Fertilization promoting peptide, a tripeptide similar to thyrotrophin-releasing hormone, stimulates the capacitation and fertilizing ability of human spermatozoa in vitro

C.M. Green¹,2, S.M. Cockle³, P.F. Watson² and I.R. Fraser¹,4

¹Anatomy and Human Biology Group, King's College London, Strand, London WC2R 2LS, ²Department of Veterinary Basic Sciences, Royal Veterinary College, London and ³Department of Biochemistry and Physiology, University of Reading, Reading, UK

*To whom correspondence should be addressed

Recent studies have demonstrated that a prostatic tripeptide similar in structure to thyrotrophin-releasing hormone (TRH) can stimulate the in-vitro capacitation and fertilizing ability of epididymal mouse spermatozoa. Therefore we have proposed that this tripeptide be referred to as fertilization promoting peptide (FPP). Using chlorotetracycline fluorescence analysis and the hamster oocyte penetration test (HOPT), we have obtained evidence that FPP can also promote the capacitation and fertilizing ability of ejaculated human spermatozoa in vitro. FPP (25–200 nM) caused a significant increase in the proportion of B-pattern capacitated cells and a decrease in the proportion of F-pattern uncapacitated cells, with no significant stimulation of acrosomal exocytosis. Comparison of FPP with two structurally similar tripeptides, TRH and pyroglutamyl phenylalanylprolineamide, at 50 nM revealed that only FPP could significantly promote capacitation. Finally, after a brief exposure to progesterone to induce acrosomal exocytosis in capacitated cells, FPP-treated suspensions penetrated a significantly higher proportion of oocytes than the untreated controls when assessed in the HOPT. The presence of FPP in human seminal plasma at concentrations similar to those used here suggests that, in vivo, FPP may play a positive role in promoting human sperm function.

Key words: capacitation/chlorotetracycline/fertilization-promoting peptide/hamster oocyte penetration test/TRH

Introduction

The tripeptide pyroglutamyl glutamylprolineamide (pGlu–Glu–ProNH₂), which differs in structure from thyrotrophin-releasing hormone (TRH), otherwise known as pyroglutamyl-histidylprolineamide (pGlu–His–ProNH₂), by the substitution of glutamic acid for histidine at position 2, has been identified in several mammalian tissues including the reproductive tract and the pituitary gland (Ashworth, 1994). Following the discovery of high levels of TRH immunoreactivity in mammalian prostate tissue and semen, which appeared to be chromatographically distinct from authentic TRH (Pekary et al., 1980, 1983), pGlu–Glu–ProNH₂ was first characterized in the rabbit prostate complex (Cockle et al., 1989a) where it was localized to the prostate gland, with little evidence of any peptide presence within other accessory glands (Thetford et al., 1992). It has since been found in rat prostate gland as well as in human prostate and semen. Its relative abundance, for a small peptide, in seminal plasma (49.5 ± 10.3 nM, mean ± SEM; Cockle et al., 1994) is suggestive of a biological activity relating to spermatozoa that would be exposed to the tripeptide at ejaculation. In the rabbit, there is an increase in the concentration of pGlu–Glu–ProNH₂ at sexual maturity which also implies a role in male fertility (Thetford et al., 1992). Although pGlu–Glu–ProNH₂ is present in semen from the rabbit and human, none has been detected in ram or bull semen, in the prostate of the boar or in porcine female reproductive tissues (Cockle et al., 1989b; Ashworth et al., 1992). In recent years, two other TRH-related tripeptides have been discovered in human seminal fluid, pyroglutamyl-glutaminoprolinaldeamid and pyroglutamyl phenylalaninolprolineamide (Khan et al., 1992). The origin of these tripeptides is unknown because their precursors and encoding genes have yet to be characterized.

