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SYNTHETIC PEPTIDES AS CHEMOATTRACTANTS FOR BULL SPERMATOZOA

STRUCTURE ACTIVITY CORRELATIONS

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SUMMARY. The ability of various synthetic peptide analogs of Formyl-Met-Leu-Phe to induce chemotaxis in bull sperm is compared using an inverted capillary assay. The formyl group is essential for chemotactic activity and corresponding t-butyloxycarbonyl tripeptides are inactive. Sequence analogs, Formyl-Met-Phe-Leu, Formyl-Leu-Met-Phe and Formyl-Leu-Phe-Met are active. Replacement of Met and Leu by Pro does not diminish activity. Formyl-Met-Leu-Phe-NH_2 is active suggesting that electrostatic interactions involving the carboxyl group may be unimportant in receptor interactions. The studies establish the importance of an amino terminal formyl group and a sequence of at least three hydrophobic residues, for inducing sperm chemotaxis.

INTRODUCTION

Formyl-Met-Leu-Phe (FMLP)[1], a synthetic tripeptide has been shown to induce chemotaxis and lysosomal enzyme release in neutrophils (1,2). Recent studies in this laboratory have established that FMLP causes acrosomal enzyme release and induces chemotaxis in bull sperm (3). These observations are of particular interest because of the possible involvement of chemical factors in the control of directed motility (4) and in the induction of the acrosome reaction in sperm (5,6). The availability of synthetic peptides with such biological activities will provide excellent model systems

[1] Abbreviations: FMLP, Formyl-Met-Leu-Phe; DCC, dicyclohexyl carbodiimide; FPLP, Formyl-Pro-Leu-Phe; BMLP, t-butyloxycarbonyl-Met-Leu-Phe; BPLP, t-butyloxycarbonyl-Pro-Leu-Phe; Boc, t-butyloxycarbonyl; KRB, Krebs-Ringer's bicarbonate buffer.

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for the in vitro study of these properties of sperm cells. In this report we examine the chemotactic activities of bull sperm towards a number of synthetic analogs of FMLP, with a view towards delineating the structural requirements for biological activity.

MATERIALS AND METHODS

All peptides listed in Table 1 were synthesised by solution phase procedures and checked for homogeneity by TLC. Structures were established by 60 MHz and 270 MHz ^1H NMR. Carboxyl activation of Boc protected amino acids was carried out using dicyclohexylcarbodiimide (DCC) in methylene chloride. Dipeptide acids were activated with DCC and 1-hydroxybenzotriazole in dimethylformamide. Boc group removal in dipeptides was accomplished with trifluoroacetic acid. Conversion of the Boc-tripeptide acids to the corresponding formyl derivatives was effected by a one pot procedure using anhydrous formic acid and acetic anhydride. Details will be published elsewhere.

Chemotaxis and motility of sperm were assayed using an inverted capillary method (7). Glass capillaries (1mm internal diameter) were sealed at one end, passed several times over a flame and plunged open end down, into a beaker containing the test solution. The capillary thus filled was then inserted into a small beaker containing the sperm suspension in egg yolk-citrate buffer (5×10^7 cells/ml) and allowed to stand for 3 hours at 37°C. The capillaries were then removed and the number of cells inside were estimated microscopically with a hemocytometer. Peptide solutions were prepared in Krebs-Ringer's bicarbonate buffer (pH 7.4).

RESULTS AND DISCUSSION

The results in Table 1 clearly show that bull sperm tend to move towards FMLP when a concentration gradient exists. This is evident from the large increase in the number of cells entering a capillary containing FMLP. Fig.1 shows the percent increase in cells in the capillary as a function of time. It can be seen that there is an increase with the time of contact between the capillary and sperm suspension. For purposes of comparison of the various peptides a contact time of 3 hours was

