Activation of Cyclic Nucleotide Phosphodiesterase from Isolated Human Peripheral Blood Lymphocytes by Mitogenic Agents

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ABSTRACT

During long-term incubation of human peripheral blood lymphocytes with phytohemagglutinin (PHA), adenosine cyclic 3':5'-monophosphate (cyclic AMP) phosphodiesterase increases about 5-fold and guanosine cyclic 3':5'-monophosphate (cyclic GMP) phosphodiesterase increases about 10-fold in specific activity. Under similar conditions, there is little change in either basal or fluoride-stimulated adenylyl cyclase activity. The increase in cyclic nucleotide phosphodiesterase activity also occurs with other mitogenic activators of blastogenesis including concanavalin A, pokeweed mitogen, and Streptolysin O. Cyclic AMP phosphodiesterase activity in PHA-stimulated lymphocytes develops an increase in maximum velocity but no change in other kinetic properties when compared to that of unstimulated lymphocytes. Linear density gradient analyses of cyclic nucleotide phosphodiesterases in mitogen-stimulated lymphocytes show an increased activity in lower (3.65) and higher (5.9S) molecular weight forms of cyclic AMP and cyclic GMP phosphodiesterase.

Hydroxyurea inhibits PHA-induced mitogenesis but has no effect on the observed increase in phosphodiesterase activity. 1-Methyl-3-isobutylxanthine inhibits PHA-induced mitogenesis and prevents the increase in cyclic GMP phosphodiesterase activity, but the increase in cyclic AMP phosphodiesterase activity is greater than that seen with PHA alone. The PHA-induced increase in cyclic AMP and cyclic GMP phosphodiesterase activity is inhibited by cycloheximide; however, Actinomycin D does not completely inhibit PHA stimulation of cyclic nucleotide phosphodiesterase activity when it is used at a concentration that inhibits the PHA-induced increase in [3H]-thymidine and [3H]uridine incorporation by greater than 90%. These results indicate that de novo protein synthesis but not mitogenesis per se is required for the increased enzyme activity induced by mitogenic agents. The results are discussed with respect to the regulation of cyclic nucleotide phosphodiesterase and to the role of these enzymes in lymphocyte proliferation.

INTRODUCTION

Treatment of lymphocytes with nonspecific mitogens such as plant lectins leads to a wide variety of biochemical and morphological alterations resulting in transformation of the lymphocytes from resting to growing cells (9, 20). Although it is not known which biochemical events are specific signals for mitogenesis, the direct site of action of the mitogens has been demonstrated to be the lymphocyte cell surface (13). Several lines of evidence have implicated cyclic nucleotides in the modulation or control of mitogen-induced lymphocyte activation. Rapid increases in cyclic AMP (32, 46) or cyclic GMP (6, 14, 15) have been reported to occur following treatment of human lymphocytes with mitogens. Moreover, lymphocyte mitogenesis, under optimal mitogenic conditions, is inhibited by cyclic AMP and by agents that increase intracellular concentrations of cyclic AMP (7, 16, 33, 42). Cyclic AMP also mediates the recovery of DNA synthesis in serum-free lymphocyte cultures suppressed by supratherapeutic concentrations (41, 44).

Previous studies from this laboratory have shown that isolated human peripheral blood lymphocytes contain distinct forms of soluble, high-affinity cyclic nucleotide phosphodiesterases (35) and that increased cyclic nucleotide phosphodiesterase activity is associated with growth in lymphoid cells (10). In this report, we describe and analyze an increase in cyclic nucleotide phosphodiesterase activity that occurs in human peripheral blood lymphocytes following long-term stimulation by mitogens.

MATERIALS AND METHODS

Isolation of Human Peripheral Blood Lymphocytes

Human peripheral blood lymphocytes were isolated from 60 to 120 ml of blood from normal donors by defibrination with glass beads and separation of the lymphocytes by Ficoll-Hypaque density gradient centrifugation (35). Isolated lymphocytes were washed twice with MEM, and the remaining erythrocytes were lysed in hypotonic buffer [280 mM NH₄Cl-30 mM Tris-Cl (pH 7.2)] by incubation at 37°C for 10 min. Final cell purity after 2 additional washes in MEM ranged from 80 to 90% lymphocytes, 8 to 20% monocytes, and 0 to 2% granulocytes, as determined by light microscope analysis.

