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Ca^{2+} IONOPHORE-INDUCED CYCLIC ADENOSINE-3′,5′-MONOPHOSPHATE ELEVATION IN HUMAN NEUTROPHILS

A CALMODULIN-DEPENDENT POTENTIATION OF ADENYLATE CYCLASE RESPONSE TO ENDOGENOUSLY PRODUCED ADENOSINE: COMPARISON TO CHEMOTACTIC AGENTS

MARIE A. IANNONE,* GERALD WOLBERG and THOMAS P. ZIMMERMAN
Division of Experimental Therapy, Wellcome Research Laboratories, Research Triangle Park, NC 27709, U.S.A.

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Abstract—The cyclic adenosine-3′,5′-monophosphate (cAMP) elevation caused by exposure of human neutrophils to the Ca^{2+} ionophore A23187 was prevented when endogenously produced adenosine was either removed by preincubation with adenosine deaminase or blocked from binding to the adenosine receptor by antagonists [theophylline or (*E*)-4-(1,2,3,6-tetrahydro-1,3-dimethyl-2,6-dioxo-9*H*-purin-8-yl)cinnamic acid]. In the absence of endogenous adenosine, A23187 potentiated the neutrophil cAMP response to 2-chloroadenosine, prostaglandin E_1, and isoproterenol. When neutrophil suspensions were preincubated with concentrations of Ro 20-1724, which appeared to maximally inhibit cAMP phosphodiesterase, A23187 was still able to substantially elevate cAMP levels, suggesting that A23187 increases cAMP by amplifying adenylate cyclase responsiveness to the agonist rather than by inhibiting cAMP phosphodiesterase. The ability of A23187 to augment the cAMP elevation caused by 2-chloroadenosine was persistent over a 10-min period. The neutrophil cAMP elevations caused by the chemoattractants leukotriene B_4, C5a, and *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP) were all prevented when endogenously produced adenosine was eliminated from the cell suspensions by the addition of adenosine deaminase. The A23187-induced cAMP elevation was inhibited completely by the calmodulin inhibitors chlorpromazine, trifluoperazine and *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide, whereas cAMP levels induced by FMLP, leukotriene B_4 and C5a were less affected. It appears that A23187 raises cAMP in human neutrophils by a calmodulin-dependent potentiation of adenylate cyclase responsiveness to endogenously produced adenosine while the chemoattractant-induced cAMP elevations (FMLP, leukotriene B_4, and C5a), although possibly Ca^{2+}dependent, are less sensitive to calmodulin inhibitors and may involve additional biochemical events.

Neutrophil activation by a variety of stimuli is often preceded by intracellular Ca^{2+} elevation [1–13] and a concomitant rise in cAMP† levels [14–23]. We have used the Ca^{2+} ionophore A23187, which elevates Ca^{2+} levels independently of receptor stimulation [16, 18, 24, 25], to investigate the role of Ca^{2+} in the mechanism of stimulus-induced cAMP elevation in human neutrophils.

MATERIALS AND METHODS

Reagents. A23187, *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP), leukotriene (LT) B_4, human recombinant C5a, chlorpromazine, trifluoperazine, W-5, W-7, theophylline, 2-chloroadenosine (Cl-Ado), prostaglandin (PG) E_1, and L-isoproterenol (ISO) were obtained from the Sigma Chemical Co. (St. Louis, MO). Calf intestine adenosine deaminase (ADA) was a product of Boehringer Mannheim Biochemicals (Indianapolis, IN). Hanks' balanced salt solution was obtained from the Grand Island Biological Co. (Gaithersberg, MD), and [8-^{3}H]cAMP (20 Ci/mmol) was a product of Schwartz Mann (Cleveland, OH). Ro 20-1724 was provided by H. Sheppard (Hoffmann-La Roche, Nutley, NJ). SQ 22,356 was obtained from E.R. Squibb & Sons (Princeton, NJ). 533U83 was synthesized by Susan Daluge of Wellcome Research Laboratories (Research Triangle Park, NC).