TABLE 1

Chemotactic effect of synthetic peptides on bull sperm*

Peptide	Concentration of peptide (μM)			
	10	20	40	80
F-Met-Leu-Phe	+75±16.5	+133±36	+170±53	+235±56
F-Met-Leu-Phe-OMe	+9±3	+33±7	+54±14	+12±6
F-Met-Leu-Phe-NH_2	+38±8	+85±18	+114±21	+175±29
F-Met-Phe-Leu	-48±9	+226±32	+255±41	+235±31
F-Leu-Phe-Met	+21±6	+150±41	+131±29	+111±45
F-Leu-Met-Phe	+65±36	+71±14	+126±46	+203±43
F-Met-Pro-Phe	+354±58	+238±29	+285±41	+228±27
F-Pro-Leu-Phe	+97±49	+106±57	+112±60	+192±57
Boc-Met-Leu-Phe	-15±2	-24±8	-39±23	-32±5
Boc-Pro-Leu-Phe	-20±4	-34±15	-47±22	-53±7
Boc-Met-Phe	-43±7	-45±11	-34±11	-29±9
Boc-Phe-Met	-11±5	-15±4	-41±16	-73±26
Boc-Phe-Leu	-44±16	-74±19	-71±26	-80±27
F-Met-Phe	-31±9	-52±17	-4±2	-4±1

* The values represent the percentage increase (+) or decrease (-) in the number of cells entering the capillary containing the peptide over the control capillary containing KRB buffer (pH 7.4).

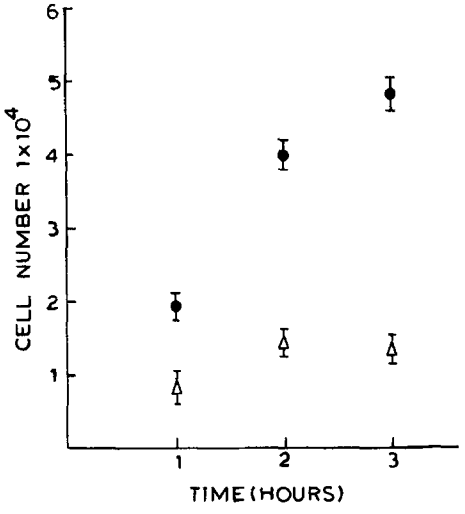

Fig.1. Time dependent chemotactic response of bull sperm to FMLP. ● capillaries contained 40 M FMLP. Δ capillaries contained only KRB pH 7.4 buffer.

chosen and the results are summarised in Table 1. While all the formyl tripeptides appeared to induce chemotaxis Boc-tripeptides and dipeptides resulted in a decrease in the number of cells entering the capillary. While the concentration dependence and the magnitude of the chemotactic response was slightly different for each tripeptide, it is clear that the various formyl analogs are biologically active. The effects observed for FMLP, Formyl-Met-Phe-Leu, Formyl-Leu-Met-Phe and Formyl-Leu-Phe-Met suggest that specific sequence may not be particularly important, but the presence of three consecutive hydrophobic residues may be critical. Studies on the effect of formyl peptides in inducing lysosomal enzyme release and chemotaxis in polymorphonuclear leukocytes (neutrophils) have shown that while FMLP is the most active peptide, Formyl-Met-Met-Phe and Formyl-Met-Met-Met-Met are also quite active (1). Further the isosteric analog obtained by replacing Met by norleucine is also active, suggesting that the sulfur atom in the position 1 side chain is not essential for inducing chemotaxis in neutrophils (8). While a direct correlation between the activities of these peptides in inducing chemotaxis in neutrophils and sperm is not possible with the available data, it is likely that both effects are mediated by interactions with a membrane bound receptor.

From Table 1 it is seen that both Formyl-Met-Pro-Phe and Formyl-Pro-Leu-Phe show high biological activity. A similar retention of activity on replacement of Met and Leu residues by Pro has also been observed in the case of the enkephalins (9). This suggests that the

flexible hydrophobic side chains of the Met and Leu residues may in fact be folded at the receptor site in a manner that can be mimicked by the rigid pyrrolidine ring of Pro. Esterification of the carboxylic acid group in FMLP results in a lowering of chemotactic activity in sperm. However the retention of some activity suggests that the carboxylate group may not be essential for receptor interactions and argues against very significant contributions from electrostatic effects. The relatively high activity observed for Formyl-Met-Leu-Phe-NH_2 supports this conclusion and suggests that hydrogen bonding between the terminal -COOH or -$CONH_2$ group of the peptide and appropriate functions on the receptor may be important. The hexapeptide Formyl-Nle-Leu-Phe-Nle-Tyr-Lys is a potent chemoattractant for human neutrophils (10), an observation that again emphasises that blocking of the Phe carboxyl group does not destroy activity. The chemotactic effect of FMLP and its analogs on bull sperm has been measured at four concentrations ranging from 10-80 μM. It may be noted that the concentration dependence varies with the structure of the peptide. This may result from aggregation of the hydrophobic peptides in aqueous solution.