We have demonstrated previously that pGlu–Glu–ProNH₂ at concentrations of ≥25 nM can significantly promote the capacitation of epididymal mouse spermatozoa in vitro (Green et al., 1994). Spermatozoa were assessed after a 40 min incubation period using the fluorescent stain chlorotetracycline (CTC). There was a significant decrease in the proportion of cells exhibiting the F pattern of CTC fluorescence, characteristic of uncapacitated cells (Fraser and McDermott, 1992), and a corresponding increase in the proportion of cells exhibiting the B pattern of fluorescence, characteristic of capacitated cells. However, there was no significant stimulation of the acrosome reaction. Comparison of pGlu–Glu–ProNH₂ with TRH revealed that TRH could stimulate capacitation to the same extent at the highest concentration tested (250 nM) but was either less effective than pGlu–Glu–ProNH₂ or ineffective at lower concentrations. These results suggested that pGlu–Glu–ProNH₂ would stimulate fertilization, and in-vitro fertilization (IVF) experiments confirmed this: there was a significantly higher proportion of fertilized oocytes in the 100 nM peptide-treated samples than in the untreated controls (peptide, 56.5% of oocytes fertilized versus controls, 26.5%). On the basis of these results we have proposed that the tripeptide pGlu–Glu–ProNH₂ be referred to as fertilization promoting peptide (FPP).

Here we have examined the effect of FPP on ejaculated human spermatozoa using a range of concentrations and incubation periods. To do this we used CTC fluorescence analysis to investigate the possibility that FPP may be able to promote the capacitation of ejaculated human spermatozoa,
and the hamster oocyte penetration test (HOPT) to determine whether the peptide can promote the fertilizing ability of human spermatozoa. For ethical reasons it was not possible to carry out IVF experiments using human oocytes. Previous studies have demonstrated that human spermatozoa can penetrate hamster oocytes but only if the zona pellucida has been removed (Yanagimachi et al., 1976). Because sperm–oocyte fusion in the absence of the zona pellucida requires acrosome-reacted cells, we exposed the sperm cells briefly to progesterone before using them in the HOPT.

Materials and methods

Media

The standard medium used was Earle’s medium (TCN Flow, High Wycombe, UK) with added benzylpenicillin (100 IU/ml; Sigma, Poole, UK) and human serum albumin (HSA; Sigma) at 4 mg/ml. For the HOPT, a modified Biggers–Whitten–Whittingham (BWW) medium (Biggers et al., 1971) supplemented with 20 mM HEPES and bovine serum albumin (Sigma) at 3 mg/ml was used.

Peptide and progesterone preparation

We dissolved 1 mg FPP (Sigma) in distilled water. Then 10 μl aliquots, containing 10 nmol each, were placed in microcentrifuge tubes, lyophilized overnight and stored at −20°C. TRH was obtained from Cambridge Research Biochemicals (Gadbrook Park, Northwich, UK) and pyrogulatamin-phenylalanylprolineamide from Peninsula Laboratories (St Helens, UK) and prepared as for FPP. Stock solutions of the peptides were prepared by dissolving the contents of one tube in 1 ml of protein-free medium and mixing well by vortexing. The peptide stock was divided into aliquots, frozen and kept for up to 4 weeks. In preparation for use, the stock was thawed and diluted with medium as required; all substocks were used at a 1 in 50 dilution.

A 10 mg/ml progesterone (Sigma) stock solution was prepared in dimethylsulphoxide (DMSO); substocks of 1.0 or 0.5 mg/ml were made by dilution in 1:1 DMSO: 0.9% NaCl. These stock solutions were diluted 1 in 100 into sperm suspensions to give the final concentration of 10 (31.8 μM) or 5 μg/ml (15.9 μM); the final concentration of DMSO was ~0.5%. In an earlier study (DasGupta et al., 1994), DMSO alone at 1.0% had no detectable effect on CTC patterns or sperm viability.