Human neutrophil isolation. Neutrophils were isolated from heparinized whole blood of healthy volunteers by one-step gradient centrifugation [26, 27]. Red blood cells were lysed with 0.2% NaCl. Neutrophils were resuspended to the desired cell concentration in HBSS. All neutrophil preparations contained less than 5% mononuclear cells and less than 1% contamination by platelets.

* Corresponding author. Tel. (919) 248-8709; FAX (919) 248-8747.

† Abbreviations: 533U83, (*E*)-4-(1,2,3,6-tetrahydro-1,3-dimethyl-2,6-dioxo-9*H*-purin-8-yl)cinnamic acid; ADA, adenosine deaminase; cAMP, cyclic adenosine-3′,5′-monophosphate; Cl-Ado, 2-chloroadenosine; FMLP, *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine; HBSS, Hanks' balanced salt solution with 10 mM Hepes; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; ISO, L-isoproterenol; PG, prostaglandin; LT, leukotriene; RIA, radioimmunoassay; Ro 20-1724, 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone; W-5, *N*-(6-aminohexyl)-1-naphthalenesulfonamide; and W-7, *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide.

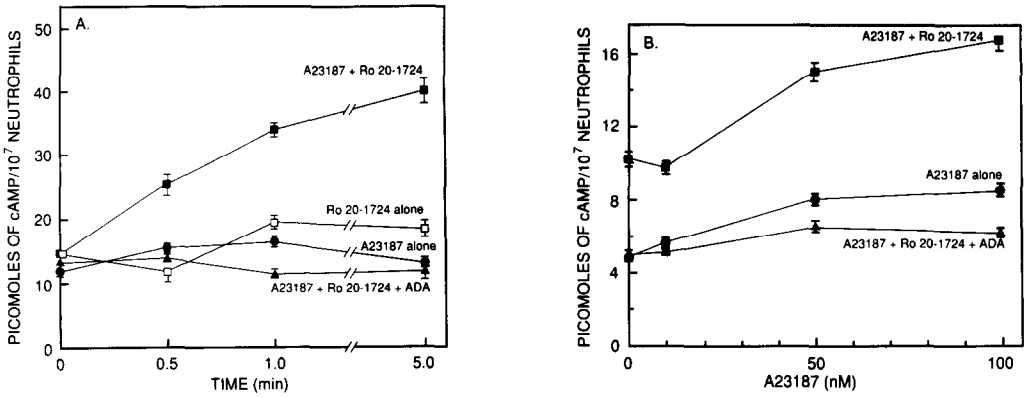

Fig. 1. Time- and concentration-dependence of A23187-induced cAMP elevation in human neutrophils. Neutrophil suspensions were incubated for 10 min at 37° with saline (●), 10 μM Ro 20-1724 (□, ■), or Ro 20-1724 in combination with ADA (1 unit/mL) (▲), prior to incubation with (closed symbols) or without (□) A23187. In panel A, neutrophils were incubated with 50 nM A23187 for the indicated period of time. In panel B, neutrophils were incubated with the indicated concentration of A23187 for 0.5 min. Neutralized acid-soluble extracts of the neutrophil suspensions were quantitated for cAMP by RIA as described in Materials and Methods. The data shown in each graph were obtained from a single representative experiment. Each point is the mean ± SEM for four determinations, two for each duplicate sample. Error bars are not shown when symbol size exceeded the range of the SEM.

cAMP determinations. Neutrophil suspensions (1.3–3.5 × 10^6 cells/1.0 mL HBSS) were incubated with specified agents for the indicated period of time at 37°. The cell suspensions were then extracted by the addition of 500 μL of cold 2.5 M trichloroacetic acid containing 8000–10,000 dpm (0.2 pmol) of [8-^{3}H]cAMP as a recovery marker. Acid-soluble extracts of the neutrophil suspensions were neutralized by extraction with 3.5 mL of 0.5 M trioctylamine in 1,1,2-trichlorotrifluoroethane (Freon 113) [28]. The cAMP present in the samples was quantitated by radioimmunoassay (RIA) [29] after purification of the extracts on columns of aluminium oxide and Dowex 1-X8 and 2'-O-succinylation. Values in each figure or table are from a single experiment which has been repeated at least once and has yielded similar results.

