Biochemical Pharmacology, Vol. 31, No. 19, pp. 3071–3076, 1982.
Printed in Great Britain.

0006-2952/82/193071-06 $03.00/0
© 1982 Pergamon Press Ltd.

CHEMOKINETIC ACTIVITY OF *N*-FORMYL-METHIONYL-LEUCYL-PHENYLALANINE ON HUMAN NEUTROPHILS, AND ITS MODULATION BY PHENYLBUTAZONE

AXEL PERIANIN, MARIE-THÉRÈSE LABRO and JACQUES HAKIM*
Laboratoire d'Immunologie et d'Hématologie, Centre Hospitalo-Universitaire Xavier Bichat, Université Paris 7, Paris, France

(*Received* 8 *February* 1982; *accepted* 14 *April* 1982)

Abstract—Phenylbutazone (PBZ) is known to inhibit the oriented migration of human polymorphonuclear leukocytes (PMNs) induced by formyl-methionyl-leucyl-phenylalanine (FMLP), and to protect these cells against the deactivation caused by their prior incubation with FMLP. To gain insight into the mechanism of these effects, we measured the oriented PMN migration under agarose induced, in the presence and absence of PBZ, by FMLP, zymosan-activated serum and *Klebsiella pneumoniae* culture supernatant. The two components of this migration, i.e. the speed (chemokinesis), and direction of locomotion (chemotaxis), were also assessed.

At concentrations ranging from 10^{-8} to 10^{-5} M, FMLP displayed similar chemotactic activity but the speed of PMN locomotion was maximal for 10^{-7} M, and lower for concentrations above and below this level. Oriented migration was proportional to the mean cell locomotion speed during the experiments. PBZ inhibited both the oriented migration and locomotion speed induced by 10^{-7} M FMLP, but did not affect its chemotactic activity. At concentrations of 10^{-6} and 10^{-5} M, PBZ increased oriented migration and locomotion speed, again without influencing FMLP chemotactic activity. Oriented migration induced by zymosan-activated serum was not affected by PBZ but the migration induced by *Klebsiella pneumoniae* culture supernatant diminished slightly. These results demonstrate that PBZ modulates the chemokinetic effect of FMLP on PMNs and thus alters oriented PMN migration.

The non steroidal anti-inflammatory drug, phenylbutazone (4-butyl-1,2-diphenyl-3,5 pyrazolidine dione, PBZ), was recently shown to antagonize the interaction of *N*-formyl-methionyl-leucyl phenylalanine peptides (FMLP) with their polymorphonuclear (PMN) leukocyte receptors [1, 2]. These results showed that PBZ affects specifically the effects of FMLP on human PMN locomotion. To further substantiate this point and to define more precisely the characteristics of these effects we analysed the *in vitro* effects of PBZ on human PMN locomotion, using the agarose method [3–5]. We began by studying the influence of PBZ on random and oriented PMN migration. The latter migration, conventionally measured by the PMN front lead of migration after a fixed incubation period [3–5], was induced by several attractants, including FMLP, zymosan-activated serum (ZAS) and *Klebsiella pneumoniae* culture supernatant (KPCS). The results reported here showed that PBZ effectively and specifically modulates FMLP-induced oriented migration. To further explore whether this modulating effect was due to the influence of PBZ on the chemokinetic effect of FMLP (locomotion speed) or on its chemotactic effect (direction of locomotion), these two effects were assessed by single cell analysis. This was done because both parameters affect the resulting oriented migration conventionally measured by the cell front lead of migration [6]. The present results demonstrate that, under our experimental conditions, PBZ modulates FMLP-induced locomotion by acting predominantly, if not exclusively, on the chemokinetic activity of FMLP.

* Author to whom all correspondence should be addressed: J. Hakim, Laboratoire d'Immunologie et d'Hématologie, C.H.U. Xavier Bichat, 46 rue Henri Huchard, 75018 Paris, France.