Sperm preparation

Semen obtained by masturbation was provided by healthy volunteer donors, several of whom were known to be fertile. The use of human semen for this research received ethical approval from the King’s College Research Ethics Committee. A research licence (licence no. R0087/1) to carry out the HOPT was obtained from the Human Fertilisation and Embryology Authority. Motile cells were obtained using mini-Percoll gradients (Ord et al., 1990). An isotonic substock solution of Percoll (Pharmacia LKB, Uppsala, Sweden) was prepared by adding 90 ml of 100% Percoll to a solution containing 10X Earle’s concentrate, 0.37 ml sodium lactate, 2 ml HEPES buffer, 3 mg sodium pyruvate and 4 mg/ml HSA (final concentration); 95, 70 and 50% v/v solutions of this substock were prepared by dilution with 1X Earle’s medium containing HSA. Discontinuous gradients were made using 300 μl of each. After centrifugation for 5 min at 600 g, the supernatant was removed and the pelletted cells were resuspended in fresh medium. The cells were then centrifuged again for 5 min at 600 g and resuspended in fresh medium. The sperm concentration was assessed using a haemocytometer and then adjusted to 5×10^6 cells/ml. A small drop of the suspension was placed on a microscope slide and an estimate was made of the proportion of cells exhibiting progressive motility; usually this was >90%. Suspensions were transferred to 10 ml centrifuge tubes, peptide was added and the cells were incubated at 37°C in 5% CO₂, 5% O₂ and 90% N₂.

Cell assessment

The live/dead status of the spermatozoa was assessed with the vital dye Hoechst bis-benzimid 33258 (Sigma). A 100 mg/ml stock solution was made up in Milli-Q water and stored at 4°C for up to 1 month. For use, the stock solution was diluted 1 in 1000 in protein-free Earle’s medium and then 1 in 100 into sperm suspension. Samples were incubated at room temperature for 2 min before being washed by centrifugation through 4 ml of 2% polyvinylpyrrolidone (PVP40; Sigma) in phosphate-buffered saline (PBS) at 900 g for 5 min. The supernatant was removed and the pellet was resuspended in Earle’s medium before being treated with CTC as described below.

The functional state of the spermatozoa was assessed using the CTC fluorescence assay method described by DasGupta et al. (1993). CTC solution was prepared on the day of use and contained 750 μM CTC (Sigma) in a buffer of 130 mM NaCl, 5 mM cysteine, 20 mM Tris–HCl; the pH was adjusted to 7.8. This solution was kept wrapped in foil at 4°C until just before use. Hoechst-treated sperm suspension (45 μl) was added to 45 μl of CTC solution at room temperature in a foil-wrapped centrifuge tube and mixed thoroughly. Cells were then fixed by adding 8 μl 12.5% w/v paraformaldehyde in 0.5 M Tris–HCl (pH 7.4). Slides were prepared by placing 10 μl of the stained, fixed suspension on a slide. One drop of 0.22 M 1,4-diazabicyclo(2.2.2)octane dissolved in glycerol:PBS (9:1) was mixed carefully to retard fading of the fluorescence. A coverslip was placed on top. The slide was compressed firmly between tissues to remove any excess fluid and to maximize the number of spermatozoa lying flat on the slide. The coverslip was then sealed with colourless nail varnish and stored wrapped in foil in the cold.

An assessment was carried out on either the same or the following day using an Olympus BHS microscope (Olympus Optical Co. (UK) Ltd, London, UK) equipped with phase-contrast and epifluorescent optics. Cells were assessed for live/dead status using ultraviolet light. The excitation beam was passed through a 334 nm bandpass filter and fluorescence emission was observed through a DM 400 dichroic mirror (Olympus Optical Co.). Cells were assessed for CTC staining using violet light. The excitation beam was passed through a 405 nm bandpass filter and fluorescence emission was observed through a DM 455 dichroic mirror. In each sample, 200 live cells were assessed for CTC staining patterns; in all cases the proportion of dead cells was very low. There are three main patterns of CTC fluorescence that can be identified: F, with uniform fluorescence over the entire head, characteristic of uncapacitated, acrosome-intact cells; B, with a fluorescence-free band in the post-acrosomal region, characteristic of capacitated, acrosome-intact cells; and AR, with dull or absent fluorescence over the sperm head, characteristic of capacitated, acrosome-reacted cells. At all three stages bright fluorescence on the midpiece could be seen.

