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RAPID CHANGES IN THE ACTIVITIES OF THE ENZYMES OF CYCLIC AMP METABOLISM AFTER ADDITION OF A23187 TO MACROPHAGES

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Summary: The ionophore A23187 stimulated adenylate cyclase activity in intact macrophages within 1 min. This action was blocked by pretreatment with indomethacin (25 μmol/l) suggesting the involvement of a prostaglandin (PG). PGE_2 (500 nmol/l) also stimulated adenylate cyclase activity in intact cells, but this was not prevented by indomethacin pretreatment. Colchicine (100 μmol/l) potentiated the increases in macrophage cyclic AMP production seen after addition of PGE_2 or A23187. The high affinity form of cyclic AMP phosphodiesterase (PDE) was activated within 1 min of the addition of A23187 to intact macrophages. The data suggest that the increase in macrophage cyclic AMP production after A23187 is a consequence of adenylate cyclase activation and not PDE inhibition. The endogenous production of a prostaglandin probably mediates this effect of A23187, emphasizing the importance of arachidonic acid metabolites in the regulation of macrophage functions.

The divalent ion ionophore A23187 stimulates many processes in rodent macrophages, including prostaglandin formation (1,2), respiration (3) and chemiluminescence (4). The ionophore has been used to study the biochemical processes which occur rapidly after the stimulation of macrophages. PGE_2 increases cyclic AMP formation in macrophages (5) and inhibits a number of macrophage functions, including tumoricidal activity (6) and phagocytosis (7). The involvement of cyclic AMP and prostaglandins in the regulation of the immune response has been reviewed (8,9). Since prostaglandins are synthesized and released by macrophages, these metabolites of arachidonic acid may act as endogenous regulators of macrophage function (1,2). Cyclic AMP concentrations within macrophages increase after the addition of A23187 (1,5); pretreatment with indomethacin, an inhibitor of prostaglandin formation, prevents this increase. It has been suggested therefore, that release of PGE mediates the effect of A23187 on the intracellular cyclic AMP concentration (1,5). The present study demonstrates that A23187 stimulates adenylate cyclase

Abbreviations: PGE_2, F, I_2: prostaglandins E_2, F, I_2. Cyclic AMP-PDE, cyclic AMP phosphodiesterase.

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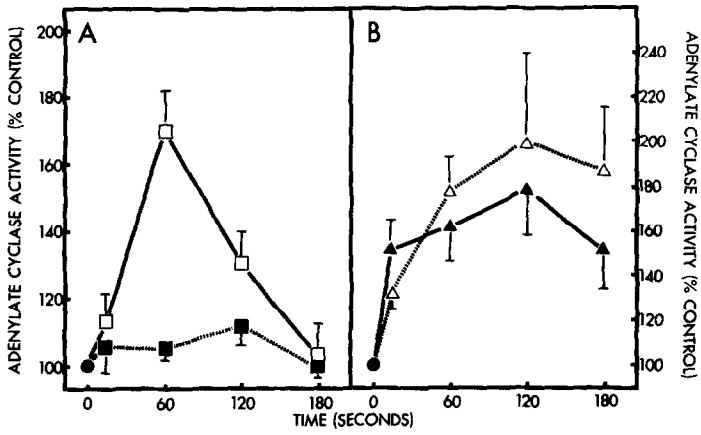

Fig. 1. Effect of A23187 (panel A) and PGE_2 (panel B) on adenylate cyclase activity in macrophages. Macrophages were incubated in buffer with (solid symbols) or without (open symbols) indomethacin (final concentration 25 μmol/l) for 5 min. A23187 (final concentration 6.7 μmol/l) or PGE_2 (final concentration 0.5 μmol/l) was added at time 0 to some incubations. Samples (1 ml) of the incubation were taken at various times and transferred rapidly into microfuge tubes. They were immediately microfuged (Eppendorf Model 5412) for 30 sec. The tubes and pellets were rinsed with cold buffer and the pellets were resuspended in buffer and homogenized (12 strokes, tight-fitting Dounce homogenizer) for assay of adenylate cyclase activity. Unstimulated and NaF-stimulated activities were measured; the latter did not change with any pre-treatment conditions. Control adenylate cyclase activity was 817 ± 60 pmol cyclic AMP produced/min/mg protein (24 observations from 6 experiments). Indomethacin pre-treatment did not affect adenylate cyclase activity (786 ± 60 p mol cyclic AMP produced/min/mg protein; 24 observations from 6 experiments). The data are expressed as a percentage of control, unstimulated adenylate cyclase activity. Values are means ± S.E.M. from 4 - 6 experiments.

activity in intact macrophages and provides evidence that this action is mediated by an endogenous metabolite of arachidonic acid.