MATERIALS AND METHODS

Drug and chemoattractants. Phenylbutazone (a gift from Ciba-Geigy, Basel, Switzerland) was dissolved in 0.5 N sodium hydroxide (mole per mole) and diluted to the desired concentrations in 0.1 M Krebs–Ringer phosphate buffer (KPB), pH 7.4, for immediate mixing with the agarose, chemoattractants or PMN suspension. Chemoattractants were FMLP, ZAS or KPCS. FMLP (Sigma Chemical Co., St. Louis, Mo., U.S.A.) was prepared as stock solution at 10^{-3} M in 0.15 N NaOH; aliquots of this solution were stored at −80° until use. When needed, an aliquot was thawed and diluted in KPB for immediate use. One batch of KPCS was obtained from growing non-virulent *Klebsiella pneumoniae* (strain No. 53133 from the Institut Pasteur, Paris, France). For this purpose, *Klebsiella* were first grown in a nutritive solid culture medium (gelose nutritive No. 54476, Institut Pasteur), and then in liquid medium (Eau peptonée No. 54175, Institut Pasteur). After high speed centrifugation, the supernatant was stored in aliquots at −80°, and was used throughout all experiments. KPCS-induced oriented migration under agarose of this batch was 1.9 mm ± 5% with control PMNs. ZAS was prepared as follows: 0.9 ml of fresh pooled human serum was added to 0.1 ml

of preboiled zymosan (Sigma Chemical Co.) at 4% in 0.9% NaCl, and shaken for 30 min at 37°. Zymosan particles were then separated from the activated serum, and the supernatant was used as chemoattractant.

Isolation of PMNs. One volume of human venous blood, heparinized at 10 IU/ml blood, was carefully layered in an inverted plastic syringe over one volume of a mixture of Dextran T 500-(Pharmacia, Uppsala, Sweden) and Radio Selectan (Sherring Lab., U.S.A.) comprising 24 vol. of 9% dextran in 0.9% NaCl, and 10 vol. of 38% Radio-Selectan. After sedimentation of the red blood cells for 40 min at room temperature, the leukocyte-rich supernatant was removed and centrifuged at 300 *g* for 8 min. The resulting cell pellet was washed twice with Ca^{2+} and Mg^{2+}-free KPB, pH 7.4, and suspended in the same medium at approximately 10^8 PMNs per ml.

Conventional technique for measurement of PMN locomotion. Spontaneous (random) and oriented migrations were measured by the previously described modified version of the agarose technique [4, 5]. Briefly, 4 ml of 0.75% (w/v) indubiose (A 37 from Industrie Biol. Française, France) in KPB, pH 6.8, containing 10% of heat-inactivated fetal calf serum, was poured into a small tissue culture Petri dish (Falcon 15 × 55 mm, Lab. Exp. Service, France). When needed, PBZ was mixed at the desired concentration with the indubiose preparation. Four sets of 3 wells, 2.5 mm in diameter and spaced 2.5 mm apart, were cut out using a plexiglass template as a guide. Five μl of the leukocyte suspension was placed in each of the middle wells (PMN wells), 5 μl of chemoattractant in the outer wells (chemoattractant wells), and 5 μl of KPB in the inner wells (control wells).

When ZAS or KPCS were used as chemoattractans, they were added to the wells 30 min before addition of PMNs, and incubated at 37° in 95% humidified air and 5% CO_2. This was done because preliminary studies (results not shown), showed that it led to more reproducible results than when these two attractants were added to the wells at the starting of the assay. This was not the case for FMLP, which was added to the wells just before PMNs and the starting of the assay. For the migration assays, plates were incubated at 37° in a 95% air/5% CO_2 humidified atmosphere, for 90 min in the case of FMLP-induced oriented migration, and for 180 min in that of ZAS or KPCS-induced oriented migration. Random and oriented migrations were measured as the front lead of migration (at least 10 PMNs) under the microscope (magnification 2.4 × 10) using a calibrated eyepiece, i.e. as the distance traversed by the cells from the border of the middle well towards the chemoattractant wells (oriented migration) or the control wells (random migration).