RESULTS

The Ca^{2+} ionophore A23187 (50 nM) alone caused a small elevation ($P < 0.001$) in human neutrophil cAMP levels which peaked about 1 min after ionophore addition (Fig. 1A). As previously noted [14, 30], neutrophil cAMP levels were enhanced when cAMP phosphodiesterase activity was inhibited. In the presence of the nonmethylxanthine phosphodiesterase inhibitor Ro 20-1724 (10 μM), A23187-induced cAMP accumulation was greatly enhanced (Fig. 1A). Human neutrophil suspensions endogenously produce 0.1 to 0.2 μM adenosine [31–33]. When the endogenously produced adenosine was converted to inosine by preincubation with ADA (1 unit/mL), the A23187-induced cAMP elevation in the presence of Ro 20-1724 was prevented (Fig. 1A). The elevation in neutrophil cAMP caused by A23187 was concentration dependent in both the presence and the absence of Ro 20-1724 (Fig. 1B). The increase in cAMP levels was greatly potentiated when Ro 20-1724 was present. Removal of endogenously produced adenosine by preincubation with ADA again prevented the rise in cAMP levels induced by A23187 in the presence of Ro 20-1724. A23187-induced cAMP elevations were also not observed when adenosine was prevented from binding to its receptor by the adenosine receptor antagonists theophylline (50 μM) or the more selective 533U83 [34] (Table 1).

In the absence of endogenously produced adenosine, and in the presence of Ro 20-1724, a 1.0-min incubation with the Ca^{2+} ionophore markedly potentiated cAMP generation induced by three adenylate cyclase agonists that bind to different receptors—PGE_1 (2.0 μM), ISO (1.0 μM), and Cl-Ado (which cannot be deaminated by ADA) (2.0 μM) (Table 2). This demonstrates that A23187 can potentiate cAMP response to different classes of adenylate cyclase agonists. The potentiating effect of A23187 on cAMP generation was reported previously for PGE_1, PGE_2 and ISO using guinea pig macrophages [35] and neutrophils [36, 37].

The potentiation of neutrophil cAMP response to agonists by A23187 may occur in one of two ways: inhibition of cAMP phosphodiesterase or activation of adenylate cyclase. To address this point, neutrophil suspensions were incubated for 1.0 min with increasing concentrations of the cAMP phosphodiesterase inhibitor Ro 20-1724 (0–100 μM) in the presence or absence of A23187 (50 nM). In the absence of A23187, maximal inhibition of neutrophil cAMP phosphodiesterase was indicated by a plateauing of neutrophil cAMP levels at concentrations of Ro 20-1724 at 10 μM and above (Table 3). Even at these concentrations of phosphodiesterase inhibitor, A23187 still elevated neutrophil cAMP levels. This suggests that A23187 potentiates cAMP response to endogenously produced adenosine by

Table 1. Effects of ADA, theophylline, or 533U83 on A23187-induced cAMP elevation in human neutrophils

Additive	cAMP (pmol/10^7 neutrophils)	
	−A23187	+A23187 (50 nM)
None	11.78 ± 1.58	22.68 ± 1.01
ADA (1 unit/mL)	10.83 ± 0.54	8.17 ± 0.63
Theophylline (50 μM)	10.89 ± 0.48	8.94 ± 0.76
533U83 (1 μM)	11.21 ± 0.80	10.79 ± 0.79
533U83 (5 μM)	10.57 ± 0.32	8.84 ± 0.90
533U83 (50 μM)	9.45 ± 0.36	9.73 ± 0.34

Prior to a final 1.0-min incubation in the presence or absence of A23187, neutrophil suspensions were preincubated for 15 min at 37° in the presence or absence of ADA, theophylline, or 533U83, followed by a 1.0-min incubation with Ro 20-1724 (10 μM). Neutralized acid-soluble extracts of the neutrophil suspensions were quantitated for cAMP by RIA as described in Materials and Methods. All incubations were performed in triplicate and each extract was radioimmunoassayed in duplicate for cAMP. Each value is the mean ± SEM of six determinations from a single representative experiment.

