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SHORT COMMUNICATION

Interaction of Cyclic GMP and Cyclic AMP during Neutrophil Migration: Involvement of Phosphodiesterase Type III

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ABSTRACT. In previous experiments, it was shown that migration of electropermeabilized human neutrophils induced by a combination of cGMP and cAMP markedly lower relative to that induced by cGMP or cAMP alone. However, when cGMP was replaced with 8-(para-chlorophenylthio-guanosine-3′,5′-cyclic monophosphate (8-pCPT-cGMP), a metabolic stable analogue of cGMP which does not affect the activity of cGMP-regulated phosphodiesterases (PDEs), migration in the presence of cAMP was enhanced in an additive way. To investigate the role of cyclic nucleotide breakdown during neutrophil migration in more detail, specific inhibitors of phosphodiesterase type III (PDE-III) (cGMP-inhibited) were used. Milrinone and cilostamide inhibited migration induced by an optimal concentration of cAMP. This revealed that inhibition of cAMP breakdown, by prolonging the action of an otherwise optimal concentration of cAMP, led to decreased migration, in accordance with the observation that the effect of cAMP on migration of electropermeabilized neutrophils was biphasic. Furthermore, it was found that a combination of 8-pCPT-cGMP and milrinone/cilostamide could substitute for cGMP in both activating cGMP-dependent protein kinase (8-pCPT-cGMP) and inhibiting PDE-III (milrinone/cilostamide). In conclusion, evidence is presented that cGMP and cAMP could interact on the level of PDE-III during neutrophil migration. BIOCHEM PHARMACOL **56**;8:1061–1063, 1998.

KEY WORDS. GMP; cAMP; cyclic nucleotide phosphodiesterases; neutrophil migration; inhibitors; cyclic nucleotide analogues

Cyclic nucleotides are considered to play an important—although yet largely undefined—role in modulating neutrophil migration [1, 2]. Recently, we showed that cGMP as well as cAMP are able to enhance migration of electropermeabilized neutrophils; the effect of both cyclic nucleotides on migration is biphasic [3]. In neutrophils, cyclic nucleotide responses are under the strict control of cyclic nucleotide-hydrolyzing PDEs,† which also provide a mechanism by which signaling pathways involving cGMP and cAMP can interact (e.g. via allosteric binding or regulation of phosphorylation [4]).

PDE-resistant cyclic nucleotide analogues are powerful tools for studying the role of cyclic nucleotide breakdown during cellular processes. In a previous study it was found that 8-pCPT-cGMP, a non-hydrolyzable cGMP analogue, enhanced migration of electropermeabilized neutrophils in the presence of cAMP in an additive way. In contrast, migration in the presence of both cGMP and cAMP was lower compared to that in the presence of either cGMP or cAMP alone [5]. These results seemed to indicate an interaction of cGMP and cAMP on the level of PDEs during neutrophil migration.

It has been shown for platelets that cGMP can potentiate the actions of cAMP via inhibition of PDE-III (cAMP-consuming, cGMP-inhibited PDE), i.e. via inhibition of cAMP breakdown leading to a prolonged intracellular cAMP response [6]. Impaired or delayed hydrolysis of cAMP in our system could potentially lead to an inhibitory effect of cAMP on migration, since the effect of cAMP on migration is biphasic [3]. On the basis of previous studies [3, 5] and some preliminary experiments, we came to the hypothesis that regulation of the concentration of cAMP by cGMP, rather than cGMP breakdown, played an essential role in cyclic nucleotide-activated migration. Therefore, the possible involvement of PDE-III in migration of electropermeabilized neutrophils induced by cGMP and cAMP was investigated, using the specific inhibitors of PDE-III, milrinone and cilostamide [7].

MATERIALS AND METHODS

Isolation of Neutrophils

Neutrophils were isolated from the buffy coat of blood of healthy donors by starch sedimentation and Ficoll centrif-

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†*Abbreviations:* fMLP, formyl-methionyl-leucyl-phenylalanine; 8-pCPT-cGMP, 8-(para-chlorophenylthio-guanosine-3′,5′-cyclic monophosphate; PDE-III, phosphodiesterase type III; and PDE-V, phosphodiesterase type V.

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ugation. The neutrophils were suspended in medium consisting of 140 mM NaCl, 5 mM KCl, 10 mM glucose, 1 mM Ca^{2+}, 1 mM Mg^{2+}, 0.5% BSA and 20 mM Hepes, pH 7.3. The final suspension during the migration experiments contained 3×10^6 neutrophils per mL.