Single cell locomotion. Single cell locomotion was assessed by adapting the agarose method to continuously monitor cell movement under the microscope. The Petri dish containing the agarose was thermostatically controlled *in situ* under the microscope by partial immersion in a circulating water bath. To avoid dessication, agarose was covered with transparent plastic sheeting 10 min after the beginning of the experiment. Cell displacement was recorded at 2-min intervals. Control experiments showed that, under these conditions, random migration after 90 min was similar to that measured by the conventional method, and that FMLP-induced oriented migration decreased by 20% at most. The 10 × 10 grid used in the eyepiece of the microscope for single cell analysis (magnification 4 × 10) allowed precise location of the position of the cells and their movement, and therefore assessment of their direction (chemotaxis) and speed (chemokinesis) under various experimental conditions. Mean locomotion speed was measured by adding the pathways covered by each cell in 4 min whatever its direction, and dividing this sum by the total period of measurement, usually 70 min, i.e. 90 min minus 20 min (the initial lag time for separation of the cells to be analysed. Among the cells which were separated from the bulk of the other cells, 2–4 cells per experiment were randomly chosen in order to be followed. The total number of cells which were required to assess locomotion speed in each of the experimental conditions are given in Table 1. Pathways covered in 4 min instead of 2 min were chosen in order to be suitably represented in Fig. 4.

Statistics. Mean and standard deviations were calculated for the different series of experiments (see Table and Figures). Paired or unpaired Student's *t*-tests were used to assess differences between control and experimental values.

RESULTS

PBZ-inhibition of FMLP-induced oriented migration

As shown in Fig. 1, FMLP-induced oriented migration of PMNs measured after 90 min was maximum when the concentration of the FMLP added to the attractant well was 10^{-7} M. Above and below this concentration, PMN migration towards the attractant well decreased and the resulting migration patterns differed as previously reported [7].

The effect of various concentrations of the PBZ in the agarose on the migration induced by 10^{-7} M FMLP (i.e. maximum migration) is illustrated in Fig. 1. FMLP-induced migration was inversely related to the concentration of the PBZ in the gel. The PBZ concentration which inhibited 50% of the oriented migration induced by 10^{-7} M FMLP was 33 μM. FMLP-oriented migration was equal to random migration for 66 μM of PBZ in the gel, but was not further reduced by increasing the PBZ to 165 μM. Random migration was not significantly affected by the various concentrations of PBZ in the gel, suggesting that this drug is not cytotoxic under our experimental conditions. To confirm this property we pretreated PMNs suspended in KPB with various PBZ concentrations ranging from 17 to 165 μM for 15 min at 37°. The PMNs were then washed and tested for random and oriented migration in response to 10^{-7} M of FMLP. No difference was observed between the PMNs incubated with PBZ and those incubated without it (paired data; results not shown). The addition to the attractant wells of PBZ concentrations ranging from 17 to 330 μM instead of FMLP did not affect PMN migration, which was similar to random migration. The results of further experiments reported below constituted additional evi-

