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# Effect of Piracetam on Polyphosphoinositide Metabolism, Cytosolic Calcium Release, and Oxidative Burst in Human Polymorphonuclear Cells: Interaction with fMLP-induced Stimulation

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**ABSTRACT.** We investigated the action of piracetam on human polymorphonuclear leukocyte (PMN) responsiveness *in vitro*. We first studied phosphoinositide metabolism and calcium release with and without fMLP (formyl-methionyl-leucyl-phenylalanine) stimulation. Piracetam at concentrations from $10^{-4}$ to $10^{-2}$ M induced a slight increase in inositol 1,4,5-trisphosphate ($IP_3$) release and phosphatidylinositol 4,5-bisphosphate ($PIP_2$) breakdown. At concentrations above $10^{-3}$ M, piracetam sensitized PMNs to subsequent stimulation by fMLP used at subliminal concentrations ($10^{-9}$ and $10^{-8}$ M), inducing a significant increase in $IP_3$ release and $PIP_2$ breakdown similar to that obtained with cells stimulated by the highest effective concentrations of fMLP ($10^{-7}$ and $10^{-6}$ M). In the same way, piracetam greatly enhanced calcium release induced by weak concentrations of fMLP. However, piracetam had no effect on oxidative metabolism. We then studied the binding of ($^3$H)fMLP to the PMN membrane in the presence of various concentrations of piracetam. We were not able to demonstrate an obvious action of piracetam either on receptor recruitment or on receptor affinity to fMLP. The difference between the actions of piracetam on phosphoinositide metabolism and calcium release on the one hand and oxidative burst on the other could be explained by an uncoupling of the triggering and activating effects of piracetam on PMNs. The enhancement by piracetam of intracellular cyclic AMP levels rapidly induced termination of the PMN response and accounted for the lack of effect on superoxide production. Thus, piracetam was able to modulate human PMN reactivity and in particular to exert a "priming effect" (rather due to structural modifications of the membrane), which might be of importance in infectious episodes given the absence of deleterious actions such as oxygen free radical production leading to tissue injury. BIOCHEM PHARMACOL **57**;2:163–170, 1999. © 1998 Elsevier Science Inc.

**KEY WORDS.** human polymorphonuclears; fMLP; piracetam; priming; transduction

Piracetam (2-oxo-1-pyrrolidine acetamide; Nootropil®) has been reported to increase phospholipase $A_2$ activity and $^{32}$P incorporation into phosphatidylinositol and phosphatidylcholine in glial and neuronal animal cells [1]. In aged mouse brain cells, it produced an elevated accumulation of inositol monophosphates, that could traduce a partial restoration of age-related deficits in the central muscarinic cholinergic receptor function [2]. Piracetam has also been shown to accelerate ATP turnover in hypoxic rat neurons,§ a property which has been used therapeutically in metabolic disorders associated with aging [3, 4]. Piracetam has also been found to accelerate thermal burn healing and to reduce the incidence of infectious complications. These effects are mainly due to an augmentation of transcutaneous oxygen exchanges [5, 6]. Piracetam is also able to enhance membrane fluidity, via a new organization of the lipids, in brain and blood cells [7–9]. These data show the effects of piracetam, particularly on human or animal neurons, but also on other cells and cell lines, that led us to study a possible activity on human PMNs.‖

The role of phosphoinositides in transduction mechanisms is now well established [10, 11]. Hydrolysis of membrane PI into InsP by phospholipase C via activation of a G-protein is part of the signal transduction mechanisms controling numerous cellular processes, particularly in PMNs. Hydrolysis of $PIP_2$ leads to the formation of two second messengers: diacylglycerol, which activates protein

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§ Gobert JG and Temmerman JJ, Piracetam-induced increase of the rat brain energetic metabolism and of the polyribosome/ribosome ratio in old rats. *4th International Meeting of the International Society for Neurochemistry*, Tokyo, 1973.

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‖ *Abbreviations:* cAMP, cyclic AMP; fMLP, formyl-methionyl-leucyl-phenylalanine; H-HBSS, Hepes-buffered Hank's balanced salt solution; $IP_3$, inositol 1,4,5-trisphosphate; PI, polyphosphoinositides; $PIP_2$, phosphatidylinositol 4,5-bisphosphate; PMN, polymorphonuclear leukocytes; and $O_{2-}$, superoxide anion.

kinase C, thereby triggering the PMN oxidative burst, and $IP_3$, which mobilizes intracellular calcium, a step in protein phosphorylation and cellular responses.

