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# STIMULATORY EFFECT OF IONOPHORES ON ADENOSINE 3′,5′-MONOPHOSPHATE CONTENT IN HUMAN MONONUCLEAR LEUKOCYTES

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**Abstract**—Ionophores A23187 and bromo-lasalocid ethanolate enhanced the cyclic AMP content in human mononuclear leukocytes. The maximum effect of A23187 with a 10-min incubation was found with 0.3–1.0 μM concentrations with or without *l*-isoproterenol (1 μM) or prostaglandin $E_1$ ($PGE_1$) (0.3 μM). The maximum effect after 5 min of incubation at 37° was observed with 0.05, 0.2 and 1 μM A23187. The effect of ionophore A23187 was enhanced by both aminophylline (1 mM) and isobutylmethylxanthine (1 mM). Calcium (1 mM), aspirin (1 mM) and indomethacin (100 μM) decreased the stimulatory action of A23187. Bromo-lasalocid ethanolate increased cyclic AMP content in cells maximally at a 3 μM concentration with or without 0.3 μM $PGE_1$.

Ionophores produce significant effects in a wide variety of biological systems [1]. In lymphocytes, ionophore A23187 has a moderately good mitogenic effect [2–4]. Although the mechanism of its action is unknown, it has been reported that this drug increases calcium influx [5] and cyclic AMP* formation in mononuclear leukocytes [5, 6]. These two effects are incompatible because studies to date have shown that calcium inhibits adenylate cyclase activity and cyclic AMP formation in most tissues [7–10]. However, the adenylate cyclases of adrenal and cerebral cortex are calcium dependent [11, 12].

Ionophore A23187 is a lipophilic compound [13], and it probably dissolves in the lipid matrix of the cell plasma membrane. This may disturb the steady state of receptors and catalytic subunits of the adenylate cyclase and may lead to a temporary rearrangement of the adenylate cyclase complex with the resulting activation of the enzyme. In addition, ionophore A23187 binds many cations [13], the influx or efflux of which may affect the activity of ion-dependent adenylate cyclase [14].

The aim of the present study was to analyze the mechanism of ionophore A23187 action on cyclic AMP production in human mononuclear leukocytes. Another ionophore, bromo-lasalocid ethanolate, was also used. Both ionophores caused an elevation of cyclic AMP content in mononuclear leukocytes. The increased level was additionally enhanced by $PGE_1$, *l*-isoproterenol and phosphodiesterase inhibitors. In contrast, calcium and prostaglandin synthesis inhibitors decreased the stimulatory action of ionophore A23187.

## MATERIALS AND METHODS

*Materials.* $^3$H-Labeled cyclic AMP (37.7 Ci/mmole) was purchased from the New England Nuclear Corp., Boston, MA. Ionophore A23187 was donated by Dr. R. J. Hosley, The Eli Lilly Co., Indianapolis, IN, and bromo-lasalocid ethanolate by Dr. W. E. Scott, Hoffman-La Roche, Inc., Nutley, NJ. $PGE_1$ was a gift of Dr. J. Pike, The Upjohn Co., Kalamazoo, MI. The buffy coats for mononuclear leukocyte isolation were purchased from the Central Blood Bank of Pittsburgh.

*Isolation of human mononuclear leukocytes.* The cells were harvested from buffy coats donated by blood donors. The precise protocol was described previously [10]. Briefly, the buffy coats were mixed with dextran solution (3 g/100 ml, 200,000–300,000 mol. wt) containing 3 units heparin/ml. The white blood cell layer was removed after 30 min and pelleted by centrifugation. The remaining erythrocytes were lysed in hypotonic NaCl, and the leukocytes were fractionated by the Ficoll–Hypaque technique [15]. The upper layer containing mononuclear leukocytes was washed twice and finally resuspended in the incubation buffer.

*Incubation of mononuclear leukocytes.* The cells were incubated in buffer consisting of: NaCl (123 mM), $KH_2PO_4$ (1.23 mM), KCl (4.9 mM), $MgSO_4$ (1.23 mM), glucose (1 mg/ml) and Tris/HCl (21 mM) (pH adjusted at room temperature to 7.4). The basal concentration of calcium in the incubation buffer was 3 μM and was determined by the method of Borle and Briggs [16]. Approximately $2 \times 10^7$ cells were incubated in duplicate or triplicate in a total volume of 2 or 3 ml. The ionophores A23187 and bromo-lasalocid ethanolate were dissolved in dimethylsulfoxide, and $PGE_1$ was dissolved in ethyl alcohol. Equal volumes of solvents were added to the control cells. The final concentration of dimethyl sulfoxide or ethyl alcohol in the incubation solution was less than 1 μl/ml. The mononuclear leukocytes were incubated with the drugs for various time intervals at 37° and after the incubation were cooled to 4° in an ice-bath and separated from the incubation medium by centrifugation (1600 *g*, 2 min at 4°). The

* Abbreviations used: cyclic AMP, adenosine 3′,5′-monophosphate; and $PGE_1$, prostaglandin $E_1$.

cell pellet was immediately homogenized by sonication (Quigley-Rochester Inc., setting 80 for 30 sec) in 0.5 ml trichloroacetic acid (6 g/100 ml).