Table 2. Potentiation of neutrophil cAMP response to PGE_1, ISO, and Cl-Ado by A23187

Additive	cAMP (pmol/10^7 neutrophils)	
	−A23187	+A23187 (50 nM)
None	8.6 ± 1.2	12.0 ± 0.6
PGE_1 (2 μM)	56.7 ± 1.6	145.5 ± 8.3
ISO (1 μM)	51.2 ± 1.9	159.9 ± 8.4
Cl-Ado (2 μM)	40.6 ± 1.8	152.7 ± 2.8

Neutrophil suspensions were incubated at 37° for 15 min with ADA, 1.0 min with or without A23187, and 2.0 min with Ro 20-1724 in the presence or absence of PGE_1, ISO, or Cl-Ado. Neutralized acid-soluble extracts of the neutrophil suspensions were quantitated for cAMP by RIA as described in Materials and Methods. All incubations were performed in triplicate and each extract was radioimmunoassayed in duplicate for cAMP. Each value is the mean ± SEM of six determinations from a single representative experiment.

amplifying adenylate cyclase response rather than by inhibiting cAMP phosphodiesterase.

The duration of the ability of A23187 to potentiate neutrophil cAMP response to Cl-Ado is shown in Fig. 2. In this experiment, Cl-Ado (2.0 μM) and Ro 20-1724 (10 μM) were added to ADA-pretreated neutrophils at various times after the addition of A23187 (50 nM). The cellular incubations were terminated 1.0 min later. A23187 amplified the cAMP response to Cl-Ado throughout the 10-min period examined.

FMLP, LTB_4, and C5a are potent neutrophil chemoattractants which, like A23187, raise intracellular Ca^{2+} [4–13] and stimulate cAMP elevation [14–23]. We have reported previously that FMLP also raises neutrophil cAMP levels by potentiating adenylate cyclase responsiveness to endogenously produced adenosine [14]. We therefore looked at the role of endogenously produced adenosine in LTB_4- and C5a-induced cAMP elevations in neutrophils. During a 1.0-min exposure of neutrophils to increasing concentrations of LTB_4 and C5a, in the presence of the cAMP phosphodiesterase inhibitor Ro 20-1724, neutrophil cAMP levels were elevated. LTB_4- and C5a-induced cAMP elevations were prevented completely when endogenously produced adenosine was removed from the cell suspensions by preincubation with ADA (Fig. 3).

To determine if Ca^{2+}-activated calmodulin played a role in the amplification of adenylate cyclase response to agonists, neutrophil suspensions were incubated with noncytotoxic concentrations (as determined by trypan blue exclusion) of the calmodulin inhibitors chlorpromazine (50 μM), trifluoperazine (20 μM) or W-7 (100 μM) prior to the addition of A23187, FMLP, LTB_4, or C5a, in the presence of Ro 20-1724. A23187-induced cAMP elevations were inhibited completely by all three calmodulin inhibitors (Table 4). In contrast to A23187-induced cAMP elevation, the effects of the calmodulin inhibitors on chemoattractant-induced cAMP levels were comparatively small (Table 5). Inhibition by chlorpromazine and trifluoperazine of cAMP elevations induced by the chemotactic agents FMLP and LTB_4 was 4–21%, while C5a-induced cAMP elevation was enhanced slightly in the presence of chlorpromazine and was unaffected by trifluoperazine. W-7, however, partially inhibited FMLP-, LTB_4, and C5a-induced cAMP elevations (41–58%) but still not as completely as A23187. W-5 (100 μM), a less active, nonchlorinated derivative of W-7, had no effect on cAMP levels induced by A23187 or FMLP (data not shown).

DISCUSSION

We have shown that the Ca^{2+} ionophore A23187 elevates cAMP by amplifying adenylate cyclase response to endogenously produced adenosine. In the absence of endogenous adenosine, or in the presence of an adenosine receptor antagonist, the

Table 3. Effect of the cAMP phosphodiesterase inhibitor Ro 20-1724 on A23187-induced cAMP elevation in human neutrophils

Concentration of Ro 20-1724 (μM)	cAMP (pmol/10^7 neutrophils)	
	−A23187	+A23187 (50 nM)
0	3.41 ± 0.29	4.79 ± 0.33
1	5.17 ± 0.43	12.26 ± 0.82
5	5.92 ± 0.18	16.17 ± 0.58
10	6.70 ± 0.26	11.45 ± 0.53
50	7.05 ± 0.30	12.66 ± 0.33
100	5.66 ± 0.27	11.03 ± 0.44