Hamster oocyte penetration test (HOPT)

Six mature oestrous female hamsters (Bantin and Kingman, Grimston, Hull, UK) were injected with 25 IU pregnant mare’s serum gonadotrophin (Folligon; Intervet, Cambridge, UK) and 52 h later with 25 IU human chorionic gonadotrophin (Chorulan; Intervet); 18 h later the oviducts were removed and the cumulus masses released into BWW medium. The oocytes were then treated with 1 mg/ml hyaluronidase (Type III from sheep testes; Sigma) for 1–2 min to dissolve the cumulus, and then washed once with BWW medium.

FPP stimulates human sperm fertilizing ability
Then the cumulus-free oocytes were treated with 1 mg/ml trypsin (Type I from bovine pancreas; Sigma) to remove the zona pellucida. The oocytes were left in the trypsin for a period of 2 min or until the zona could be seen to be dissolving. These zona-free oocytes were then washed twice in fresh BWW medium and transferred to dishes containing the sperm preparations.

The sperm suspensions were prepared as described below (series IV and V) before being diluted to give a final concentration of $5 \times 10^5$ cells/ml. The gametes were coincubated for 3 h. Oocytes were then washed free of any loosely adhering spermatozoa, transferred to fresh medium and fixed with buffered formalin. The fixed oocytes were stained with 0.75% aceto-orcein, mounted and assessed for penetration. The number of decondensing sperm heads and the number of attached spermatozoa were recorded for each oocyte assessed.

**Statistics**

CTC results were analysed using Cochran's modification of the $\chi^2$ test (Snedecor and Cochran, 1980). Each treatment sample was compared with the appropriate control sample. Results from the HOPT were analysed using another modification of the $\chi^2$ test (Maxwell, 1964).

**Results**

**Series I. Does FPP cause a time-dependent change in CTC patterns?**

To determine whether or not FPP could modulate the capacitation of ejaculated human spermatozoa, FPP at a concentration of 100 nM was added to sperm suspensions and the cells were assessed with CTC after 1, 3, 5 and 22 h of incubation. Untreated control suspensions were assessed at the same time points; seven replicates were carried out ($n = 7$). A concentration of 100 nM FPP was used because this was the concentration found to be most effective at stimulating capacitation in epididymal mouse spermatozoa (Green et al., 1994).

The results indicated a significant stimulation of capacitation in the FPP-treated cells after 1 and 3 h ($P < 0.01$ and $P < 0.05$ respectively) when compared with the untreated controls at the same time points, as shown by a decrease in the proportion of F-pattern cells and an increase in the proportion of B-pattern cells (Figure 1). There was no significant stimulation of acrosomal exocytosis observed after the addition of FPP. By 5 h the differences seen between the FPP-treated suspensions and the corresponding control suspensions were smaller and therefore not significant. Presumably this reflects the progress of capacitation in control suspensions; similar changes later in capacitation have been noted in mouse sperm suspensions (C.Green, unpublished results). By 22 h there was no detectable difference between the two groups. In all subsequent experiments, suspensions were incubated for a period of 1 h in the presence of FPP or related peptides before being assessed by CTC. In these and all other experiments, the motility of FPP-treated samples was at least equivalent to that in the untreated controls.

**Series II. Is the response to FPP concentration dependent?**

FPP at concentrations between 25 and 200 nM was added to sperm suspensions which were then incubated for 1 h before being assessed by CTC ($n = 8$). Control samples were assessed at 1 and 22 h. The latter time was included to demonstrate that with time, in the absence of FPP, a significant proportion of the cells had undergone capacitation and acrosomal exocytosis (see DasGupta et al., 1993).

