Calcium ionophore A23187 induction of spermidine/spermine N^1-acetyltransferase activity in bovine lymphocytes

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The divalent cation ionophore A23187 increased the activity in bovine lymphocytes of spemidine/spermine N^1-acetyltransferase, a rate-limiting enzyme in polyamine biodegradation. The enzyme was induced in a dose- and time-dependent manner. Induction was suppressed by indomethacin, but not by trifluoperazine (TFP), *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7) or palmitoylcarnitine. These results suggest that the activation of phospholipase A_2 involves the induction of spermidine/spermine N^1-acetyltransferase. Ornithine decarboxylase, a rate-limiting enzyme in polyamine biosynthesis, was not suppressed by indomethacin but was by TFP and W-7. The molecular mechanism of the induction of spermidine/spermine N^1-acetyltransferase and ornithine decarboxylase may be different.

Calcium ionophore *Spermidine/spermini* N^1*-acetyltransferase* *Ornithine decarboxylase* *Enzyme induction* *Indomethacin* *Bovine lymphocyte*

1. INTRODUCTION

Ca^{2+} is an important second messenger for the initiation of a variety of intracellular processes in many animal cells [1]. Excitation of cells transiently increases intracellular Ca^{2+} from resting concentrations of around 0.01–1.0 μM to 10 μM or higher [2]. A rise in the concentrations of Ca^{2+} seems to initiate various physiological responses. The divalent cation ionophore A23187, which is thought to act primarily by increasing the rate of Ca^{2+} transport across the cell membrane [3], induces DNA synthesis of lymphocytes [4,5]. Mitogenic lectins such as phytohemagglutinin and concanavalin A also increase the rate of uptake of Ca^{2+} by lymphocytes [6,7].

There is a large increase in the activity of spermidine/spermine N^1-acetyltransferase in bovine lymphocytes after exposure to phytohemagglutinin [7,8]; this enzyme is a rate-limiting enzyme of polyamine biodegradation [10–14]. The activity of the pathway for polyamine degradation is regulated by the amount of this enzyme present [13,14]. It is therefore important to understand the mechanism of its induction. Here, we studied the effects of A23187 on spermidine/spermine N^1-acetyltransferase in bovine lymphocytes.

2. MATERIALS AND METHODS

2.1. *Reagents*

Verapamil hydrochloride and trifluoperazine (TFP) were kindly supplied by Eisai, Tokyo and Yoshitomi Pharmaceutical Industries, Osaka, respectively. The divalent cation ionophore A23187 was purchased from the Calbiochem-Behring, La Jolla, CA. Indomethacin and palmitoylcarnitine were obtained from Sigma, St. Louis, MO. *N*-(6-Aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7) was obtained from Rikaken, Nagoya. Horse serum was from the Commonwealth Serum Laboratories, Victoria.

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[*acetyl*-1-^{14}C]Acetyl-CoA (55.0 mCi/mmol) was purchased from Amersham International, Amersham.

2.2. *Lymphocytes*

Bovine pharyngeal lymph nodes were kindly provided by the Osaka City Meat Inspection Laboratory, Osaka. The lymphocytes were separated from other cells as in [15] and suspended in Eagle's minimum essential medium containing 1% (v/v) horse serum at a concentration of 1×10^7 cells/ml.

2.3. *Assay of spermidine/spermine* N^1*-acetyltransferase activity*

Preparation of the enzyme solution from cells was described in [8]. Spermidine/spermine N^1-acetyltransferase was assayed by following the incorporation of [*acetyl*-1-^{14}C]acetyl-CoA into monoacetylspermidine [10]. Incubation was for 10 min at 37°C. Protein was measured by the method of Lowry et al. [16] using bovine serum albumin as the standard.

3. RESULTS AND DISCUSSION

When various concentrations of A23187 were added to the lymphocyte suspensions and the activity of spermidine/spermine N^1-acetyltransferase was assayed after 24 h, the enzyme activity had increased in a dose-dependent manner (fig.1). The optimum concentration of A23187 was 0.1 μg/ml. Enzyme activity increased with the length of exposure to A23187 (fig.2). To further evaluate the kinetics of the increase in enzyme activity caused by A23187, we exposed lymphocytes to A23187 for various periods. Exposure of the lymphocytes for 9 h caused maximum enhancement of spermidine/spermine N^1-acetyltransferase activity (fig.3). The elevation of enzyme activity was much less when cycloheximide or actinomycin D was added to the cell culture with the A23187 (not shown). These results suggest that synthesis of new protein and RNA is required for the enhancement of spermidine/spermine N^1-acetyltransferase activity in bovine lymphocytes treated with A23187.