FMLP induces rapid hydrolysis of $PIP_2$ and stimulates the respiratory burst in PMNs, which bear fMLP-specific membrane receptors. Two types of effects of fMLP have been described according to the concentration used ($10^{-10}$ to $10^{-9}$ M or $>10^{-8}$ M): rapid "triggering", and more sustained "activation" [12, 13]. "Triggering" involves a first series of rapid events (generation of $IP_3$ and diacylglycerol and mobilization of intracellular $Ca^{2+}$) which lead to actin polymerization and PMN degranulation. "Activation" requires a second step of intracellular signal generation by continuous receptor occupancy (a second wave of diacylglycerol release, major translocation of protein kinase C, calcium influx) and leads to $O_{2-}$ generation. These activities are regulated by at least two types of receptors of low- and high-affinity on PMN plasma membranes, or by a single receptor class in intact PMNs. These receptors on the surface of viable leukocytes can be converted from a low- to a high-affinity form, the latter becoming inactive and internalized [14–16]. It has been proposed that fMLP receptors on intact PMN and plasma membranes may consist of at least three components with different affinities for fMLP [17]. At concentrations from $10^{-7}$ to $10^{-6}$ M, fMLP triggers the oxidative burst, but lower concentrations can prepare PMNs for subsequent activation, a phenomenon known as the "priming" effect.

The aim of this study was to identify a possible effect of piracetam on human PMN polyphosphoinositide breakdown, cytosolic calcium release, and oxidative metabolism and to investigate the mechanisms of a possible interaction with fMLP stimulation.

## MATERIALS AND METHODS

### *Materials*

Piracetam ($C_6H_{10}N_2O_2$) was from UCB Laboratories and Ficoll-Hypaque was from Pharmacia Fine Chemicals. fMLP, BSA, sodium tetraborate, ammonium formate, formic acid, boric acid, lithium chloride, and polyvinylic alcohol were from Sigma Chemical Co. Dowex AG1-X8 ion-exchange resin (200–400 mesh, formate form) was from BioRad; myo (2-$^3$H) inositol (740 GBq/mmol) was from Amersham International; H-HBSS was from Gibco; and the scintillation cocktail Hisafe 3 was from Pharmacia LKB. Cyclic AMP was assayed using an Elisa kit from Cascade Biochem. All other reagents were of the highest quality available commercially.

### *Preparation of Blood PMNs*

Blood was collected on heparin (final concentration 5 units/mL) from healthy subjects, in accordance with the Helsinki declaration.

PMNs were isolated by a two-step procedure using Ficoll-Hypaque and polyvinyl alcohol [18], and gently resuspended in 0.015 M of PBS pH 7.4. Purity was 99% (Giemsa staining) and viability more than 95% (trypan blue exclusion). The cells were used immediately after isolation.

### *Phosphoinositide Breakdown Analysis*

We used a method developed in our laboratory [19]. In brief, the PMN suspension was adjusted to $10^8$ cells/10 mL/tube, labeled with 200 μci of myo-(2-$^3$H) inositol/tube and incubated for 16–20 hr with gentle shaking. After washing, cells were counted and adjusted to a density of $10^7$/600 μL in Eppendorf microtubes and incubated for 10 min at 37°. The test agents were then added to the tubes in duplicate, as follows:

- PBS for 15 sec (controls)
- fMLP from $10^{-9}$ to $10^{-6}$ M for 15 sec
- Piracetam at $3 \times 10^{-4}$, $10^{-3}$, $3 \times 10^{-3}$, and $10^{-2}$ M, for 30 min at 37°
- Piracetam at the same concentrations as above, 30 min before the addition of fMLP for 15 sec at the same concentrations as above.

The reactions were stopped by adding perchloric acid, followed by three cycles of freezing-thawing. After centrifugation, the water-soluble perchloric acid supernatants containing inositol phosphates were diluted and neutralized. Inositol lipids were extracted from the perchloric acid-insoluble pellets, and deacylated [20]. ($^3$H)-Inositol phosphates and ($^3$H)-glycerophosphorylesters were separated by anion-exchange chromatography on Dowex AG1-X8 columns using the buffer system described [21, 22].

### *Measurement of Calcium Mobilization*

Changes in the cytosolic free calcium concentration were measured as described [23] in PMN loaded with $10^{-6}$ M Fura-2/acetoxymethylester at 37° for 1 hr. Cells were then washed and resuspended in 20 mM of H-HBSS. Fura-2 fluorescence assays were performed with aliquots of $5 \times 10^6$ PMNs in 2 mL of HBSS, using a fluorimeter (Jobin Yvon 3D) equipped with a thermally controled cuvette holder and a magnetic stirrer. Excitation and emission wavelengths for Fura-2 fluorescence assays were 340 and 510 nm, respectively. Cytosolic calcium concentrations were calculated as described [24]. Tracings were reproduced and scanned using an Agfa Snap CAM with version F-3.0 Color It software (Apple).

### *Chemiluminescence Assay*

PMN chemiluminescence response was measured using a Packard Picolite® luminometer [25]. The PMN suspension (100 μL, $5 \times 10^6$ cells/mL) was distributed into 6 × 50 mm borosilicate tissue-culture tubes, which were placed in the apparatus for 5 min in the dark at 37° to equilibrate. Then, 20 μL of luminol solution was added at a final concentra-

tion of $4 \times 10^{-5}$ M. When background light emission had stabilized, 150 μL of fMLP solution ($10^{-7}$ M) was added and light emission was recorded for 20 min.

