involvement of different adenosine receptors during the two different phases.

than are required to stimulate capacitation in uncapacitated mouse and human sperm cells ($\geq$ 25 nmol l^{-1} 16,17). These differences in sensitivity to the same ligands would be consistent with the involvement of two different adenosine receptors. The various effects of FPP on mammalian spermatozoa are summarized in table 1**.**

6. FUTURE DEVELOPMENTS

Current evidence indicates that subfertility is a reasonably common problem in the general population, affecting probably 10% or more of couples (34). In some instances, a female factor can be identified as a possible cause and in others, a male factor. When there appears to be a male factor contributing to subfertility, sometimes the possible problem is fairly obvious, i.e., low sperm concentration often in conjunction with poor quality spermatozoa (poor motility and/or poor morphology). In other cases, there may be no obvious defects in sperm quality but yet spermatozoa appear to be dysfunctional. Although no studies on samples from subfertile men have yet been undertaken, it is plausible that in at least some such cases of subfertility, deficiencies/abnormalities involving FPP and/or TCP11 could contribute to dysfunction. For example, men with prostatic dysfunction have less FPP and more related peptides, the latter possibly being either less effective or competitive inhibitors of FPP (e.g., Gln-FPP; 9). As we learn more about FPP's possible role in modulating fertility *in vivo* it may be possible to develop new therapeutic strategies to alleviate subfertility in at least some men. Furthermore, FPP and TCP11 might also provide suitable targets for future male contraceptives.

7. ACKNOWLEDGEMENTS

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