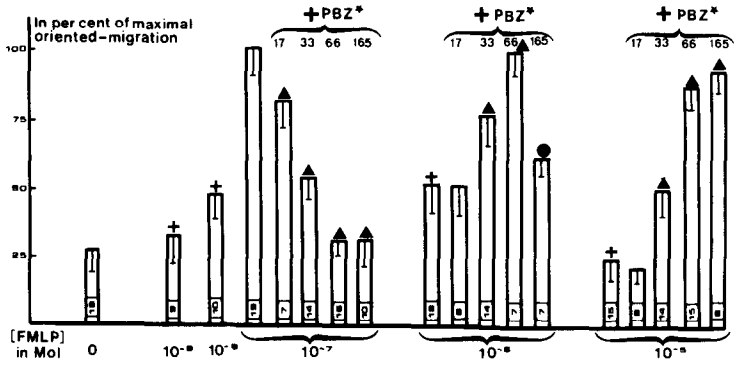

Fig. 1. Effect of phenylbutazone on FMLP-induced PMN migration. Maximal oriented migration (100%) obtained with 10^{-7} M FMLP added to the attractant well, corresponds to a cell migration of 2.16 ± 0.34 mm. Each bar indicates the mean ± S.D. of the per cent of maximal oriented migration. The number of migrations measured in each assay is indicated in the bars. Phenylbutazone (★) is incorporated into the agarose gel at the final concentrations indicated (μM). Significant differences ($P < 0.001$) between migrations obtained in the absence of drug, compared to maximal control values (FMLP = 10^{-7} M) are designated by +. A statistically significant difference in the effects of PBZ was observed between oriented migration in the presence and absence of the drug in the same series of migration experiments, and is designated by ▲ ($P < 0.001$) and by ● ($P < 0.01$).

dence that the effect of PBZ on PMNs was not cytotoxic. Taken together, the above results suggested that PBZ inhibited oriented migration induced by 10^{-7} M FMLP, but had no effect on movement *per se*. They did not show, however, whether the PBZ acted on the chemotactic or the chemokinetic effect of FMLP. This point is further analysed below.

PBZ stimulation of FMLP-induced oriented migration

In contrast to its inhibitory effect on the oriented migration induced by 10^{-7} M FMLP, PBZ restored optimal migration at higher FMLP concentrations (10^{-6} or 10^{-5} M) which were less effective than 10^{-7} M (Fig. 1). When there was no PBZ in the gel, the migration induced by 10^{-6} M FMLP was approximately half that observed with 10^{-7} M. Concentrations of 33 or 66 μM PBZ in the gel respectively increased oriented migration to 75 and 100% of the one observed with 10^{-7} M FMLP. Raising the PBZ concentration in the gel to 165 μM was less effective (Fig. 1), suggesting that the ratio of FMLP to PBZ might crucially affect the action of PBZ on FMLP-induced oriented migration of PMNs. This was confirmed when FMLP was used at 10^{-5} M in the attractant well. Figure 1 shows that the oriented migration induced by 10^{-5} M FMLP gradually rose with the PBZ concentration in the agarose gel. This migration was close to the one obtained with 10^{-7} M in the control experiment in the absence of PBZ. It was also the one observed when the PBZ concentration was 165 μM, the maximum tested here. To exclude the possibility that PBZ might exert a direct effect on FMLP, the two substances were mixed at all the concentrations used in such a way as to obtain the same final concentration as that of the preceding experiments, and incubated for 10 min at 37° before being added to the attractant well. PMNs migration was not significantly different under these conditions from that observed with FMLP but without PBZ (paired data; results not shown).

Effect of PBZ on oriented migration induced by ZAS or KPCS

The observed ability of PBZ to modulate FMLP-induced oriented migration suggested either that PBZ might interfere nonspecifically with the response of PMNs to an attractant, or that it might be specific to FMLP and related compounds. To test these possibilities indirectly, and to find out how PMNs interact with a complement-derived attractant—$C5a_{des\text{-}Arg}$ obtained from zymosan-activated serum (ZAS)—and with the little studied microbial culture supernatant of *Klebsiella pneumoniae* (KPCS), we measured the effect of PBZ on PMN-oriented migration in response to ZAS and KPCS. When PBZ was included in the gel at concentrations ranging from 33 to 330 μM it had no significant effect on either random or ZAS-oriented migration. In contrast, increasing PBZ concentrations in the gel progressively inhibited KPCS-induced oriented migration (Fig. 2), which, however, remained higher than random migration, even when the PBZ concentration in the gel reached 330 μM. PBZ inhibited oriented migration less markedly than FMLP. The PBZ concentration which inhibited 50% of the KPCS-induced oriented migration was 165 μM, i.e. five times higher than that required for FMLP inhibition. Preincubation of KPCS with PBZ before its addition to the attractant well had no effect on KPCS induced migration.