All concentrations of FPP caused a significant stimulation of capacitation ($P < 0.05 - P < 0.01$), as shown by a decrease in the proportion of F-pattern cells and an increase in the proportion of B-pattern cells when compared with the control group assessed at the same time point (Figure 2). As before, there was no detectable change in the proportion of AR-pattern cells. Thus, the response to FPP did not appear to be strongly concentration dependent. The changes in CTC patterns seen in suspensions incubated for 1 h in the presence of FPP were not as marked as those seen in control suspensions incubated for 22 h.

**Series III. Are structurally similar tripeptides as effective as FPP in promoting capacitation?**

The effect on capacitation of two peptides structurally related to FPP (pGlu-Glu-ProNH$_2$), TRH (pGlu-His-ProNH$_2$) and pyroglutamyl phenylalanylprolineamide (pGlu-Phe-ProNH$_2$), was compared with that of FPP, again using CTC analysis. We have already evaluated the responses of mouse spermatozoa to a range of concentrations of TRH and pGlu-Phe-ProNH$_2$ and observed significant responses only at $\geq 100$ nM (TRH, Green et al., 1994; pGlu-Phe-ProNH$_2$, C.Green, unpublished results). Because FPP stimulates both mouse and human sperm cells at $\geq 25$ nM, it was felt that human and mouse spermatozoa would probably show similar concentration-dependent responses to the related tripeptides. Therefore, we chose to
Figure 2. Changes in chlortetracycline (CTC) patterns in human sperm suspensions incubated for 1 h in the presence of 25–200 nM fertilization promoting peptide (FPP). Data are presented as means ± SEM (n = 8). White bars = F; black bars = B; shaded bars = AR. (*) P < 0.05; (**) P < 0.025; (***) P < 0.01; compared with untreated control suspensions incubated for 1 h (Con-22h).

Figure 3. Changes in chlortetracycline (CTC) patterns in human sperm suspensions incubated for 1 h in the presence of 50 and 100 nM fertilization-promoting peptide (F), thyrotrophin-releasing hormone (T) or pGlu–Phe–ProNH₂ (P). Data are presented as means ± SEM (n = 5). White bars = F; black bars = B; shaded bars = AR. (*) P < 0.05; (**) P < 0.025; (***) P < 0.01; compared with untreated control suspensions incubated for 1 h.

Series IV. Does FPP treatment increase acrosomal exocytosis in response to progesterone?

Before trying the HOPT, it was necessary to determine whether FPP-treated spermatozoa could be stimulated to undergo acrosomal exocytosis. To do this we tried progesterone, a component of follicular fluid and a natural agonist known to stimulate acrosomal exocytosis in human spermatozoa (Osman et al., 1989; DasGupta et al., 1994). Sperm suspensions (from known fertile donors) were prepared as described above and divided in two. One suspension received FPP at 100 nM and the other received only medium. Suspensions were pre-incubated for 1 h, at which point a sample from each was treated with 10 µg/ml progesterone. After the addition of progesterone, incubation was continued for a further 15 min (n = 7).

As in the previous series of experiments, FPP treatment stimulated capacitation but not acrosomal exocytosis (P < 0.01). However, the subsequent addition of progesterone resulted in a significant increase in the proportion of AR-pattern cells when compared with the untreated controls (P < 0.01; Figure 4). Progesterone also promoted a slight stimulation of capacitation, consistent with responses reported by DasGupta et al. (1994).

Series V. Are FPP-treated cells more fertile in the HOPT?

Sperm suspensions (from known fertile donors) were prepared as described in series IV. Once again, one suspension received FPP at 100 nM and the other received only medium. These suspensions were then pre-incubated for 1 h, at which point a sample from each was treated with 5 µg/ml progesterone. We used 5 µg/ml progesterone instead of 10 µg/ml progesterone as in series IV so as to increase the difference in the response obtained in suspensions treated with FPP plus progesterone when compared with those treated with progesterone alone. The resulting four suspensions were then incubated for a
Further 15 min, at which point a CTC analysis of the suspensions was carried out. They were then diluted to give a final concentration of $5 \times 10^5$ cells/ml. This sperm concentration was chosen deliberately to ensure that the number of acrosome-reacted cells would be below that needed to obtain maximal penetration; in this way, differences in the proportions of functionally competent cells could be detected most readily. Zona-free hamster oocytes were added and the gametes were coincubated for 3 h ($n = 4$).