*Determination of cyclic AMP.* The technique of Gilman [17] was employed; the precise protocol for cyclic AMP determination was described previously [10]. Cyclic AMP was determined in an aliquot of the ether-extracted trichloroacetic acid supernatant fluid. The assay was performed in duplicate in a total volume of 0.2 ml and the bound $^3$H-labeled cyclic AMP was determined in a liquid scintillation counter. A standard curve with non-radioactive cyclic AMP was included in each experiment.

## RESULTS

Figure 1 shows the effects of various concentrations of A23187 on cyclic AMP content in human mononuclear leukocytes. The additional stimulatory effect of $PGE_1$ (0.3 $\mu$M) or *l*-isoproterenol (1 $\mu$M) is also shown. The maximum stimulatory effect of A23187 was produced by a 1 $\mu$M concentration, at which the cyclic AMP concentration in the cells was approximately four times above the basal level. The addition of $PGE_1$ or *l*-isoproterenol did not change significantly the concentration of A23187 that produced the maximum effect. A23187, at the 10 $\mu$M level, did not have any effect on cyclic AMP content in mononuclear leukocytes; the same pattern was found in the cells treated simultaneously with ionophore A23187 and the two agonists.

The time dependence of the effect of various concentrations of A23187 on cyclic AMP content is shown in Fig. 2. A23187 at 0.05, 0.2 and 1 $\mu$M concentrations maximally stimulated cyclic AMP content at 5 min of incubation. There was an abrupt decrease of cyclic AMP level after this time interval, and after 120 min of incubation the cyclic AMP levels in the control and ionophore-treated cells were almost identical.

The effect of A23187 was further enhanced by the phosphodiesterase inhibitors aminophylline and isobutylmethylxanthine, both of which approximately doubled the basal cyclic AMP level and increased the A23187 effect 5- to 6-fold (Table 1). The maximum effect, however, was found when aminophylline (1 mM), $PGE_1$ (0.3 $\mu$M) and A23187 (1 $\mu$M) were added simultaneously to the mononuclear leukocytes. Although calcium (1 mM) increased the cyclic AMP content in control mononuclear leukocytes, it had an inhibitory effect on cyclic AMP content when the cells were incubated simultaneously with it and A23817.

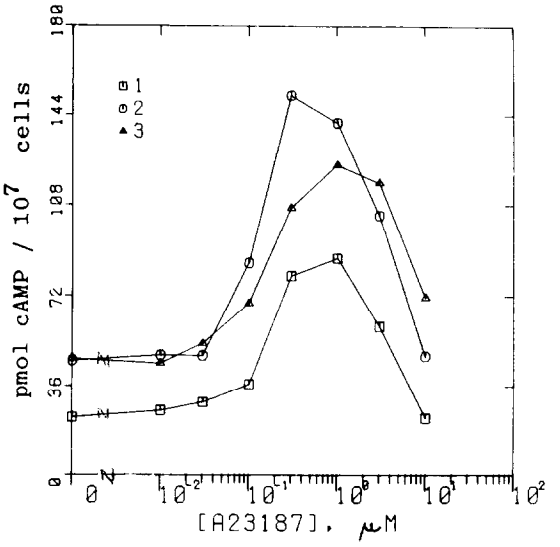


Fig. 1. Effect of ionophore A23187 on cyclic AMP formation in mononuclear leukocytes in the presence of agonists. The cells were incubated without (1) or with 0.3 $\mu$M $PGE_1$ (2) or 1.0 $\mu$M *l*-isoproterenol (3) for 10 min at 37°. Mean of five experiments.

As shown in Table 2, the effect of A23187 was decreased by about 50 per cent when the cells were preincubated before the ionophore addition with aspirin (1 mM) or indomethacin (100 $\mu$M).

Bromo-lasalocid ethanolate also increased the cyclic AMP content in mononuclear leukocytes (Table 3). The maximum effect was found at the 3 $\mu$M level. The addition of 0.3 $\mu$M $PGE_1$ increased further the cyclic AMP content in the cells but had

Table 1. Cyclic AMP formation in mononuclear leukocytes*

| Group | Additions | Cyclic AMP (pmoles/$10^7$ cells) Control | A23187 (1 $\mu$M) |
|---|---|---|---|
| 1 | None | 26.9 ± 2.49 | 43.8 ± 4.47 |
| | Aminophylline (1 mM) | 43.8 ± 4.47 | 248.0 ± 35.0 |
| | $PGE_1$ (0.3 $\mu$M) | 216.0 ± 38.7 | 368.0 ± 17.0 |
| | Aminophylline + $PGE_1$ | 315.0 ± 19.0 | 538.0 ± 26.2 |
| 2 | None | 16.5 ± 5.36 | 56.2 ± 8.32 |
| | Isobutylmethylxanthine (1 mM) | 43.2 ± 16.5 | 280.0 ± 27.2 |
| 3 | None | 27.6 ± 2.17 | 85.2 ± 7.31 |
| | Calcium (1 mM) | 41.3 ± 5.18 | 67.3 ± 5.68 |

* Cells were incubated with drugs for 10 min at 37°. The averages of each of six experiments ± S.D. are shown.