The induction of spermidine/spermine N^1-acetyltransferase was inhibited by the addition of EGTA to the cell cultures (table 1). Verapamil, which interacts with Ca^{2+} binding sites at cell surfaces [17],

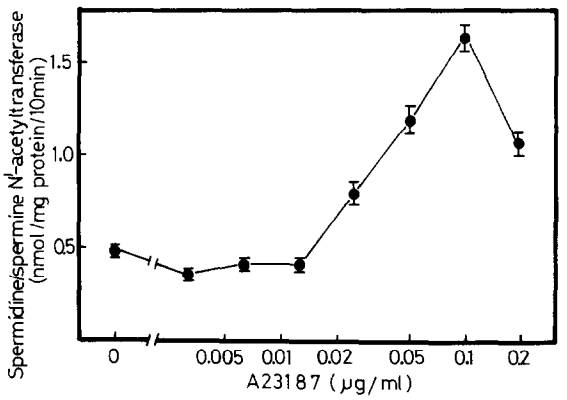

Fig.1. Effect of various concentrations of A23187 on spermidine/spermine N^1-acetyltransferase activity. Bovine lymphocytes (10^7 cells/ml) were treated with various concentrations of A23187 for 24 h. Spermidine/spermine N^1-acetyltransferase activity was measured as described [8]. The results are the means ± SE of 3 experiments.

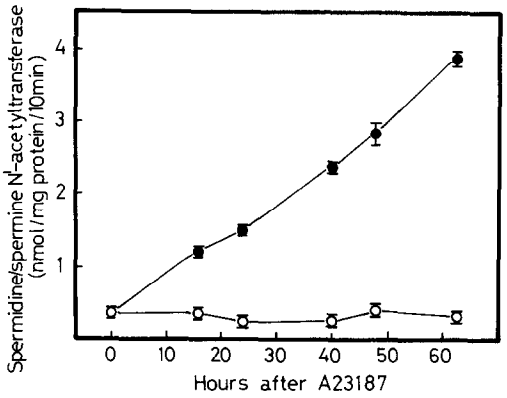

Fig.2. Effect of A23187 on spermidine/spermine N^1-acetyltransferase activity. Bovine lymphocytes were treated with A23187 (0.1 μg/ml) and harvested at the times shown. (○) Untreated cells, (●) A23187-treated cells. The results are the means ± SE of 3 experiments.

also inhibited enzyme induction. These results show that the elevation of intracellular Ca^{2+} is involved in the induction of spermidine/spermine N^1-acetyltransferase.

Ca^{2+} increases in the cytosol probably initiate various physiological responses by interaction with calmodulin, which is a specific intracellular Ca^{2+}-binding protein [2], by activation of either phospholipid-sensitive Ca^{2+}-dependent protein kinase [18] or phospholipase A_2 [19], with the

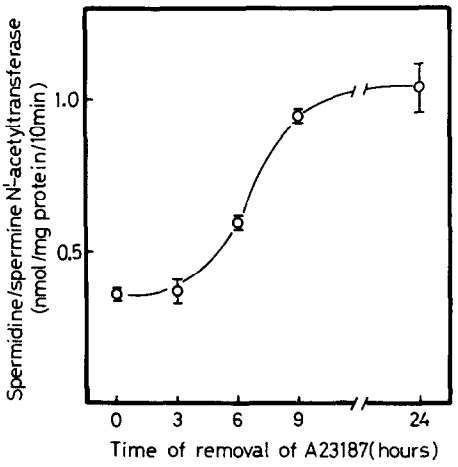

Fig.3. Effects of varying the time of removal of A23187 on the induction of spermidine/spermine N^1-acetyltransferase activity. A23187 was added to cultures at time zero. At the times indicated, cultures were centrifuged, the supernatant media decanted, and the cells resuspended in medium without A23187. The cells were harvested at 24 h after the addition of A23187. The results are the means ± SE of 3 experiments.