The results indicated that the presence of FPP and progesterone consistently and significantly stimulated fertilizing ability in the HOPT. There was a significantly higher proportion of penetrated oocytes in the suspensions treated with both FPP and progesterone when compared with the untreated control suspensions ($P < 0.001$; Figure 5a). There was also a significantly higher proportion of penetrated oocytes when the suspensions treated with FPP plus progesterone were compared with those treated with FPP or progesterone alone ($P < 0.001$).

CTC analysis of the sperm suspensions after the addition of progesterone indicated responses similar to those seen in the previous series of experiments (Figure 5b). There was a significant increase in the proportion of AR-pattern cells in FPP-treated suspensions in response to progesterone when compared with the untreated control suspensions ($P < 0.025$).

The results did not reveal any differences in the incidence of polyspermy between the different treatment groups. The mean number of penetrating spermatozoa per penetrated oocyte was in the range 1.0–1.3. There were some detectable differences between treatment groups in the mean number of spermatozoa attached to the penetrated oocytes; these seemed to reflect the proportion of acrosome-reacted cells, as seen in the CTC results. The mean number of attached spermatozoa per penetrated oocyte was 1.3 in untreated controls, 7.9 in suspensions treated with progesterone only, 3.8 in suspensions treated with FPP only and 16.0 in suspensions treated with FPP plus progesterone.

Discussion

Our previous data have demonstrated that a TRH-like tripeptide with the structure pyroglutamyl glutamylprolineamide could stimulate the capacitation and fertilizing ability of epididymal mouse spermatozoa in vitro. On the basis of these results, we have proposed that the peptide be referred to as fertilization promoting peptide (FPP). Here we have investigated the effect of FPP on the functional status of ejaculated human spermatozoa in vitro. Because it was necessary to use ejaculated cells, i.e. those already exposed to FPP, we were uncertain as to whether we would observe any effects. The manipulations used to prepare motile sperm suspensions, which included centrifugation steps, might have removed some or all of the FPP from the cells, but this was unknown.

Despite these potential difficulties, FPP proved to have a positive effect on human sperm cells. The experimental results obtained were very similar to those seen with mouse spermatozoa, and suggest that FPP can also stimulate the capacitation and fertilizing ability of human spermatozoa in vitro. As in the mouse, FPP had a significant stimulatory effect on human sperm capacitation at all concentrations used between 25 and 200 nM. This was shown by a shift from the F pattern of CTC staining, characteristic of uncapacitated, acrosome-intact cells, to the B pattern of staining, characteristic of capacitated, acrosome-intact cells. The response was seen early, within 1–3 h of the start of incubation, and did not appear to be concentration dependent; there was no significant stimulation of acrosomal exocytosis. This lack of stimulation of acrosomal exocytosis is of functional importance because in vivo the acrosome reaction would be triggered by oocyte-associated agonists. Given that the stimulation of capacitation occurred at a range of concentrations similar to the concentration of FPP detected in human seminal plasma (~50 nM; Cockle et al.,
With reference to the CTC analysis of human spermatozoa, a recent study (Perry et al., 1995) reported a range of CTC patterns which differ considerably from those that we observed and have used here. These authors suggested that the discrepancies might reflect differences in the methods used for initial sperm preparation (swim-up versus mini-Percoll) and differences in the fertility status of the donors. We have compared the two methods of sample preparation and have observed the same CTC patterns described originally (DasGupta et al., 1993) and the same distribution of patterns in both samples (S.DasGupta, unpublished observations). Furthermore, all the donors used in our study had good semen profiles, and those used in series IV and V were known fertile donors.