### *$O_{2-}$ Generation*

Superoxide ($O_{2-}$) generation was measured in terms of ferricytochrome C reduction (horse heart, type III), as described [26]. PMNs ($2 \times 10^6$ cells/mL) and 150 μL of 0.4 mM ferricytochrome C solution were incubated for 15 min at 37° with or without various concentrations of piracetam. FMLP ($10^{-10}$ to $10^{-7}$ M) was then added and the final volume of the reaction mixture was adjusted to 1 mL. The reaction was stopped after 5 min by placing the tubes in an ice-water bath, followed by centrifugation at 800 *g* for 10 min at 4°. The amount of $O_{2-}$ produced was calculated from the difference in absorbance before and after incubation, using an extinction coefficient of 20 $nM^{-1}$ $cm^{-1}$ at 550 nm. The results are expressed in nmoles of $O_{2-}$-released/min/$10^6$ cells. The specificity of the reaction was checked by adding superoxide dismutase, which inhibited at least 90% of fMLP stimulation.

### *Binding Study*

Aliquots of $10^7$ PMNs (in duplicate) were suspended in 1 mL of Dulbecco's PBS with and without (controls) piracetam ($3 \times 10^{-4}$ to $10^{-2}$ M). The mixture was incubated for 30 min at 37°. ($^3$H)fMLP ($1.5 \times 10^{-9}$ to $5 \times 10^{-8}$ M) was then added for 60 min at 4°, with a large excess of unlabeled fMLP ($5 \times 10^{-4}$ M) to evaluate specific binding, or without unlabeled fMLP to estimate total binding. The reaction was stopped by adding 500 μL of ice-cold PBS, followed by centrifugation (10,000 *g* for 15 sec). The supernatant was discarded and the microtubes were rinsed twice with 200 μL of ice-cold PBS, without resuspending the cells, and centrifuged again. The pellet was homogenized with 250 μL of PBS + 25 μL of acetic acid, and 3 mL of scintillation cocktail was added to measure radioactivity.

### *Intracellular cAMP Assay*

Aliquots of $10^7$ PMNs (tested in duplicate) were incubated with or without piracetam ($10^{-3}$ to $10^{-2}$ M) for 10 or 30 min. Reactions were stopped by freezing in liquid nitrogen. Cyclic AMP was extracted by disrupting cell walls by sonication in 1 M of perchloric acid. After centrifugation (20,000 *g*, 10 min), supernatants were adjusted to pH 6.2. Acetylation of samples and the following steps were carried out as recommended by the manufacturers of the Elisa kit (Cascade Biochem).

### *Analysis of the Results*

Since tritiated inositol incorporation into phosphoinositides varies from one pool of cells to another, $IP_3$ and $PIP_2$ data are expressed as means ± SEM percentages of PBS control values. The statistical significance of differences between means was estimated using the Mann–Whitney U-test for percentages, two-way analysis of variance (Anova), and post hoc Student's paired *t*-test for cpm. Data for $O_{2-}$ generation, chemiluminescence, and cAMP concentrations were analyzed by Anova and then by Student's unpaired *t*-test.

## RESULTS

### *Direct Effect of Piracetam on Phosphoinositide Metabolism*

We only report variations in $IP_3$ and $PIP_2$ content, as these two metabolites are of particular interest in cell activation,