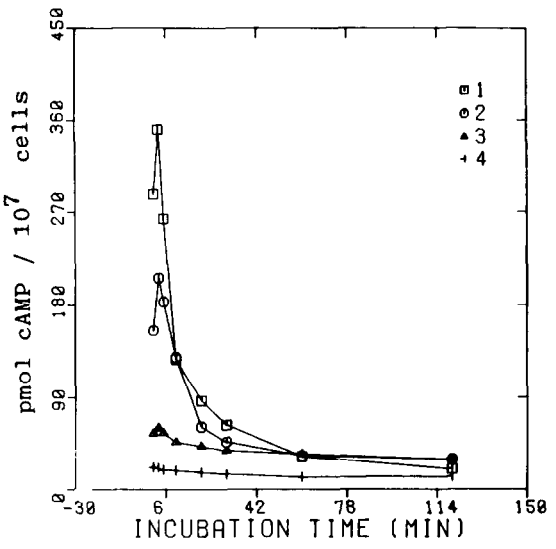


Fig. 2. Effect of ionophore A23187 on cyclic AMP formation in mononuclear leukocytes in relation to dose and time of incubation. The cells were incubated at 37° with 1 (1), 0.2 (2) and 0.05 μM A23187 (3). The cyclic AMP in the control cells is also shown (4). Mean of four experiments.

little or no effect on the ionophore concentration that maximally stimulated the cyclic AMP content in the cells.

## DISCUSSION

Ionophores A23187 and bromo-lasalocid ethanolate increased the cyclic AMP content in human circulating mononuclear leukocytes. The ionophore A23187, in a mitogenic dose, caused a transient, sharp increase of the cyclic AMP content in mononuclear leukocytes that peaked 5 min after its addition. However, over a period of 30–60 min, the cyclic AMP content declined to control values. Similar findings have also been reported by Greene *et al.* [5]. In contrast, Gallin *et al.* [6] have found an increased cyclic AMP formation that persisted for 30 min in mononuclear leukocytes after the addition of ionophore A23187. This discrepancy may be caused by the methodological procedure that was used, because Gallin *et al.* [6] analyzed the cyclic AMP levels in the cells plus the medium, whereas we determined the cyclic AMP content in the cells only. The possibility of cyclic AMP efflux from the stimulated mononuclear leukocytes into the incubation medium cannot be ruled out.

Table 2. Effect of prostaglandin synthesis inhibitors on cyclic AMP*

| Addition | Cyclic AMP (pmoles/$10^7$ cells) | |
|---|---|---|
| | Control | A23187 |
| None | 16.7 ± 1.33 | 79.7 ± 9.52 |
| Aspirin (1 mM) | 20.0 ± 1.23 | 39.9 ± 6.16 |
| Indomethacin (100 μM) | 20.9 ± 2.49 | 38.7 ± 6.23 |

* Mononuclear leukocytes were preincubated for 15 min at 37° with aspirin or indomethacin, and then ionophore A23187 (1 μM) was added for 10 min. Mean ± S.D. for seven experiments.

It has been reported recently that A23187 stimulates prostaglandin synthesis in rat and human tissues [18]. We can support this observation indirectly as the stimulatory effect of ionophore A23187 was partially abolished in the presence of the prostaglandin synthesis inhibitors aspirin and indomethacin.

Whether or not ionophore A23187 has a specific or general stimulatory effect on cyclic AMP formation, its stimulation of cyclic AMP formation appears to be related to mononuclear leukocytes only [2, 5, 19, and this report]. No effect was found un umbilical artery [20], bone organ culture medium [21], brain tissue [22], tracheal smooth muscle [23], renal tissue [24] or human granulocytes [25]. The reason for this specific stimulatory action of ionophore A23187 on cyclic AMP formation is unknown, but it may be related to the intrinsic organization of the mononuclear leukocyte plasma membrane.

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Table 3. Effect of bromo-lasalocid ethanolate on cyclic AMP*

| Bromo-lasalocid ethanolate (μM) | Cyclic AMP (pmoles/$10^7$ cells) | |
|---|---|---|
| | Control | $PGE_1$ (0.3 μM) |
| None | 20.8 ± 0.56 | 180 ± 12.5 |
| 0.01 | 23.1 ± 0.70 | 185 ± 13.5 |
| 0.03 | 20.6 ± 0.71 | 176 ± 14.8 |
| 0.1 | 27.3 ± 1.09 | 197 ± 12.8 |
| 0.3 | 30.5 ± 1.78 | 205 ± 10.6 |
| 1.0 | 44.9 ± 3.45 | 250 ± 20.6 |
| 3.0 | 51.5 ± 4.22 | 293 ± 23.3 |
| 10.0 | 41.3 ± 2.79 | 220 ± 19.8 |

* Mononuclear leukocytes were incubated with ionophore and $PGE_1$ for 10 min at 37°. Mean ± S.D. for seven experiments.

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