Table 1

Effect of EGTA and verapamil on spermidine/spermine N^1-acetyltransferase activity in A23187-treated lymphocytes

Treatment	Spermidine/spermine N^1-acetyltransferase activity (nmol/mg protein per 10 min)
None	0.53 ± 0.03
A23187, 0.1 μg/ml	2.00 ± 0.10
A23187 + EGTA, 1.5 mM	1.41 ± 0.06
+ EGTA, 3.0 mM	0.63 ± 0.05
+ verapamil, 50 μM	1.32 ± 0.02
+ verapamil, 100 μM	0.53 ± 0.06

A23187, EGTA and verapamil were added to the cell culture at 0 h and incubated for 24 h at 37°C. The results are the means ± SE of 3 experiments

Table 2

Effect of the calmodulin inhibitors, palmitoylcarnitine and indomethacin on spermidine/spermine N^1-acetyltransferase and ornithine decarboxylase activity

Treatment	Spermidine/spermine N^1-acetyltranferase activity (nmol/mg protein per 10 min)	Ornithine decarboxylase activity (nmol/mg protein per h)
None	0.34 ± 0.02[b]	0.48 ± 0.09[a]
A23187, 0.1 μg/ml	1.63 ± 0.07[b]	2.16 ± 0.32[a]
+ TFP, 3.125 μM	1.68 ± 0.03[b]	1.65 ± 0.08[a]
+ TFP, 6.25 μM	2.02 ± 0.02[b]	1.42 ± 0.10[a]
+ W-7, 6.25 μM	1.56 ± 0.07[b]	1.68 ± 0.14[a]
+ W-7, 12.5 μM	1.73 ± 0.10[b]	0.92 ± 0.11[a]
A23187, 0.1 μg/ml	1.34 ±0.01[b]	–
+ palmitoylcarnitine, 2.5 μM	1.46 ± 0.19[b]	–
+ palmitoylcarnitine, 5 μM	1.64 ± 0.06[b]	–
A23187, 0.1 μg/ml	1.06 ± 0.06[b]	3.00 ± 0.07[a]
+ indomethacin, 25 μM	0.38 ± 0.03[b]	2.78 ± 0.15[a]
+ indomethacin, 50 μM	0.34 ± 0.04[b]	3.05 ± 0.46[a]
A23187, 0.1 μg/ml	0.94 ± 0.02[a]	–
+ indomethacin, 25 μM	0.33 ± 0.05[a]	–

A23187, trifluoperazine (TFP), *N*-(6-aminohexyl)-5-chloronaphthalenesulfonamide (W-7), palmitoylcarnitine and indomethacin were added to the cell culture at 0 h. The cells were harvested at 16 ([a]) or 24 ([b]) h after the additions. The results are the means ± SE of 3 experiments

resultant arachidonic acid cascade. To ascertain which process lies on the pathway leading to the induction of spermidine/spermine N^1-acetyltransferase, the cells were treated with TFP, W-7, palmitoyl carnitine, or indomethacin.

Table 2 shows that TFP and W-7, which are calmodulin inhibitors [20,21], and palmitoylcarnitine, an inhibitor of phospholipid-sensitive Ca^{2+}-dependent protein kinase [22], had little effect on the induction of spermidine/spermine N^1-acetyltransferase activity. Indomethacin, a cyclooxygenase inhibitor, completely suppressed the enzyme induction. Sodium *n*-butyrate, an inducer of cyclooxygenase in many cells [23,24], induces spermidine/spermine N^1-acetyltransferase in bovine lymphocytes [8]. These results suggest that activation of the arachidonic acid cascade is involved in the enzyme induction in A23187-stimulated bovine lymphocytes. We have demonstrated that spermidine/spermine N^1-acetyltransferase is induced after exposure to a number of stimuli that induce ornithine decarboxylase [8–14], a rate-limiting enzyme in polyamine biosynthesis. So we also examined the effect of inhibitors on the induction of ornithine decarboxylase. Ornithine decarboxylase activity was suppressed by TFP and W-7 but not by indomethacin. These results suggest that the molecular mechanism of induction of spermidine/spermine N^1-acetyltransferase differs from that of ornithine decarboxylase in A23187-stimulated bovine lymphocytes.

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