The fact that PBZ did not affect ZAS-induced oriented migration confirmed that it was not cytotoxic and did not act on FMLP-induced migration through modification of the agarose, and suggested that PBZ did not exert any non-specific action on the machinery of PMN movement. The results observed with KPCS suggested that this attractant,

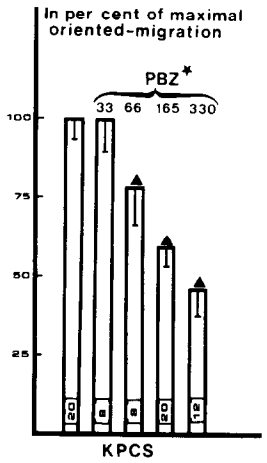

Fig. 2. Effect of PBZ on KPCS-induced PMN migration. Results are expressed as the per cent of untreated controls. Maximal oriented migrations measured after incubation for 180 min was 1.92 ± 0.12 mm. Mean and S.D. values were calculated from the number of migrations indicated in the bars. PBZ (★), included in the agarose gel, was used at the indicated concentration (μM). A significant difference between oriented migration obtained in absence and in presence of PBZ is designated by ▲ ($P < 0.001$).

like FMLP, lost some of its effect on PMNs through the action of PBZ. Further studies are needed to define the factors involed in KPCS attractant properties; some of these factors might also apply to FMLP.

Effect of the combined addition of PBZ and PMNs to PMN wells

PMNs were mixed with various concentrations of PBZ and preincubated for 10 min at 37° before being added, still suspended with PBZ in KPB, to the PMN wells. Results of these experiments are reported in Fig. 3. The oriented migrations induced by 10^{-7} M FMLP and KPCS were both reduced by PBZ but to a much lesser extent than when PBZ was included in the gel (Figs 1 and 2). No effect was observed either on random migration or on ZAS-induced oriented migration. These results suggested that if the PBZ acted by binding to PMN membrane receptors; as recently reported [1, 2], such binding was reversible. This was confirmed by washing the suspension of PBZ and PMNs before its addition to the wells, thereby eliminating the inhibitory effect of PBZ.

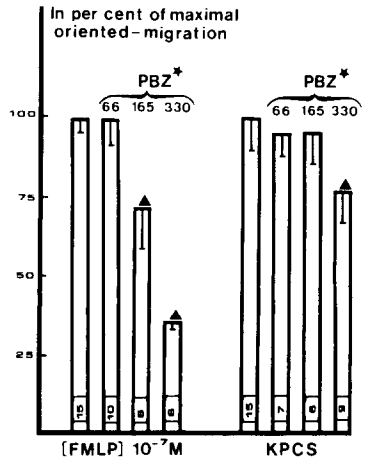

Fig. 3. Effect of PBZ added to the PMN suspension on FMLP and KPCS-induced migration. Results are expressed as the per cent of untreated controls. PMNs were treated for 10 min at 37° with the drug at different concentrations (★), and added to the PMN wells which still contained the drug. Bars denote the mean ± S.D. of the per cent of maximal oriented migration (control). Maximal FMLP induced migration was 2.13 ± 0.22 mm after incubation for 90 min. The maximum migration induced by KPCS was 1.77 ± 0.20 mm after incubation for 180 min. The number of migrations measured in each assay is indicated in the bars. A significant difference between oriented migration obtained in absence and in presence of PBZ is designated by ▲ ($P < 0.001$).

Effect of PBZ on FMLP-induced oriented migration, studied by single PMN locomotion analysis