Enhanced movement in motile cells induced by chemical stimuli may be the result of chemotactic (directional movement down a chemical concentration gradient) or chemokinetic (enhanced random movement) effects (11). Table 2 summarises the results of capillary assay performed under different conditions for FMLP, Formyl-Pro-Leu-Phe and the corresponding Boc analogs. The enhancement in the number

TABLE 2

Response of bull sperm to peptides under various conditions[+]

Assay conditions		% Cells entering capillary	Assay conditions		% Cells entering capillary
Sperm suspension	Capillary		Sperm suspension	Capillary	
KRB	KRB	100 (25)	BMLP (20 μM)	BMLP (20 μM)	82±9 (9)
FMLP (20 μM)	FMLP (20 μM)	130±20(9)	BMLP (40 μM)	BMLP (40 μM)	73±9 (12)
FMLP (40 μM)	FMLP (40 μM)	140±20(9)	BMLP (20 μM)	KRB	65±22(15)
FMLP (20 μM)	KRB	195±41(12)	BMLP (40 μM)	KRB	76±18(15)
FMLP (40 μM)	KRB	129±31(12)	KRB	BMLP (20 μM)	76±8 (15)
KRB	FMLP (20 μM)	233±36(25)	KRB	BMLP (40 μM)	61±23(15)
KRB	FMLP (40 μM)	270±53(25)	BPLP (20 μM)	BPLP (20 μM)	72±16(9)
FPLP (20 μM)	FPLP (20 μM)	137±19(9)	BPLP (40 μM)	BPLP (40 μM)	64±6 (9)
FPLP (40 μM)	FPLP (40 μM)	150±22(9)	BPLP (20 μM)	KRB	89±8 (9)
FPLP (20 μM)	KRB	154±14(12)	BPLP (40 μM)	KRB	81±9 (9)
FPLP (40 μM)	KRB	141±13(12)	KRB	BPLP (20 μM)	66±15(12)
KRB	FPLP (20 μM)	206±57(18)	KRB	BPLP (40 μM)	53±22(12)
KRB	FPLP (40 μM)	212±60(18)			

[+] Approximately 1×10^5 cells entered the control capillary (case 1). Values are an average over the number of experiments indicated in the parentheses.

of cells entering the capillary compared to buffer controls when FMLP is present both inside and outside the capillary or when FMLP is present only in the suspension may be due to enhanced random motility. This chemokinetic effect is also exhibited by Formyl-Pro-Leu-Phe. However it is significant that the number of cells entering the capillary is substantially greater when these peptides are present only in the capillary. This strongly suggests that the sperm cells are indeed sensing a concentration gradient and that the chemotactic effect is superimposed on a general chemokinetic effect. Both Boc-Met-Leu-Phe and Boc-Pro-Leu-Phe cause a decrease in the number of cells entering the capillary as compared to the control value in all cases. This may reflect a motility inhibiting effect as well as a chemorepellent activity towards bull sperm.

The results described above establish that Formyl-Met-Leu-Phe and related formyl tripeptides are chemoattractants for bull sperm. Chemotactic activity is abolished on replacement of the formyl group by a Boc group. Conversion of the terminal carboxyl group to an amide results in retention of activity as does interchange of the Met, Leu and Phe residues. The replacement of Met and Leu residues by Pro yields highly active analogs. These observations suggest that hydrophobic formyl peptides may be capable of interacting with receptors on the sperm cell surface. The stereochemical requirements for peptide cell interaction would be better defined by a study of conformationally constrained analogs. Studies in this direction are presently in progress.

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