Electropermeabilization

Electropermeabilization was performed as described previously [3] in medium consisting of 140 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM glucose, 20 mM Hepes, pH 7.0, and 0.5% (w/v) BSA. Electropermeabilization permits the introduction into the cell of a fixed concentration of cyclic nucleotides, determined by the concentration in the medium (which is in equilibrium with the cell interior when the cells are open). The pores of electropermeabilized neutrophils are unstable and will close again after several minutes [8]. Cyclic nucleotides and other reagents were added before electropermeabilization. Both random migration and chemoattractant-activated migration is somewhat less for electropermeabilized cells as compared with control cells. While random migration and fMLP-activated migration for intact cells were 49.2 ± 2.1 and 101.3 ± 2.0 μm, respectively, the corresponding values for electroporated cells were 30.7 ± 1.9 and 70.6 ± 1.6 μm.

Migration Measurements

Migration was measured with the Boyden Chamber technique, as described previously [9]. The filters used were from Millipore and consisted of cellulose acetate (mixed cellulose esters: nitrate, acetate), pore size: 3 μm.

Statistical Analysis

Measurements of five different filter sites were averaged to obtain a value representative of the distance migrated by cells into each filter. Data from three independent experiments (two filters each) were taken, recalculated as percentage of controls (i.e. random migration) and expressed as means ± SEM. Comparisons between means of multiple groups were analyzed by ANOVA and Scheffé's multiple comparison test; $P < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Both cGMP and cAMP (Sigma), as well as 8-pCPT-cGMP (BIOLOG Life Science Inst.), have a biphasic effect on migration of electropermeabilized neutrophils; optimal stimulation is achieved with 5 μM, 1 μM and 0.1 μM, respectively [3]. Replacement of cGMP with 8-pCPT-cGMP dramatically altered the effect on the migration of electropermeabilized neutrophils, when combined with cAMP [5] (Fig. 1). It has been reported that the activity of PDE-V, which is present in neutrophils [10], can be enhanced by the catalytic subunit of cAMP-dependent protein kinase (A-kinase) [11]. It is therefore tempting to speculate that the mechanism of the inhibitory effect on neutrophil migration in the presence of both cGMP and cAMP (Fig. 1) is related to cAMP-enhanced breakdown of cGMP by PDE-V. However, cAMP inhibited migration in the presence of cGMP to an even lesser extent than that of cAMP alone (5 μM cGMP: 219 ± 12%; 1 μM cAMP: 156 ± 7%; 5 μM cGMP + 1 μM cAMP: 140 ± 9%, $P < 0.05$ as compared to cAMP alone). Furthermore, increasing concentrations of cAMP progressively inhibited migration in the presence of cGMP (Fig. 1). Inhibition by cAMP of migration in the presence of 8-pCPT-cGMP could be achieved as well, but only at high concentrations of cAMP (Fig. 1). These observations led to the hypothesis that, rather than cGMP breakdown, (regulation of) the concentration of cAMP by cGMP might play an essential role.

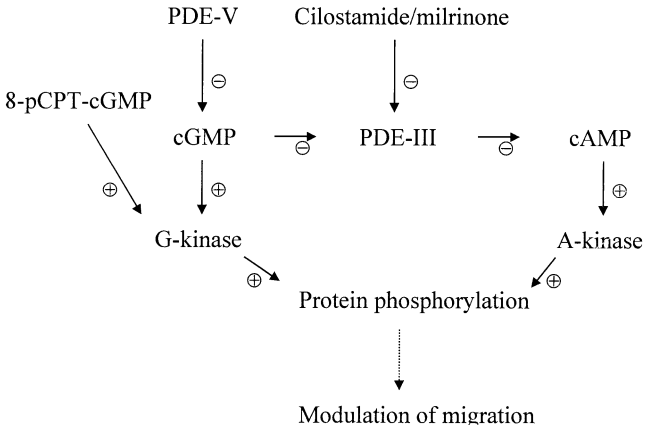

FIG. 1. Model showing the proposed interactions of cGMP, 8-pCPT-cGMP and cAMP, at the level of phosphodiesterases, during neutrophil migration. For details, see text.