All the preceding results led to the conclusion that PBZ specifically modulated FMLP-induced oriented migration, as a function of its concentration in the agarose and of the concentration of the FMLP added to the attractant wells. It was not, however, possible to ascertain whether this modulation influenced the chemotactic and/or chemokinetic effects of FMLP on the PMNs. We attempted to elucidate this question by defining and continuously measuring, under the microscope, the effects of 0, 33, 66 and 165 μM of PBZ in the gel on the direction and on the oriented or unoriented speed of locomotion of single PMNs when submitted to the attractant activity of FMLP at concentrations ranging from 10^{-8} to 10^{-5} M. As observed under the microscope, the direction taken by the PMNs in the absence of PBZ appeared to be similar at FMLP concentrations ranging from 10^{-8} to 10^{-6} M. This was more difficult to assess precisely for 10^{-5} M, because of the crowding of PMNs which rapidly occurred in the vicinity of the cell studied. The direction which the PMNs were seen to take suggested that the chemotactic effect of FMLP was independent of its concentration within a wide range extending from at least 10^{-8} to 10^{-6} M. This finding, combined with the observation that the oriented migration measured by end-point migration after 90 min (Fig. 1) diminished at FMLP concentrations higher and lower than 10^{-7} M compared to the migration measured at 10^{-7} M, suggested that the chemokinetic effect of FMLP on the PMNs was concentration-dependent. This hypothesis was confirmed by measurements of the locomotion speed of PMNs either in the absence of chemoattractants or under the action of various FMLP concentrations. Figure 4 shows the mean pathways followed by the PMNs regardless of their direction and plotted at intervals of 4 min during the 90 min of the experiment. The same figures indicates that the speed of random locomotion is fairly constant throughout the experiment. In the presence of 10^{-7} M FMLP, the locomotion speed first increased and then decreased with time. When rising concentrations of FMLP were used the decline occurred earlier at the 35th and 20th min of the experiments for concentrations of 10^{-6} and 10^{-5} M, respectively. At 10^{-8} M of FMLP, the locomotion speed began to drop at the 40th min after the beginning of the experiments.

These results supplied further evidence for the

dependence of the chemokinetic activity of FMLP on its concentration [8] in the vicinity of the PMNs. The mean locomotion speeds throughout the experiments as a function of concentrations of FMLP added to PMN wells are reported in Table 1. It can be seen that the average increase in locomotion speed induced by 10^{-7} M FMLP was about 30% of the random locomotion speed, while 10^{-8}, 10^{-6} or 10^{-5} M had a negative effect. Table 1 also shows that the mean speeds of PMN locomotion at 10^{-8} and 10^{-6} M FMLP are, respectively, 53 and 61% of the speed obtained with 10^{-7} M. Taking into account that these mean speeds are calculated from measurements made 20 min after the beginning of the experiment, they appear to correlate with the oriented migrations measured (Fig. 1) which were, respectively, 48 and 54% of the migration measured at 10^{-7} M.

The modulating effect of PBZ on FMLP-induced oriented migration (Fig. 1) was also observed on the locomotion speed of the PMNs (Fig. 4 and Table 1). Table 1 indicates that 33 μM PBZ in the gel reduced the mean locomotion speed by 32%, a proportion in the same range as the decrease in oriented migration reported in Fig. 1. This provides further evidence that PBZ affects the chemokinetic but not the chemotactic activity of FMLP. A similar conclusion can be drawn from comparing Fig. 1 with Table 1, as regards the effects of 66 and 165 μM of PBZ on the chemokinetic activities of 10^{-6} and 10^{-5} M of FMLP, respectively.