Mitogenic Stimulation of Lymphocytes

A. Macroculture Method. Prior to culturing, lymphocytes, isolated as described above, were resuspended in RPMI Medium 1640 supplemented with 10% autologous serum; penicillin, 100 units/ml; streptomycin, 100 μg/ml; and glutamine, 0.4%.
temperature. Cells were then aliquoted for incubation in a humidified atmosphere of 5% CO₂ in air, as 1 x 10⁶ lymphocytes/ml (10 ml) in 25-sq-cm Falcon No. 3012 tissue culture flasks (37°C) or as 0.33 x 10⁶ lymphocytes/ml (3 ml) in 16 x 125-mm Falcon No. 3033 or glass tissue culture tubes. Mitogens or solvent controls were added as indicated.

B. Microculture Method. Conditions were the same as those used for macroculture except that lymphocyte cells were dispensed with a sterile Hamilton gastight syringe into Falcon No. 3040 Micro-Test II tissue culture plates as 1.5 x 10⁵ lymphocytes/well.

Measurement of [³H]Leucine, [³H]Uridine, and [³H]Thymidine Incorporation

A. Macroculture. Five µCi of [³H]thymidine (specific activity, 1.9 Ci/mmol) were added to lymphocytes in macroculture 3 hr before the termination of cell incubation. Cell incubation was terminated by cooling cells to 4°C, washing with 0.9% NaCl solution (4°), precipitating with 5% trichloroacetic acid (30 min, 4°), and then washing an additional time with trichloroacetic acid and then with methanol. Acid-precipitable material was dissolved in 0.5 ml of Soluene (30 min, 60°), and radioactivity was measured in 10 ml of PPO-POPOP in toluene scintillation fluid.

B. Microculture. One µCi of [³H]thymidine (specific activity, 1.9 Ci/mmol), [³H]uridine (specific activity, 28 Ci/mmol), or [³H]leucine (specific activity, 15 Ci/mmol) was added to each microwell 8 hr before termination of the cell incubation. Cells were collected on glass fiber filters and washed 15 times with 0.9% NaCl solution using a Multiple Automatic Sample Harvester (37). Filters were dried, and radioactivity was determined using liquid scintillation techniques. Incorporation of all radioactivity was linear for 8 hr.

Assay of Adenylyl Cyclase Activity

Lymphocytes in culture were pelleted and suspended at 10⁷/ml in 0.32 M sucrose-50 mm Tris-Cl (pH 7.4) and sonicated in 0.45-ml volumes for 10 sec with the microprobe of a Biosonic IV sonicator (Bromwill Scientific, Rochester, N.Y.) at a setting of 5 (~5 watts). As shown for other cell systems (2), these cells appear intact by light microscope examination and have activities which are greater than those obtained by homogenization of the cells.

Reaction mixtures (0.25 ml) contained 50 mm Tris-Cl (pH 7.4); phosphocreatine, 0.4 mg/ml; creatine phosphokinase, 0.2 mg/ml; 4 mm MgCl₂; 0.8 mm cyclic AMP; BSA, 0.6 mg/ml; [α-³₂P]ATP (20 µm; 1 x 10⁶ cpm); 1 x 10⁶ sonicated cells; and, where indicated, 10 mm NaF. Reactions were incubated for 15 min at 30° and terminated by the addition of 50 µl of 1 M acetic acid with vigorous stirring. Cyclic [³H]AMP (9000 cpm) was added (0.7 µl) to estimate recovery (~80%), and the reaction mixtures were adsorbed on 0.5- x 8-cm columns of alumina (bottom, 6 cm) and MnO₂ (top, 2 cm). Cyclic [³P]AMP and cyclic [³H]AMP were eluted with 1.6 ml of 50 mm Tris-Cl (pH 7.4). Radioactivity in the total column eluate was determined by liquid scintillation techniques. One unit of activity is defined as the formation of 1 pmol of cyclic [³P]AMP per min from [³²P]ATP.

The concentrations of phosphocreatine and creatine phosphokinase were optimized as an ATP-regenerating system for these cells. Although the ATP-regenerating system was necessary for linearity, use of a cyclic nucleotide phosphodiesterase inhibitor did not affect the production or preservation of the reaction product formed in the presence of the cyclic AMP "trapping" system.

Assay of Cyclic Nucleotide Phosphodiesterase Activity

Cyclic nucleotide phosphodiesterase activity was measured as described previously (10). MEM-washed cell pellets were suspended in 40 mm Tris-Cl (pH 8.0)-5 mm 2-mercaptoethanol and sonically disrupted for 10 sec at setting 50 (80 watts) with the microprobe of a Biosonic IV sonicator. Microscopic examination showed >99% cell breakage.