Neutrophil suspensions were prewarmed at 37° for 15 min. Ro 20-1724 (at the indicated concentration) was added with or without A23187. The cell suspensions were incubated for an additional 1.0 min before termination by the addition of cold acid. Neutralized extracts of the neutrophil suspensions were quantified for cAMP by RIA as described in Materials and Methods. All incubations were performed in triplicate and each extract was radioimmunoassayed in duplicate for cAMP. Each value is the mean ± SEM of six determinations from a single representative experiment.

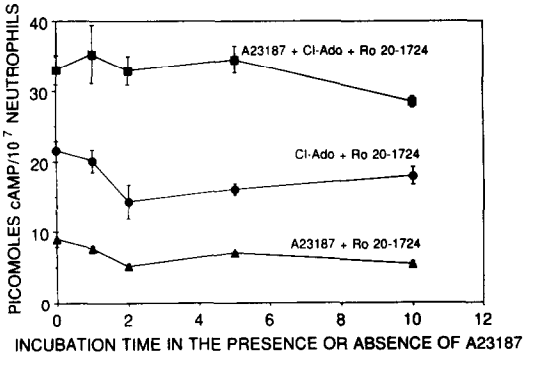

Fig. 2. Persistent nature of A23187-enhanced cAMP response to Cl-Ado. Neutrophil suspensions were incubated for 15 min at 37° with ADA (1 unit/mL) and then for the indicated period of time in the absence (●) or presence (■, ▲) of A23187 (50 nM). The cells were then incubated for 1.0 min with Ro 20-1724 (10 μM) (▲) or with Cl-Ado (2.0 μM) in combination with Ro 20-1724 (●, ■). Neutralized acid-soluble extracts of the neutrophil suspensions were quantitated for cAMP by RIA as described under Materials and Methods. The basal level of cAMP in control neutrophil suspensions that were incubated for 16 min at 37° was 2.63 ± 0.15 mol cAMP/10^7 neutrophils. Each point is the mean ± SEM for six determinations, two for each triplicate sample. The data in the graph were obtained from a single representative experiment. Error bars are not shown when symbol size exceeded the range of the SEM.

cAMP response induced by A23187 was abrogated. The A23187 potentiation of cAMP response was not just specific for adenosine but was also demonstrated in the absence of endogenously produced adenosine, with other adenylate cyclase agonists that bind to three distinct receptors. The ability of A23187 to potentiate adenylate cyclase response to Cl-Ado was constant over a 10-min period.

FMLP, LTB_4, and C5a are potent chemotactic stimuli which elevate neutrophil cAMP levels [14–23]. These cAMP elevations appear to be a chemotactant-mediated amplification of adenylate cyclase response to endogenously produced adenosine [14] (Fig. 3), rather than events necessary for signal transduction [25].

There is considerable evidence for the involvement of Ca^{2+} in chemotactant-potentiated neutrophil cAMP response to agonists. FMLP-induced cAMP elevation is inhibited when cells are in Ca^{2+}-deficient medium or in the presence of TMB-8, a putative Ca^{2+} antagonist [15, 16, 36]. The onset and duration of amplified cAMP accumulation correlate with the timing of intracellular Ca^{2+} elevation; exposure of neutrophils to FMLP, LTB_4, or C5a results in a rapid, transient Ca^{2+} burst [4–14] and cAMP response [14–23], whereas exposure to ionophore yields a prolonged elevation of intracellular Ca^{2+} [6, 7, 38] and a persistently potentiated cAMP response (Fig. 2).