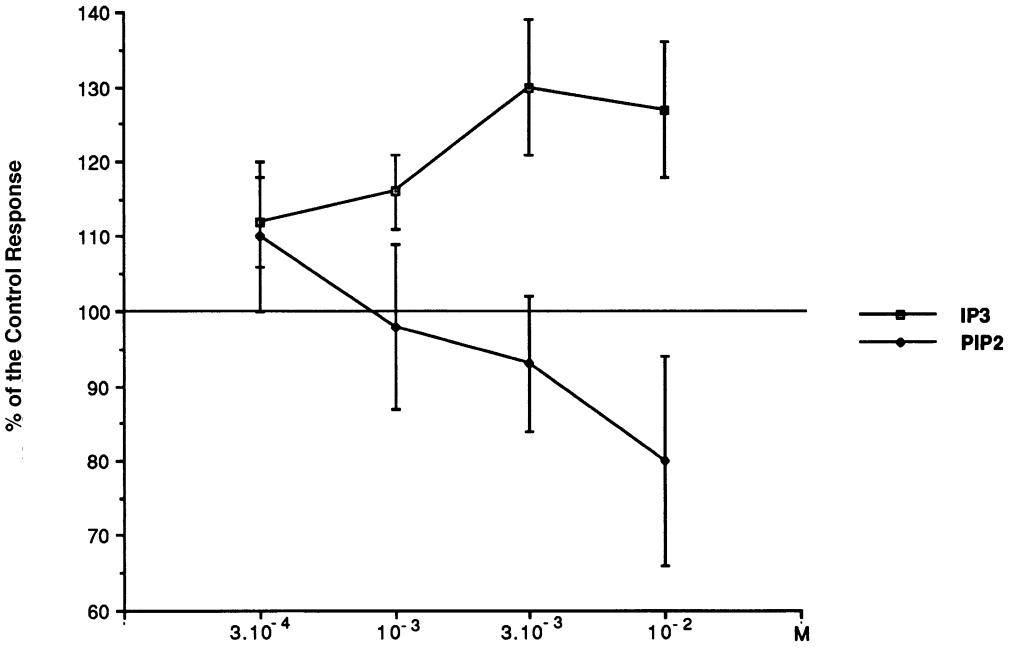


**FIG. 1. *In vitro* effect of piracetam ($3 \times 10^{-4}$ to $10^{-2}$ M, 30 min) on $IP_3$ release and $PIP_2$ breakdown by human PMNs. After labeling for 16 hr, and washing, PMN were resuspended in H-HBSS at the concentration of $10^7$ cells/aliquot and placed in a water bath for 10 min at 37°. Then piracetam was added to the test tubes for 30 min at 37° at various concentrations. No stimulation was applied unlike experiments shown in Figs. 2 and 3, where fMLP was added 30 min after piracetam for 15 sec. The data are means ± SEM of 5 independent experiments and are expressed as a percentage of control values (100%). Control values: $IP_3$ = 339 ± 55 cpm; $PIP_2$ = 416 ± 103 cpm.**

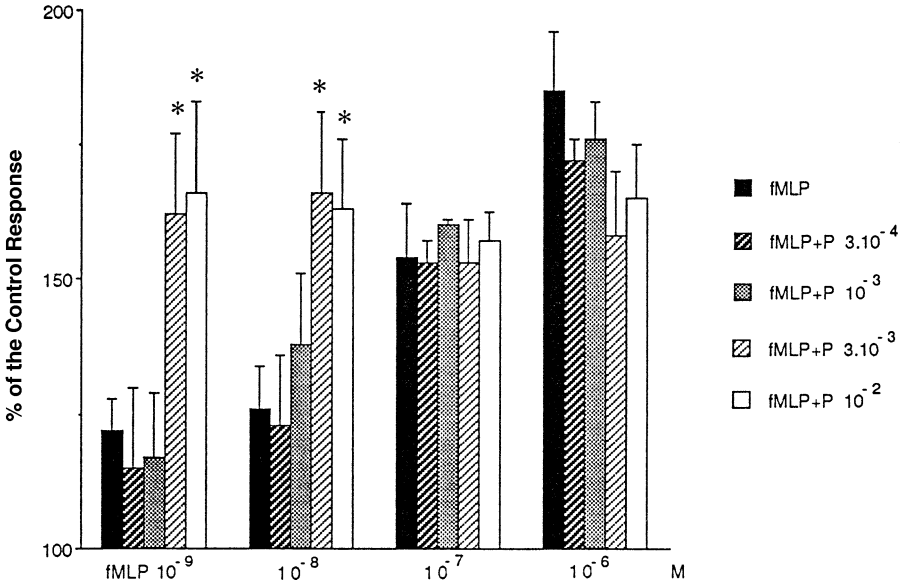


**FIG. 2.** ***In vitro*** **effect of piracetam (P: 3 × $10^{-4}$ to $10^{-2}$ M) on PMN $IP_3$ release induced by fMLP ($10^{-9}$ to $10^{-6}$ M). fMLP was added for 15 sec, 30 min after piracetam. Mean ± SEM of 3–6 independent experiments. * = $P < 0.05$ versus effect of fMLP alone (Mann–Whitney U-test and Student's paired *t*-test). Control value: 339 ± 55 cpm (100%). Piracetam alone: P 3 × $10^{-4}$ M: 342 ± 87 cpm (112 ± 6%); P $10^{-3}$ M: 362 ± 100 cpm (116 ± 5%); P 3 × $10^{-3}$ M: 477 ± 105 cpm (130 ± 9%); and P $10^{-2}$ M: 484 ± 107 cpm (127 ± 9%).**

and as the other InsP and inositol lipid levels showed similar variations. Piracetam concentrations higher than $10^{-3}$ M slightly increased $IP_3$ release and $PIP_2$ breakdown. However, the differences were not significant either between values obtained with the various concentrations of piracetam or versus the control values (Fig. 1).

### *Interaction of Piracetam and fMLP on Phosphoinositide Metabolism*

FMLP ($10^{-6}$ and $10^{-7}$ M) increased the production of InsP, particularly $IP_3$, by PMNs *in vitro*, with a corresponding fall in the amounts of PI, particularly $PIP_2$. The effect of fMLP was concentration-dependent (data not shown).