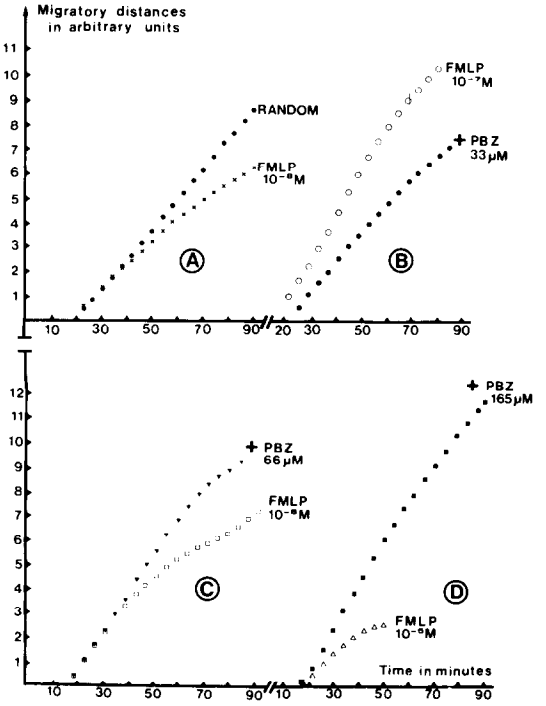

Fig. 4. Effect of PBZ on FMLP-induced locomotion speed studied by single PMN analysis. Each symbol represented was obtained by adding the mean pathways covered by the cells in 4 min, whatever their direction, after a lag time of 20 min. One unit corresponds to 156 μm. The number of cells followed in each assay was 2–4 and their total numbers are reported in Table 1. Random and oriented migration induced by 10^{-8} M FMLP in the absence of PBZ are given in part A of the figure. In the other cases, FMLP-induced migration at concentrations of 10^{-7}–10^{-5} M was assessed in the absence (○, □, △) and in presence of PBZ (●, ■, ▲) at the identical concentrations in B, C and D parts of the figure.

DISCUSSION

The present findings confirm earlier findings [7] that FMLP induces oriented PMN migration as measured after 90 min of incubation which is maximum for 10^{-7} M FMLP and declines at higher or lower concentrations ranging from 10^{-9} to 10^{-5} M. They show that the differences in oriented migration are predominantly due to variations in the chemokinetic activity of FMLP, depending on its concentration and not on its chemotactic activity. This was indicated by examination of the direction taken by the PMNs, which appeared to be similar with various concentrations of FMLP and was indirectly confirmed by comparing the oriented migration (Fig. 1) measured after 90 min as the end point (front lead) of migration with the mean speed of cell locomotion during the experiment at various FMLP concentra-

Table 1. Effects of FMLP and PBZ on mean PMN locomotion speed

FMLP (M)	PBZ (μM)	Mean locomotion speed ± S.D. (μm/min)	% of maximal control speed	n
0	0	18.1 ± 2.3	78	14
10^{-8}	0	12.5 ± 2.3 ★	54	12
10^{-7}	0	23.3 ± 1.9 ★	100	12
10^{-7}	33	15.8 ± 3.1 ★	68	14
10^{-6}	0	14.2 ± 4.5 ★	61	25
10^{-6}	66	21.4 ± 3 ★	92	10
10^{-5}	0	14 ± 2.7 ★	60	12
10^{-5}	165	25.8 ± 4.8 ★	111	16

Mean locomotion speed and S.D., calculated from the data in Fig. 4, were obtained by dividing the total migratory distances by the corresponding time of migration. The number of cells followed in each assay is indicated by *n*.

Statistical significance ($P < 0.001$) of differences between the two groups reported in the table by the square brackets are designated by ★.

tions (Table 1). The observation that the ratios of these two values were similar for the different FMLP concentrations tested was taken as evidence for the absence of change in the chemotactic effect of FMLP on PMN migration at the various concentrations tested. This conclusion does not exclude slight variations in the chemotactic effect of FMLP, depending on its concentration, but supports the possibility that low or high FMLP concentrations have similar chemotactic effects and that the differences observed in the measurements of the oriented migrations (Fig. 1) are mainly due to variations in the chemokinetic effects (Fig. 4 and Table 1). The data indicating the effect of PBZ on FMLP, ZAS and KPCS-induced oriented migration provide further evidence showing that PBZ modulates FMLP-induced oriented migration as a function of its concentration in the agarose (Fig. 1) by acting predominantly and perhaps exclusively on the positive or negative chemokinetic effect of FMLP (Fig. 4, Table 1). As previously reported the influence of PBZ was observed [5] on FMLP (Fig. 1) and not on ZAS-induced oriented migration. This confirms that PBZ is specific for FMLP action and did not affect migration through unspecific activity such as changing the consistency of the agarose. Partial inhibition by PBZ of KPCS-induced oriented migration suggests that KPCS contains components which act similarly to FMLP [9, 10] and components which act differently from it. KPCS chemoattractant activity has been little studied until now and needs further investigation. PBZ was previously reported to interfere with the binding of FMLP [1, 2] to its specific receptors [11] and thus to inhibit PMN chemotaxis which was assessed by measuring the oriented migrations only [1, 2]. PBZ was also shown to inhibit the deactivation of PMNs by their incubation with FMLP prior to the measurement of the oriented migration induced by FMLP [1, 2]. Both these reports [1, 2] suggested that PBZ was a competitive inhibitor of FMLP binding to its specific PMN receptors. This, combined with our data showing that FMLP-oriented migration levels are dependent on variations in the FMLP chemokinetic effect, might be an explanation of our results. If the chemokinetic effect of FMLP depends on the number of PMN sites to which it is bound, and if its chemotactic effect is induced when a few of these sites are occupied, then PBZ will compete with FMLP to alter the action of the latter by modulating its chemokinetic effect. For example, when, at 10^{-7} M of FMLP, the optimal number of PMN binding sites for FMLP is occupied, the oriented migration and locomotion speed of PMNs are maximal. Consequently, addition of PBZ to the agarose will reduce the number of PMN binding sites occupied by FMLP and PMN oriented migration will decrease with the speed of locomotion (Fig. 1, Table 1). In contrast, if, at 10^{-6} M or 10^{-5} M FMLP, the number of PMN sites occupied by FMLP exceeds that occupied under optimal conditions, chemokinetic negative activity will be observed (Table 1), leading to a decrease in the oriented migration (Fig. 1). By displacing some of the FMLP molecules bound to their receptors, PBZ then restore the positive chemokinetic activity of FMLP and the oriented migration.