Materials and Methods: A23187 was kindly provided by Dr. R. L. Hamill (Eli Lilly) and PGE by Dr. J. Pike (Upjohn Co., Kalamazoo, Mich.). Indomethacin was from Sigma. Complete descriptions of the procedures used for the assays of adenylate cyclase activity (5,10), cyclic AMP-PDE activity (11) and cyclic AMP concentration (5,12) have been given elsewhere. All of these assays were carried out in triplicate. Rat macrophages, elicited with Brewer's thioglycollate medium, were prepared as described previously (5). Indomethacin (25 μmol/l) was added to incubation 5 min prior to the addition of A23187 (6.7 μmol/l) in some experiments.

Results and Discussion: Both A23187 and zymosan particles are known to cause a rapid increase in cyclic AMP concentration in macrophages, this effect being blocked by pretreatment with indomethacin (1,5). Intracellular cyclic AMP concentrations can increase as a result of activation of adenylate cyclase or inhibition of cyclic AMP-PDE activity, or a combination of both. When intact macrophages were incubated with A23187, and then homogenized for the assay of adenylate cyclase activity, significant ($P < 0.01$) enzyme activation was observed after 1 min of preincubation with the ionophore (Fig. 1A). This

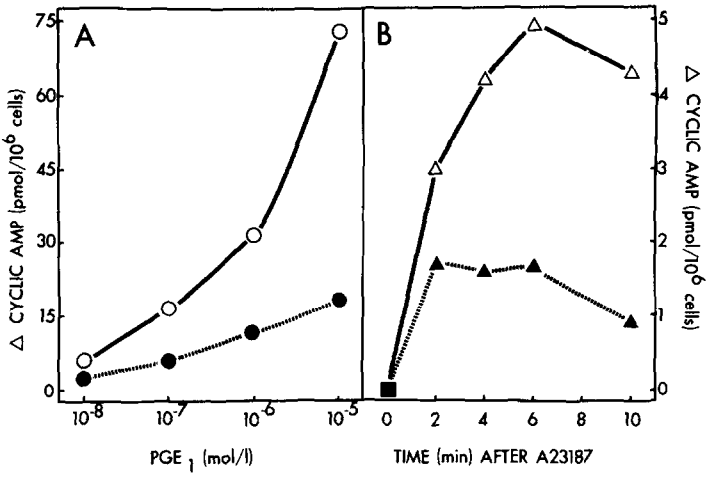

Fig. 2. Effect of colchicine upon the increases in cyclic AMP concentration produced by PGE_2 (panel A) and A23187 (panel B) in macrophage incubations. Panel A: Macrophages were incubated in buffer alone, buffer + colchicine (100 μmol/l), PGE_2 or PGE_2 + colchicine. 3-Isobutyl-1-methylxanthine (100 μmol/l, an inhibitor of cyclic AMP phosphodiesterase activity) was present in all incubations. The incubations (volume 400 μl) were terminated after 10 min by boiling for 2 min. Panel B: Macrophages were incubated in buffer alone, buffer + colchicine, A23187 (6.7 μmol/l) or A23187 + colchicine. At various times, 500 μl aliquots of incubations were withdrawn and boiled for 2 min. The data are expressed as the increase in cyclic AMP concentration produced by PGE_2 (panel A) or A23187 (panel B). Solid symbols represent increases in the absence of colchicine; open symbols represent increases in the presence of colchicine. Points are means from 2 experiments, each performed in triplicate.

result suggested that the ionophore had stimulated adenylate cyclase activity in intact cells, allowing the detection of this activation after homogenization. It should be noted that A23187 does not stimulate adenylate cyclase activity in homogenized cells (5). Preincubation of macrophages with indomethacin prevented the activation of adenylate cyclase by A23187 (Fig. 1A). This inhibitory effect of indomethacin suggested that the production of a prostaglandin might be important, as an intermediate step, in the activation of adenylate cyclase by A23187 in intact macrophages. Preincubation of macrophages with exogenous PGE_2, followed by homogenization, produced activation of adenylate cyclase in a manner analogous to that of A23187 (Fig. 1B). Indomethacin did not inhibit this effect of PGE_2 (Fig. 1B).