We focused on the interaction of piracetam with fMLP-induced $IP_3$ production and $PIP_2$ breakdown. The lowest concentrations (3 × $10^{-4}$ and $10^{-3}$ M) of piracetam did not modify fMLP-induced $IP_3$ production, while the highest concentrations (3 × $10^{-3}$ M and $10^{-2}$ M) of piracetam significantly increased the PMN response to the lowest (but not the highest) concentrations of fMLP ($10^{-9}$ and $10^{-8}$ M). $IP_3$ release was then of the same order as that observed with the highest concentrations ($10^{-6}$ M) of fMLP (Fig. 2).

Correlatively, $PIP_2$ content fell significantly when PMNs were preincubated with the highest concentrations of piracetam before stimulation with subliminal concentrations of fMLP, which themselves were ineffective (Fig. 3).

### *Effect on Cytosolic Calcium Release*

Piracetam had no effect on intracellular calcium release, but from 3 × $10^{-3}$ to $10^{-2}$ M (after 10 or 30 min incubation) increased the PMN response to low concentrations of fMLP ($10^{-12}$ to $10^{-8}$ M). Calcium release could be of the same order as that obtained with the highest concentrations of fMLP ($10^{-6}$ and $10^{-7}$ M). (Fig. 4).

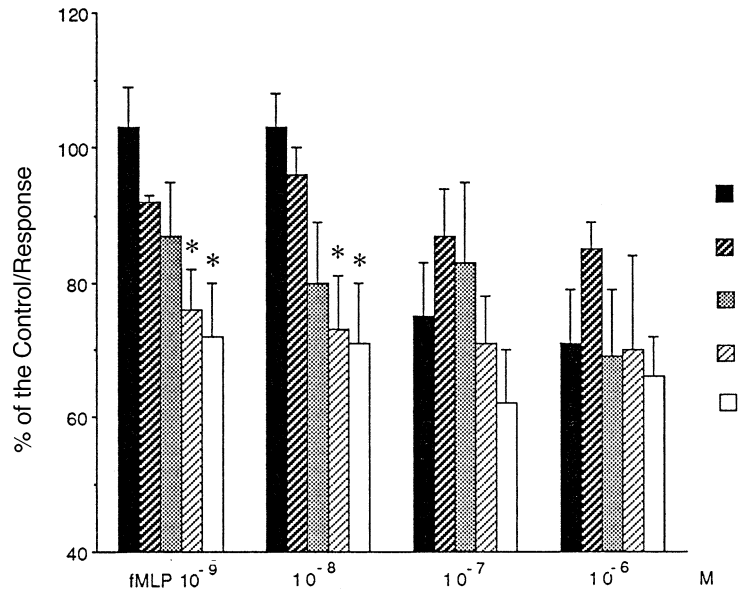


**FIG. 3.** ***In vitro*** **effect of piracetam (P: 3 × $10^{-4}$ to $10^{-2}$ M, 30 min) on PMN $PIP_2$ breakdown induced by fMLP ($10^{-9}$ to $10^{-6}$ M, 15 sec). Mean ± SEM of 3–6 independent experiments. * = $P < 0.05$ versus effect of fMLP alone (Mann–Whitney U-test and Student's paired *t*-test). Control values: 416 ± 103 cpm (100%). Piracetam alone: P 3 × $10^{-4}$ M: 449 ± 60 cpm (110 ± 10%); P $10^{-3}$ M: 406 ± 144 cpm (98 ± 11%); P 3 × $10^{-3}$ M: 321 ± 95 cpm (93 ± 9%); and P $10^{-2}$ M: 352 ± 127 cpm (80 ± 14%).**