A comparison of FPP with two structurally similar tripeptides, TRH and pyroglutamyl phenylalanine-prolineamide, at 50 nM (the mean concentration of FPP in human seminal plasma) indicated that only FPP could stimulate capacitation significantly. In somatic cells, TRH is known to exert its effects via receptor-mediated signal transduction pathways (e.g. Gershengorn, 1989); given the structural similarities of TRH and FPP, it is plausible that FPP acts in a similar manner. These results, however, suggest that FPP may be acting at a receptor distinct from that for TRH. This hypothesis is supported by other studies showing that it is the central histidine residue of TRH which is important for the binding of TRH to its specific receptor (Hinkle, 1989) and that FPP cannot displace [³H]TRH or [³H]MelTRH from GH3 cells (growth hormone-secreting cells of pituitary origin; Ashworth et al., 1993). There are two proposed mechanisms of action for TRH: (i) the activation of polyphosphoinositide-specific phospholipase C hydrolysis of phosphatidylinositol-4,5-bisphosphate to give inositol-1,4,5-trisphosphate, which in somatic cells stimulates the release of stored Ca²⁺, and diacylglycerol, which activates protein kinase C in somatic cells (Gershengorn, 1989); and (ii) the direct activation of adenylyl cyclase with a resulting increase in the formation of cyclic AMP (Gordeladze et al., 1988). At present, little is known about the mode of action of FPP.

From the results indicating a stimulatory effect of FPP on human sperm capacitation, we predicted that FPP-treated cells would become fertile more quickly. This was tested using the HOPT. Because this assay requires acrosome-reacted cells, we introduced progesterone to FPP-treated suspensions to trigger the acrosome reaction. Cells treated with FPP plus progesterone proved to be significantly more able to penetrate oocytes than their untreated counterparts when used in the HOPT. While it is true that the HOPT does not completely mimic all the events involved in the fertilization of zona pellucida-intact human oocytes, the fact that FPP can significantly stimulate both fertilizing ability in mouse spermatozoa and penetrating ability in human spermatozoa suggests that the peptide could play a similar stimulatory role in vivo in both species. It has been argued that capacitation may be important in preventing mammalian spermatozoa from becoming fertile too quickly, given that sperm cells are deposited in the female reproductive tract at a considerable distance from the site of fertilization (Bedford, 1983). Current evidence indicates that spermatozoa acquire inhibitory surface-associated molecules (decapacitation factors) of an either epididymal or seminal plasma origin which are then lost during capacitation, resulting in the acquisition of fertilizing ability (Oliphant et al., 1985).

Since FPP is also of a seminal plasma origin, what is a plausible, biologically relevant role for this tripeptide? Because FPP treatment accelerates the F to B transition in CTC patterns but does not rapidly transform an uncapacitated population into a fully capacitated one, we feel that FPP helps to promote the various early events involved in capacitation. Like decapacitation factors, FPP may well bind to the sperm cells when they contact seminal plasma and remain bound following ejaculation. In the female tract, decapacitation factors are lost, leaving FPP to exert a stimulatory effect on capacitation. Further stimuli are needed before cells can undergo the acrosome reaction.

Because of the relatively high concentrations of FPP found in seminal plasma, we suggest that FPP may provide human spermatozoa with a stimulus that promotes capacitation following deposition in the female tract. In some men, impaired fertility might reflect insufficient FPP or a defective FPP–sperm interaction.

Acknowledgements

We would like to thank Dr Eileen McLaughlin for advice on protocols for the HOPT. This study was supported by Wellcome Project Grant 036810 awarded to P.F.W., L.R.F. and S.M.C.

References


Gordeladze, J.O., Bjørø, T., østberg, B.C. et al. (1988) Phorbol esters and thyroliberin have distinct actions regarding stimulation of prolactin secretion
and activation of adenylate cyclase in rat pituitary tumour cells (GH4C1 cells). Biochem. Pharmacol., 37, 3133–3138.


Received on October 4, 1995; accepted on January 16, 1996