Colchicine potentiates the effect of PGE_2 upon cyclic AMP production in macrophages (13, 14). Similarly, the increase in macrophage cyclic AMP produced by addition of PGE_2 was enhanced by colchicine (Fig. 2A). If the effect of A23187 upon cyclic AMP production is mediated by a prostaglandin, colchicine would be expected to potentiate this action of the ionophore. Colchicine does have this effect (Fig. 2B) and since it does not affect

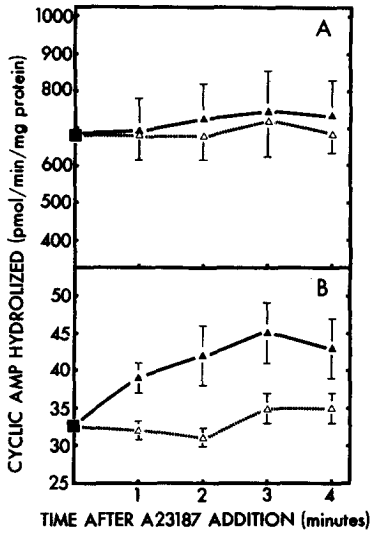

Fig. 3. Effects of A23187 on cyclic AMP-PDE activities in intact macrophages. Macrophages were incubated with A23187 (solid symbols) or buffer alone (open symbols). At various times, 1 ml aliquots were withdrawn and immediately microfuged (see legend to Fig. 1). After rinsing, the pellet was diluted and homogenized (12 strokes, tight-fitting Dounce homogenizer) for assay of cyclic AMP-PDE activity. Substrate concentrations were 100 µmol/l cyclic AMP (panel A) of 1 µmol/l cyclic AMP (panel B). Points are mean ± S.E.M. from 3 experiments.

cyclic AMP-PDE activity in macrophages (13), its actions are probably due to an influence upon adenylate cyclase.

Cyclic AMP concentrations in macrophage incubations decline to basal levels 20 min after their initial increase in response to A23187 (5). This suggests that cyclic AMP is being metabolized by cyclic AMP-PDE. To examine this possibility, macrophages were incubated with A23187 for various times and then homogenized for assay of cyclic AMP-PDE activity. Preliminary studies (not shown) have demonstrated non-linear plots of [S]/v vs. [S], consistent with the presence of high and low affinity forms of the enzyme. The activity of the high affinity form increased after the incubation of intact cells with A23187 (Fig. 4B). Pre-treatment with indomethacin did not prevent the activation of the enzyme (data not shown), suggesting that prostaglandin production was not a prerequisite for this action of A23187. The low affinity form of cyclic AMP-PDE was not activated by A23187 treatment (Fig.4A). In macrophage homogenates, neither A23187 nor indomethacin affected the activity of cyclic AMP-PDE (Table 1).

Considered together, these observations suggest that A23187 increases cyclic AMP formation by indirectly causing activation of adenylate cyclase.

Table 1: Effects of A23187 and indomethacin on cyclic AMP phosphodiesterase activity in macrophage homogenates.

Macrophages were homogenized by twelve strokes of a tight-fitting Dounce homogenizer. The homogenates were diluted and assayed for cyclic AMP phosphodiesterase activity at 1 μmol/l cyclic AMP substrate (incubation time 10 or 20 min) and at 100 μmol/l cyclic AMP substrate (incubation time 90 min). Values are mean ± S.E.M. from 5-8 experiments.