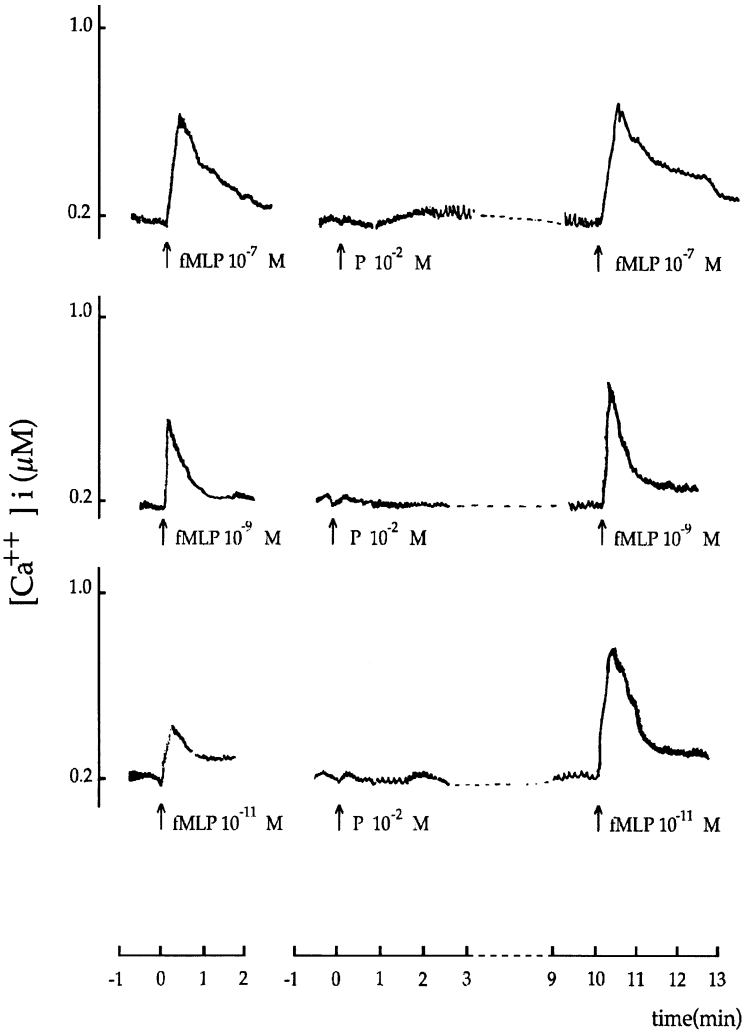


**FIG. 4.** ***In vitro*** **effect of piracetam ($10^{-2}$ M, 10 min) on calcium release by fMLP ($10^{-7}$, $10^{-9}$, $10^{-11}$ M) in human PMNs. Tracings are shown for one population of cells and are representative of at least 4 to 6 experiments. The calcium signal has been quantified as maximal increase [$\Delta(Ca^{++})_i$] over baseline with the formula: $(Ca^{++})_i$ nM = 224 (f-fmin)/(Fmax-f) where Fmax is the increase due to 0.1% Triton X-100 and fmin is the decrease due to $10^{-4}$ M of $Mn^{++}$.**

| | |
|---|---|
| fMLP $10^{-7}$ M: | $(Ca^{++})_i$ = 0.736 ± 0.081 μM |
| P $10^{-2}$ M + fMLP $10^{-7}$ M: | $(Ca^{++})_i$ = 0.703 ± 0.091 μM |
| fMLP $10^{-9}$ M: | $(Ca^{++})_i$ = 0.458 ± 0.046 μM |
| P $10^{-2}$ M + fMLP $10^{-9}$ M: | $(Ca^{++})_i$ = 0.723 ± 0.108 μM; $P < 0.05$ versus fMLP $10^{-9}$ M |
| fMLP $10^{-11}$ M: | $(Ca^{++})_i$ = 0.363 ± 0.024 μM |
| P $10^{-2}$ M + fMLP $10^{-11}$ M: | $(Ca^{++})_i$ = 0.711 ± 0.086 μM; $P < 0.01$ versus fMLP $10^{-11}$ M (Anova + Student's unpaired *t*-test). |

### *Action of Piracetam on fMLP Membrane Receptors*

To investigate the possible interaction of piracetam with fMLP on receptor expression, we measured total and specific binding of radiolabeled fMLP as a function of its concentration in the medium, with or without piracetam, using a range of ($^3$H) fMLP concentrations from $1.5 \times 10^{-9}$ to $5 \times 10^{-8}$ M.

The number of binding sites was dependent on the initial state of cells, which were isolated from various subjects over a six-month period. Scatchard plots have similar slopes and cross the x-axis at very close points (Fig. 5). Thus, receptor affinity and the number of binding sites remained unchanged after piracetam treatment.

### *Effect of Piracetam on Intracellular cAMP Levels*

Intracellular cAMP concentrations increased in PMNs incubated with piracetam for 10 or 30 min, in a time- and concentration-dependent manner (Fig. 6).

### *Effect of Piracetam on PMN Oxidative Metabolism*

CHEMILUMINESCENCE. Incubation (10 or 30 min) with piracetam ($10^{-5}$ to $10^{-3}$ M) did not significantly modify PMN chemiluminescence. Pretreatment with piracetam (10 min or 30 min; $3 \times 10^{-4}$ to $10^{-2}$ M) did not significantly modify the effect of fMLP, although an inhibitory effect appeared to occur at higher concentrations ($>10^{-4}$ M) (data not shown).

$O_{2-}$-GENERATION. From $3 \times 10^{-5}$ to $3 \times 10^{-3}$ M, piracetam did not induce PMN $O_{2-}$-production either directly or after PMN preincubation for 10 or 30 min before addition of ineffective concentrations of fMLP ($10^{-10}$ to $10^{-9}$ M). The stimulation induced by $10^{-7}$ M of fMLP was not significantly enhanced by the lowest concentrations of piracetam, but concentrations higher than $10^{-3}$ M had a slight inhibitory effect (data not shown).

## DISCUSSION

Neutrophils which are involved in the nonspecific immunological surveillance against microbial invaders and in pathological processes can be in various activity states: resting, activated, primed, or deactivated [27].