Unless otherwise indicated, reaction mixtures (0.4 ml) contained 40 mm Tris-Cl (pH 8.0), 10 mm MgCl₂, 5 mm 2-mercaptoethanol, cyclic [³H]AMP (0.25 µm; ~100,000 cpm) or cyclic [³H]GMP (0.1 µm; ~100,000 cpm), and 0 to 0.2 ml (0 to 2 x 10⁵ cell equivalents) of cell sonicate. Reactions were incubated at 30°, usually for 10, 20, and 30 min, and terminated by boiling for 1 min. After cooling, 0.1 ml of snake (Ophiophagus han nah) venom (0.5 mg/ml) was added, and the samples were incubated for 10 min at 30°. One ml of methanol was added to each sample and [³H]adenosine or [³H]guanosine was separated from unreacted cyclic [³H]AMP or cyclic [³H]GMP by chromatography on Dowex 1-X8 anion-exchange resin. Under these conditions, recovery of [³H]adenosine and [³H]guanosine was ±80% and blank values were ±1.5%. One unit of activity is defined as the formation of 1 pmol of [³H]adenosine or [³H]guanosine per min. Assay linearity was verified from 0 to 30 min and from 0 to 2 x 10⁶ cell equivalents.

Protein was determined either by the method of Lowry et al. (21) or by the fluorometric method of Udenfriend et al. (39). DNA was determined by measuring enhancement of ethidium bromide fluorescence as described by Karsten and Wollenberger (17).

Sucrose Density Gradient Centrifugation

Five-ml linear (5 to 20%) sucrose containing 40 mm Tris-Cl (pH 8.0), 5 mm 2-mercaptoethanol, and 10 mm MgCl₂ were prepared with a Buchler density gradient mixer. Cell pellets containing 10 to 75 x 10⁶ cells were sonicated in 0.2 ml of the same buffer and layered on the gradients. Three mg of BSA, layered on a separate gradient, were used as a standard. Gradients were centrifuged for 15 to 16 h (4°) at 192,000 x g in Beckman SW 50.1 rotors. Fractions were collected using a Buchler density gradient piercing unit (4°). The BSA peak was determined by absorbance measurement at 280 nm. Aliquots (10 to 140 µl of each fraction) were assayed for cyclic AMP and cyclic GMP phosphodiesterase activity to obtain activity profiles. A value of 4.3S was assigned to BSA for calculation of other sedimentation coefficients.

Materials

Cyclic [³H]AMP (specific activity, 25 Ci/mmol), cyclic [³H]GMP (specific activity, 8.4 Ci/mmol), [methyl-³H]thymidine (specific activity, 1.9 Ci/mmol), and [³H]uridine (specific activity, 23 Ci/mmol) were purchased from Schwarz/Mann.
Cyc!ic Nuc!eotide Phosphodiesterase Activation

Orangeburg, N.Y. Cyclic [3H]AMP and cyclic [3H]GMP were purified by Dowex 1-X8 (200 to 400 mesh) anion-exchange chromatography and stored at -20° in acidic 50% ethanol. Snake (Ophiophagus hannah) venom, cyclic AMP, cyclic GMP, RNase, and pronase were from Sigma Chemical Co., St. Louis, Mo.; 2,7-diamo-no-10-ethyl-9-phenylphenanthridium bromide (ethidium bromide), Actinomycyin D, and cycloheximide were from Calbiochem, San Diego, Calif.; Ficoll and Con A were from Pharmacia Fine Chemicals, Inc., Piscatway, N. J.; Dowex 1-X8 (200 to 400 mesh) was from Bio-Rad Laboratories, Richmond, Calif.; Hypaque-M, 90%, was from Winthrop Laboratories, New York, N.Y.; enzyme grade sucrose was from Merck and Co., Inc., Rahway, N.J.; RPMI Medium 1640, MEM, I-glutamine, penicillin-streptomycin solution, and PWM were from Grand Island Biological Co., Grand Island, N.Y.; glass beads (3 mm diameter) were from Scientific Products, Houston, Texas; 4-phenylspiro[furan-2(3H), 1'-phthalan]-3,3'-dione (Fluram) was from Roche Diagnostics, Nutley, N.J.; Soluene was from Packard Instrument Co., Downers Grove, Ill.; PHA (PHA-M), and SLO were from Difco Laboratories, Inc., Detroit, Mich. PHA was weighed, dissolved in RPMI Medium 1640, as 10 mg/ml, filter sterilized, and stored frozen for use. PWM and SLO were rehydrated with RPMI medium according to manufacturer’s specifications and stored frozen. Other purchased chemicals were of commercial analytical grade quality.