The failure of PBZ to affect ZAS-oriented migration also suggests that the drug alters FMLP-oriented migration by some action occurring at the site of FMLP binding to the PMNs, and not by acting on the locomotion machinery.

In conclusion, this study shows that when investigating the effect of a drug on oriented PMN migration, it is important to analyse the speed of cell locomotion before assessing the drug's mechanism of action. It also suggests that the chemotactic effect of FMLP is similar for low and high concentrations of this chemoattractant but that the chemokinetic effect of FMLP is concentration-dependent and might be either chemokinetic-positive or chemokinetic-negative. Lastly, the present findings also demonstrate that PBZ modulates the chemokinetic activity of FMLP, probably by displacing the latter from its binding sites, a point which requires confirmation by further studies.

Acknowledgements—We thank Miss C. Delpont for her excellent secretarial assistance. This work was supported by a grant from INSERM (ATP no. 69.78.101).

REFERENCES

1. C. Dahinden and J. Fehr, *J. clin. Invest.* **66**, 884 (1980).
2. D. R. Nelson, J. M. Gracyk, V. D. Fiegel, M. J. Herron and D. E. Chenoweth, *Blood* **58**, 752 (1981).
3. J. E. Cutler, *Proc. Soc. exp. Biol. Med.* **147**, 471 (1974).
4. R. D. Nelson, P. G. Quie and R. L. Simmons, *J. Immun.* **115**, 1650 (1975).
5. E. Feliu, M. A. Gougerot, J. Hakim, E. Cramer, C. Auclair, B. Rueff and P. Boivin, *Eur. J. clin. Invest.* **7**, 571 (1977).
6. H. U. Keller, P. C. Wilkinson, M. Abercrombie, E. L. Becker, J. G. Hirsh, M. E. Miller, S. W. Ramsey and S. H. Zigmond, *Clin. Exp. Immun.* **27**, 377 (1977).
7. D. E. Chenoweth, J. G. Rowe and T. E. Hugli, *J. Immun. Meth.* **25**, 337 (1979).
8. H. U. Keller, J. H. Wissler and B. Damerau, *Immunology* **42**, 379 (1981).
9. E. Schiffmann, B. A. Corcoran and S. M. Wahl, *Proc. natn. Acad. Sci. U.S.A.* **72**, 1059 (1975).
10. E. Schiffmann, H. J. Showell, B. A. Corcoran, P. A. Ward, E. Smith and E. L. Becker, *J. Immun.* **114**, 1831 (1975).
11. L. T. Williams, R. Snyderman, M. C. Pike and R. J. Lefkowitz, *Proc. natn. Acad. Sci. U.S.A.* **74**, 1204 (1977).