Piracetam at concentrations from $10^{-4}$ to $10^{-2}$ M did not significantly modify PMN phosphoinositide metabo-

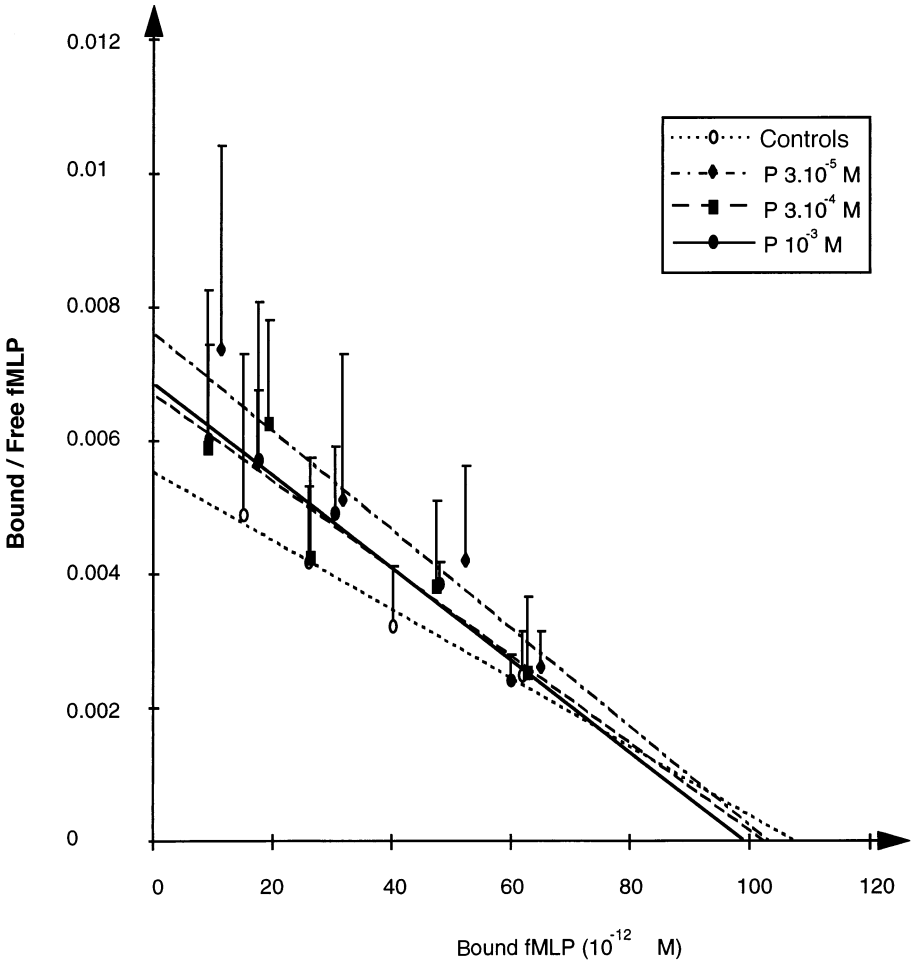


**FIG. 5. *In vitro* effect of piracetam (P: $3 \times 10^{-5}$ to $10^{-3}$ M) on ($^3$H)fMLP binding to resting human PMNs. Cells were incubated for 30 min at 37° without (controls) or with $3 \times 10^{-5}$, $3 \times 10^{-4}$ or $10^{-3}$ M of piracetam. After washings, cells were placed for 1 hr at 4° in the presence of 1.56, 3.12, 6.25, 12.5, 25 or $50 \times 10^{-9}$ M of radiolabeled fMLP. After three more washings, the total radioactivity was counted. At the same time, this procedure was duplicated with $5 \times 10^{-4}$ M of additional unlabeled fMLP to evaluate specific binding (bound ($^3$H)fMLP). Free ($^3$H)fMLP was calculated as the difference between total and specific bindings. Means ± SEM of 3 independent experiments are illustrated as Scatchard representations with regression curves (see dotted lines in legend).**

lism, although a slight increase in $IP_3$ release and a slight decrease in $PIP_2$ breakdown were observed. However, at concentrations from $3 \times 10^{-3}$ M, piracetam sensitized PMNs to subsequent stimulation by fMLP at subliminal concentrations ($10^{-9}$ and $10^{-8}$ M). This might reflect a "priming" effect of piracetam and was particularly evident in terms of $IP_3$ release.

In the same way, piracetam by itself had no effect on calcium release, but from $10^{-3}$ M and still more at $3 \times 10^{-3}$ and $10^{-2}$ M increased this release induced by weak concentrations ($10^{-12}$ to $10^{-8}$ M) of fMLP.

The concentrations used *in vitro* are of the same order as those reported by authors [28–30] who recently demonstrated that piracetam, from $10^{-3}$ to $2 \times 10^{-2}$ M, might be able to regulate $Ca^{2+}$ channels and perhaps other ion transport (sodium, potassium) in isolated snail neurons.