RESULTS

Stimulation of Lymphocyte Phosphodiesterase Activity by Mitogen. Mitogenic stimulation of human peripheral blood lymphocytes by PHA resulted in 5- to 10-fold increases in the specific activities of cyclic AMP and cyclic GMP phosphodiesterases. Cyclic nucleotide phosphodiesterase activity in PHA-treated lymphocytes began to increase only after 24 hr in culture and was near maximum at about 3 days, which corresponded to the time of maximum [3H]thymidine incorporation (Chart 1, A and C). Long-term increases in lymphocyte phosphodiesterases were consistently observed in separate experiments, although the magnitude of the increase varied.

Adenylyl cyclase activity fluctuated during the culture period, but no activation in PHA-treated cultures was apparent (Chart 1B). Both control and PHA-treated lymphocytes showed a 2- to 3-fold decrease in activity in basal and fluoride-stimulated adenylyl cyclase activity after 24 hr in culture. Partial restoration of activity occurred in PHA-treated cultures at 3 days, but the specific activity still remained below the zero time value.

Kinetic and Physical Analysis of Increased Phosphodiesterase Activity. Comparison of the kinetics of cyclic AMP phosphodiesterase in PHA-activated and resting peripheral blood lymphocytes indicated an increase in the Vmax of the PHA-treated cells with no change in the Km (Chart 2). The increased phosphodiesterase activity in 3-day PHA-stimulated lymphocytes was further examined by linear sucrose density gradient centrifugation. A general increase in both the high (5.8S) and low (3.6S) molecular-weight forms of cyclic AMP and cyclic GMP phosphodiesterase was observed, as compared to control cells cultured for 3 days in the absence of PHA (Chart 3). An increase in both molecular weight forms of cyclic AMP phosphodiesterase was also seen in cells stimulated by Con A, PWM, and SLO (data not shown). These data suggest that the increase in enzyme activity is due not to a change in the kinetic parameters of the enzyme system or to the appearance of a new enzyme form, but rather to a general increase in the activation of the same enzyme forms which are already present in the cell.

Webb et al. (43) demonstrated that lymphocytes can be activated by lectins in the absence of serum. Since serum by itself can influence phosphodiesterase in other cell systems (26-28), PHA was tested for its ability to stimulate lymphocyte cyclic AMP phosphodiesterase in the absence of serum. A 4-fold increase in both forms of cyclic AMP phosphodiesterase occurred in the absence of serum, as compared to control cells not treated with PHA (data not shown). Although this increase...
was less than that observed in the presence of serum, it nevertheless indicates that phosphodiesterase activity can be activated by lectins even in the absence of serum. Both high (330 µg/ml) and low (33 µg/ml) doses of PHA appeared to stimulate each form of cyclic AMP phosphodiesterase to the same extent (data not shown), indicating that at either dose of PHA a similar type of activation occurs.

**Relationship of Increased Phosphodiesterase Activity to Mitogenesis.** Temporally, the sharp increase in phosphodiesterase activity occurred at about the time of the onset of mitogenesis, as indicated by the enhanced rate of DNA synthesis (Chart 1). The relationship between the increase in phosphodiesterase activity and DNA synthesis was further examined by comparing the effect of different doses of PHA on these 2 processes. At varying doses of PHA, increased cyclic nucleotide phosphodiesterase activity appeared to correlate with increased DNA synthesis; the increase in phosphodiesterase activity was dependent on the dose of PHA used and was optimal at the same doses of PHA which produced an optimal mitogenic response (Chart 4).

Four different mitogens (PHA, Con A, PWM, and SLO) were tested for their effect on cyclic nucleotide phosphodiesterase activities, and increases in both cyclic AMP and cyclic GMP phosphodiesterase activities were seen for all 4 mitogens (Table 1). The increase in cyclic AMP phosphodiesterase specific activity ranged from 7- to 10-fold, and the increase in cyclic GMP phosphodiesterase specific activity ranged from 5- to 13-fold. Although increased phosphodiesterase activity occurred in response to each mitogen, the magnitude of the increases did not closely correlate with the degree of stimulation of \(^{3}H\)thymidine incorporation. Insulin (10^(-7) m) produced no detectable increase in phosphodiesterase activity or \(^{3}H\)thymidine incorporation and had no effect on PHA activation of lymphocytes when added in culture with PHA for 3 days.