Treatment	Cyclic AMP hydrolyzed (p/mol/min/mg protein)	
	1 μmol/l cyclic AMP	100 μmol/l cyclic AMP
Control	32.4 ± 1.2	352.3 ± 29.9
A23187 (6.7 μmol/l)	30.1 ± 2.2	337.0 ± 39.9
Indomethacin (25 μmol/l)	29.6 ± 1.3	333.1 ± 36.6

The inhibitory effects of indomethacin (1,5; Fig. 1) implicate a prostaglandin in this process. The increased production of prostaglandins after addition of A23187 has been well documented in a number of types of macrophages (1,2,15). Our own studies (16) have demonstrated greatly increased production of PGE, PGF and 6-keto $PGF_{1\alpha}$, a stable metabolite of PGI_2, after addition of the ionophore to elicited rat peritoneal macrophages. PGE has been suggested to be the most likely intermediary in the increased rate of cyclic AMP production (1) but PGI_2 may be involved also, as this prostaglandin can increase cyclic AMP concentrations in macrophages (16).

The present studies eliminate the possibility that A23187 increases macrophage cyclic AMP by inhibiting cyclic AMP-PDE activity. Indeed, the activity of the high affinity form of the enzyme was stimulated by the ionophore (Fig. 3). This may explain why the cyclic AMP concentration decreases from the peak values seen after addition of A23187 to macrophages (5). The effect of the ionophore on cyclic AMP-PDE activity is seen only in intact cells and does not appear to depend upon the formation of cyclooxygenation products of arachidonic acid.

Macrophages are the major producers of prostaglandins among the cells of the immune system (17). Prostaglandins influence a number of immune functions (9) and may act as local regulators of these processes. Since macrophages respond to certain prostaglandins, usually by a diminution of activity, endogenous production of prostaglandins may act as a self-limiting mechanism. Cyclic AMP may be the intracellular mediator of this phenomenon.

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REFERENCES

1. Gemsa, D., Seitz, M., Kramer, W., Grimm, W., Till, G and Resch, K. (1979). Exp. Cell Res. 118, 55-62.
2. Weidemann, M.J., Peskar, B.A., Wrogemann, K., Rietschel, E.Th., Staudinger, H. and Fischer, H. (1978). FEBS Lett. 89, 136-140.
3. Hand, W.L., King, N.L., Johnson, J.D. and Lowe, D.A. (1977). Nature 265, 543-544.
4. Smith, R.L. and Weidemann, M.J. (1980). Biochem. Biophys. Res. Commun. 97, 973-980.
5. Smith, R.L., Hunt, N.H., Merritt, J.E., Evans, T. and Weidemann, M.J. (1980). Biochem. Biophys. Res. Commun. 96, 1079-1087.
6. Schultz, R.M., Stoychkov, J.N., Pavlidis, N., Chirigos, M.A. and Olkowski, S.L. (1979). J. Reticuloendothelial Soc. 26, 93-102.
7. Oropenza-Rendon, R.L., Speth, V., Hiller, G., Weber, K. and Fischer, H. (1979). Exp. Cell Res. 119, 365-371.
8. Bourne, H. R., Lichtenstein, L.M., Melmon, K.L., Henney, C.S., Weinstein,Y. and Shearer, G.M. (1974). Science 184, 19-28.
9. Goodwin, J.S. and Webb, D.R. (1980). Regulation of the immune response by prostaglandins. Clin. Immunol. Immunopathol. 15, 106-122.
10. Hunt, N.H., Martin, T.J., Michelangeli, V.P. and Eisman, J.A. (1976). J. Endocr. 69, 401-412.
11. Hunt, N.H. and Evans, T. (1980). Biochim. Biophys. Acta 613, 499-506.
12. Hunt, N.H., Smith, B. and Pembrey, R. (1980). Clin. Sci. Mol. Med. 58, 463-467.
13. Grunspan-Swirsky, A. and Pick, E. (1978). Immunopharmacol. 1, 71-82.
14. Gemsa, D., Steggemann, L., Till, G. and Resch, K. (1977). J. Immunol. 119, 524-529.
15. Bonney, R.J., Wightman, P.D., Davies, P., Sadowski, S.J., Kuehl, F.A. and Humes, J.L. (1978). Biochem. J. 176, 433-442.
16. Lim, L.K., Hunt, N.H., Smith, R.L. and Weidemann, M.J. (1981). Proc. Austr. Biochem. Soc. 11,62.
17. Morley, J., Bray, M.A., Jones, R.W., Nugteren, D.H. and Van Dorp, D.A. (1979). Prostaglandins 17, 729-736.