The effect of cAMP on neutrophil migration was largely concentration-dependent [3] (Fig. 1, insert). It is conceivable that interference with cAMP breakdown, by altering the time course of the decrease in $[cAMP]_i$ after the cells have closed again (see Materials and Methods), will alter the effect of cAMP on migration. Thus, impaired or delayed hydrolysis of an otherwise optimal concentration of cAMP could potentially lead to an inhibitory effect on migration. Cyclic GMP may decrease cAMP hydrolysis via inhibition of PDE-III [4]. To test the hypothesis that inhibition of cAMP hydrolysis via PDE-III is involved in the inhibitory effect on migration in the presence of both cGMP and cAMP, we studied the effect of two inhibitors of PDE-III. Milrinone (1 μM; Sigma) and cilostamide (20 nM; Tocris Cookson) had marginal effects on random migration or migration induced by 8-pCPT-cGMP, but the compounds markedly inhibited migration induced by an optimal concentration of cAMP (Table 1). Unfortunately, measurement of $[cAMP]_i$ in (resealed) electropermeabilized neutrophils, stimulated with cAMP, could not be carried out. Because a high concentration of cAMP is present in the medium, cells have to be sedimented and extensively washed to remove cAMP in the interstitial space, during

TABLE 1. The effect of two inhibitors of PDE-III, milrinone (1 μM) and cilostamide (20 nM), on migration induced by cAMP (1 μM) and/or 8-pCPT-cGMP (0.1 μM)

	Migration (% of control)		
	—	Milrinone	Cilostamide
—	100 ± 4	92 ± 3*	85 ± 5*
8-pCPT-cGMP	158 ± 10	148 ± 8	143 ± 9
cAMP	154 ± 7	105 ± 6*	93 ± 8*
8-pCPT-cGMP + cAMP	250 ± 12	104 ± 10*	99 ± 8*

*$P < 0.05$ as compared with control (without inhibitor).

which rapid cAMP hydrolysis by e.g. PDE-IV interferes with the assay.

Because 8-pCPT-cGMP only mimics cGMP in being an activator of cGMP-dependent protein kinase, but does not inhibit PDE-III [12], it was hypothesized that 8-pCPT-cGMP + milrinone/cilostamide should be able to substitute for cGMP in both activating G-kinase and inhibiting PDE-III. In line with this hypothesis, addition of milrinone or cilostamide abolished the enhancing effect induced by a combination of 8-pCPT-cGMP and cAMP (Table 1; compare with Fig. 1).

In conclusion, modulation of the activity of PDE-III provides an explanation for the interaction of cGMP and cAMP during migration of electropermeabilized neutrophils, resulting in inhibition of migration. This observation, as well as the enhancement of migration in the presence of 8-pCPT-cGMP and cAMP, can be described as a model which is shown in Fig. 2:

(a) cGMP, cilostamide and milrinone, but not 8-pCPT-cGMP, may prolong the action of cAMP via inhibition of PDE-III;

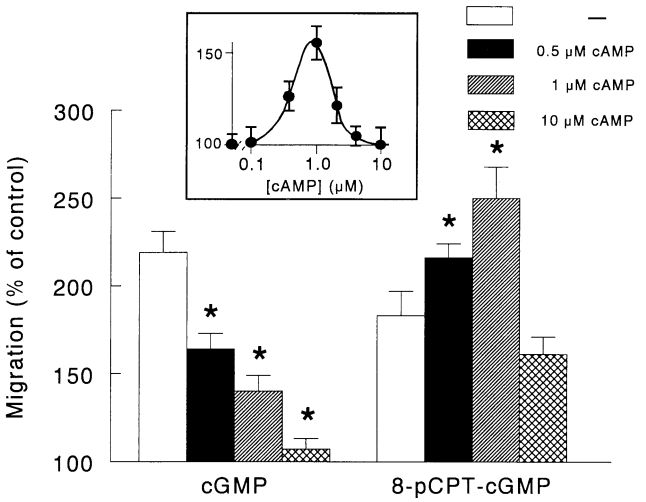

FIG. 2. Migration of electropermeabilized neutrophils in the presence of different cyclic nucleotides: the effect of different concentrations of cAMP on migration in the presence of cGMP (5 μM) and 8-pCPT-cGMP (0.1 μM). Insert: migration induced by different concentrations of cAMP alone. Results were expressed as percent of control (random migration), which ranged from 30.2 to 35.2 μm, depending on the experiment. *$P < 0.05$ as compared with control (without cAMP).

(b) Cilostamide/milrinone + 8-pCPT-cGMP can substitute for cGMP in both inhibiting PDE-III and activating cGMP-dependent protein kinase.

Although PDE-IV (cAMP-specific) and PDE-V appear to be the predominant isoforms in neutrophils [10, 13], indirect evidence is presented in this study that cAMP-hydrolyzing activity by PDE-III is present in this cell type as well. Migration by neutrophils is an important property of these cells, allowing them to reach sites of infection and inflammation. Cyclic nucleotides affect neutrophil migration. The present study shows that interactions between cGMP and cAMP on the level of PDEs may regulate neutrophil migration, and may thus modulate infection and inflammation.

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