Piracetam had no significant effect on PMN oxidative metabolism (chemiluminescence and superoxide generation), but the highest concentrations tended to inhibit it. The difference between the actions of piracetam on phosphoinositide and oxidative metabolism was not surprising, since active concentrations of chemoattractants (and probably other stimulants) can vary according to the effects analyzed. These differences confirm the distinction between "triggering" and "activation" of neutrophils [12, 13], responses that appear to be induced by different concentrations of stimulants.

Piracetam is a small hydrophilic molecule which may induce structural modifications of the membrane [8, 9] and/or act directly at the cell surface. Studies of ($^3$H)fMLP binding to PMN membranes were done to determine if piracetam primed the PMN response to fMLP by inducing receptor externalization or simply by modifying receptor structure.

Our experiments failed to show an obvious action of piracetam either on receptor recruitment or on their affinity to fMLP, contradicting Stoll's hypothesis [2] that piracetam might enhance the available number of receptors on aged mouse brain membranes by preventing their sequestration and desensitization.

The "priming" effect of piracetam could rather be due to structural modifications of the membrane. As demonstrated by Peuvot *et al.* [8], the drug modifies lipid organization by surrounding the polar head of the phospholipids and this physical and aspecific action, leading to improved cell function, could be related to various changes observed in many kinds of cells.

On the other hand, we observed an increase in intracellular cAMP levels in PMNs after treatment with the highest concentrations of piracetam. cAMP is generally an

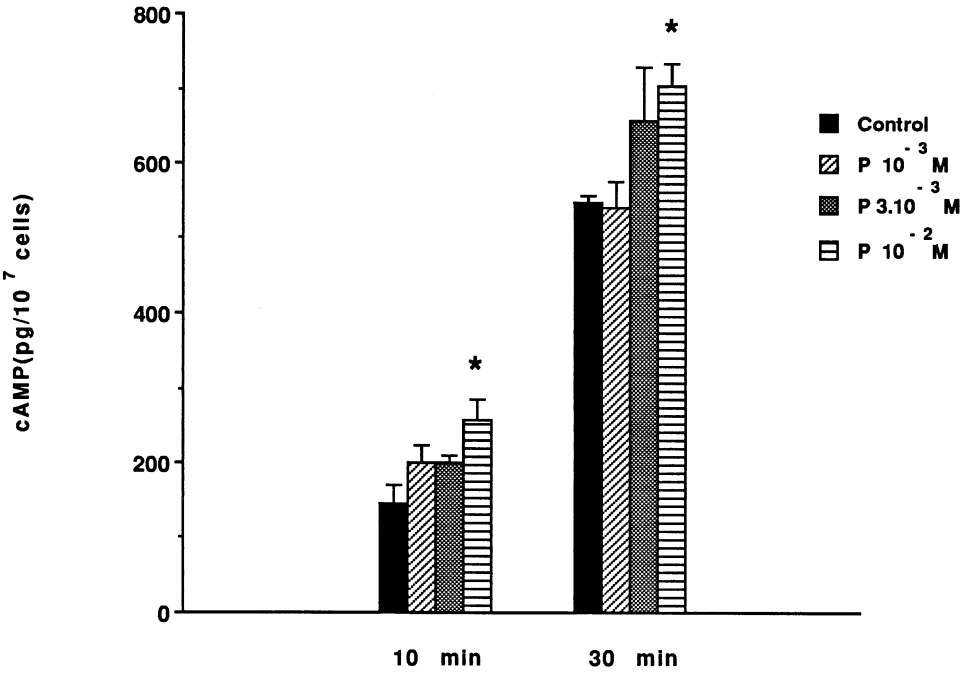


**FIG. 6.** ***In vitro*** **effect of piracetam (P: $10^{-3}$ to $10^{-2}$ M) on cAMP intracellular concentrations in human PMNs. Mean ± SEM of 3–4 independent experiments. * = $P < 0.05$ versus controls (Anova + Student' unpaired *t*-test).**

inhibitory mediator for PMNs and its effects are mediated by a serine/threonine kinase, named cyclic AMP-dependent protein kinase or PKA [13, 31]. An uncoupling of the two types of PMN responses, rapid or sustained, could occur at various steps of signal transduction. An enhancement of cAMP concentrations or occupation of adenosine receptors may provide an autoregulatory termination mechanism for PMN responses [12, 13]. This is in keeping with previous results [32] suggesting that the inhibitory action of cAMP did not alter fMLP binding or rapid responses of PMNs but could terminate their activation and thus inhibit superoxide production. These data confirm the possibility of piracetam being able to modify the signal in PMN.

In conclusion, this study shows that piracetam can modulate human PMN reactivity *in vitro*, and particularly the response to fMLP, and that the effects differ according to the concentrations used and the events considered. The main effect was on PMN "priming", which might be of importance in infectious episodes. Piracetam did not markedly stimulate oxygen free radical production, but sensitized PMN to stimulant events; it might thus enhance nonspecific defenses against infection without enhancing free radical-induced tissue damage.

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