The elevation of intracellular Ca^{2+} has several biochemical consequences, one of which is the activation of calmodulin, a ubiquitous Ca^{2+}-dependent regulatory protein. Calmodulin has been reported to regulate cAMP metabolism in a manner dependent upon intracellular Ca^{2+} concentration [39, 40]. We therefore investigated whether the cAMP elevations induced by A23187, FMLP, LTB_4, and C5a involve a Ca^{2+}-regulated calmodulin-dependent activation of adenylate cyclase. A23187-induced cAMP elevation was prevented completely by preincubation with the three different calmodulin inhibitors W-7, chlorpromazine, and trifluoperazine. These results are in agreement with an earlier report citing the involvement of calmodulin in A23187-potentiated cAMP response to PGE_1 in guinea pig neutrophils [36]. The same calmodulin inhibitors were less effective at preventing FMLP-, LTB_4-, and C5a-induced cAMP elevation. It is unclear why W-7 was more effective than the other calmodulin inhibitors in preventing cAMP generation among

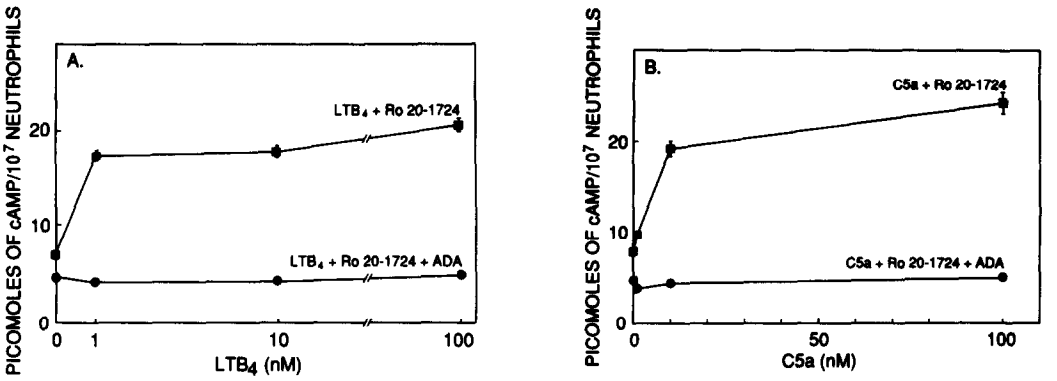

Fig. 3. Effect of endogenously produced adenosine on LTB_4- and C5a-induced cAMP elevation in human neutrophils. Neutrophil suspensions were incubated for 15 min at 37° in the presence (●) or absence (■) of ADA (1 unit/mL) prior to incubation with LTB_4 (panel A) or C5a (panel B), in combination with Ro 20-1724 (10 μM), for 1.0 min at the indicated concentration. Neutralized acid-soluble extracts of the neutrophil suspensions were quantitated for cAMP by RIA as described under Materials and Methods. The data shown in each graph were obtained from a single representative experiment. Each point is the mean ± SEM for six determinations, two for each triplicate sample. Error bars are not shown when symbol size exceeded the range of the SEM.

Table 4. Effects of calmodulin inhibitors on A23187-induced cAMP levels in human neutrophils

	cAMP (pmol/10^7 neutrophils)	
Additive	−A23187	+A23187 (50 nM)
None	4.30 ± 0.23	12.41 ± 0.30
Chlorpromazine (50 μM)	3.84 ± 0.27	3.25 ± 0.23
Trifluoperazine (20 μM)	2.97 ± 0.42	2.48 ± 0.26
W-7 (100 μM)	3.04 ± 0.11	3.02 ± 0.27

Neutrophil suspensions were preincubated for 14 min at 37° in the presence or absence of the specified calmodulin inhibitor followed by 1.0 min with Ro 20-1724 (10 μM). The cell suspensions were then incubated for 1.0 min in the presence or absence of A23187. Neutralized acid-soluble extracts of the neutrophil suspensions were quantitated for cAMP by RIA as described in Materials and Methods. All incubations were performed in triplicate and each extract was radioimmunoassayed in duplicate for cAMP. Each value is the mean ± SEM of six determinations from a single representative experiment.

Table 5. Effects of calmodulin inhibitors on FMLP-, C5a-, and LTB_4-induced cAMP levels in human neutrophils

	cAMP (pmol/10^7 neutrophils)			
Additive	Saline	FMLP (10 nM)	LTB_4 (1 nM)	C5a (10 nM)
None	7.55 ± 0.16	40.79 ± 2.35	26.18 ± 0.86	32.38 ± 1.10
Chlorpromazine (50 μM)	5.95 ± 0.25	37.91 ± 1.27	21.71 ± 0.92	43.96 ± 2.36
Trifluoperazine (20 μM)	4.87 ± 0.30	33.84 ± 1.53	19.56 ± 0.83	32.07 ± 0.67
W-7 (100 μM)	4.21 ± 0.16	18.83 ± 0.44	12.07 ± 0.34	18.87 ± 0.51

Neutrophil suspensions were preincubated for 14 min at 37° in the presence or absence of the specified calmodulin inhibitor followed by 1.0 min with Ro 20-1724 (10 μM). The cell suspensions were then incubated for 1.0 min in the absence or presence of FMLP, LTB_4, or C5a. Neutralized acid-soluble extracts of the neutrophil suspensions were quantitated for cAMP by RIA as described in Materials and Methods. All incubations were performed in triplicate and each extract was radioimmunoassayed in duplicate for cAMP. Each value is the mean ± SEM of six determinations from a single representative experiment.

the chemoattractants. It appears that A23187 potentiates adenylate cyclase responsiveness to agonists by a calmodulin-dependent process while the chemoattractant-induced cAMP elevations, although apparently Ca^{2+} dependent, are not as sensitive to calmodulin inhibitors. The chemoattractant-induced cAMP responses may involve other Ca^{2+}-mediated biochemical events such as changes in cytoskeletal components or activation of protein kinase C. In fact, cAMP elevations in human neutrophil suspensions induced by phorbol 12-myristate 13-acetate, a protein kinase C activator, were prevented when endogenously produced adenosine was removed by preincubation with ADA (unpublished observations).

In addition to effects on calmodulin, chlorpromazine, trifluoperazine, and W-7 have been reported to inhibit protein kinases [41–44] and the Ca^{2+}-dependent protease calpain I [45]. In the experiments reported here, inhibition of these calmodulin-independent activities is unlikely, since IC_{50} values determined using isolated enzyme preparations exceed concentrations we find active using whole cells. The cAMP elevations induced by the Ca^{2+}-independent protein kinase C activator phorbol 12-myristate 13-acetate were not inhibited by these agents (data not shown).

Several selective and nonselective phosphodiesterase inhibitors have been examined for their effect on cAMP metabolism in human neutrophils by Wright *et al.* [46]. Only nonselective and cAMP-specific, cGMP-insensitive phosphodiesterase inhibitors (including Ro 20-1724) were effective in causing cAMP elevation in human neutrophils exposed to FMLP. Inhibitors of other phosphodiesterase subtypes did not cause statistically significant increases in cAMP response to FMLP, these included calmodulin-dependent; cGMP-specific; cGMP-stimulated; and cAMP-specific, cGMP-inhibited phosphodiesterases. It appears that these other phosphodiesterase subtypes are not operative in chemoattractant-stimulated human neutrophils.

It may appear contradictory that cell activators such as A23187, FMLP, LTB_4, and C5a generate an inhibitory cell signal such as cAMP. The cAMP burst appears not to be a necessary component of cell activation but rather a response of the activated cell to endogenously produced adenosine. In fact, superoxide anion generation stimulated by A23187 or FMLP was enhanced when endogenously produced adenosine was removed by preincubation with ADA (unpublished observations). The extent of neutrophil involvement in an inflammatory response may be regulated by chemoattractants, which, by elevating intracellular Ca^{2+} levels, increase neutrophil sensitivity to physiological adenylate cyclase agonists.

In summary, A23187 appeared to increase cAMP levels in the human neutrophil by amplifying the responsiveness of adenylate cyclase to endogenously produced adenosine. This effect of A23187 on cAMP metabolism was persistent and was not specific for adenosine. The biochemical mechanism by which adenylate cyclase responsiveness was amplified by A23187 appears to be calmodulin-dependent. The chemotactic stimuli FMLP, LTB_4, and C5a also appeared to raise cAMP in human neutrophils by amplifying the responsiveness of adenylate cyclase to endogenously produced adenosine. Ca^{2+} may play a role in this process; however, the chemoattractant-induced cAMP elevation was less sensitive to calmodulin inhibitors than the A23187-induced response.

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