The relationship between phosphodiesterase and mitogenesis was further tested by using specific enzyme inhibitors. We found that IMBX is a potent inhibitor of lymphocyte cyclic AMP phosphodiesterase (K_i = 6 µM). When 1 mm IMBX was added with PHA at the start of lymphocyte activation, it completely inhibited DNA synthesis and the increase in cyclic GMP phosphodiesterase activity (Table 2); however, the increase in cyclic AMP phosphodiesterase was still observed. At 0.1 mm IMBX, DNA synthesis was inhibited by about 50%, and the increase in cyclic GMP phosphodiesterase was inhibited by about 35%, but cyclic AMP phosphodiesterase activity increased to an even greater extent with the addition of IMBX than with PHA alone (Table 2). In contrast to PHA, which increases the activity of both forms of cyclic AMP and cyclic GMP phosphodiesterase, as analyzed by sucrose gradient fractionation (see above), we found that incubation of lymphocytes with IMBX alone specifically leads to a long-term increase in the lower-molecular-weight (3.6S) form of cyclic AMP phosphodiesterase, with no change in cyclic GMP phosphodiesterase (34). When IMBX and PHA are added in culture together, they appear to be synergistic in their activation of the lower-molecular-weight form of cyclic AMP phosphodiesterase (data not shown).

**Effect of Inhibitors of Macromolecular Synthesis on Mitogen Stimulation of Phosphodiesterase Activity.** When hydroxyurea, a specific inhibitor of deoxyribonucleotide reductase (38), was added with PHA to lymphocyte cultures at concentrations that inhibited DNA synthesis (Table 2), activation of cyclic AMP and cyclic GMP phosphodiesterase still
occurred. Hence, activation of phosphodiesterase activities can occur under conditions where mitogenesis is completely inhibited.

Other inhibitors of macromolecular synthesis were examined for their effects on PHA activation of lymphocyte phosphodiesterase. Cycloheximide at 2 and 20 μg/ml completely inhibited PHA stimulation of both cyclic AMP and cyclic GMP phosphodiesterase activity and [3H]thymidine incorporation (Table 2). At 0.2 μg/ml, cycloheximide inhibited [3H]thymidine incorporation by 93% and inhibited the increase in both cyclic AMP and cyclic GMP phosphodiesterase activities by 90%. This suggests that the PHA-induced increase in phosphodiesterase activity is dependent on de novo protein synthesis.

Actinomycin D at low concentrations (0.5 to 50 ng/ml) was also tested for its effects on macromolecular synthesis and phosphodiesterase activation in response to PHA (Chart 5). As shown previously by others (18), at 50 ng/ml, actinomycin D completely inhibited [3H]uridine and [3H]thymidine incorporation in PHA-stimulated cultures. At this concentration, actinomycin D also completely inhibited the PHA-induced increase in both cyclic AMP and cyclic GMP phosphodiesterase. However, at concentrations below 50 ng/ml, the inhibition of [3H]uridine and [3H]thymidine incorporation by actinomycin D was more pronounced than the inhibition of phosphodiesterase activation (Chart 5, inset).

DISCUSSION

Most mammalian cells appear to contain multiple molecular forms of cyclic nucleotide phosphodiesterase differing in molecular weight, kinetic properties, and affinity for substrate (1). The functional significance and interrelationship between these different forms is not well understood. One means of understanding this enzyme system better is to study the factors and conditions which regulate changes in its activities. Evidence has accumulated to indicate that many mammalian phosphodiesterases, mostly membrane-bound forms, are under hormonal control (36). We have previously demonstrated the existence of high-affinity forms of soluble phosphodiesterases in human peripheral blood lymphocytes (10, 35). In this paper, we demonstrate severalfold activation of both cyclic AMP and cyclic GMP phosphodiesterase in lymphocytes following treatment of the cells with mitogens.

Lectins have been shown previously to affect enzymes involved in cyclic nucleotide metabolism. PHA has been reported to activate human lymphocyte adenylyl cyclase in vitro (32) and to decrease murine thymocyte adenylyl cyclase when added to intact cells (22). Activation of membrane-bound cyclic AMP phosphodiesterase has been described in the cellular slime mold, Dictyostelium discoideum (12), and in skeletal muscle (8) following treatment with Con A. Monahan et al. (23) reported that cyclic AMP phosphodiesterase activity in lymphocytes from normal donors and from chronic lymphocytic leukemia patients is increased approximately 2-fold at 3 to 5 days following treatment with PHA. These results confirm and extend the observation of Monahan et al. and provide the first full description of activation of mammalian cyclic nucleotide phosphodiesterases by plant lectins; hence, these agents must be added to the growing list of effectors of mammalian phosphodiesterases.
Increase in cyclic nucleotide phosphodiesterase activity following mitogen stimulation of human peripheral blood lymphocytes

Cells were cultured for 7 days by the macroculture method in 16- x 125-mm glass culture tubes, at 1 x 10^6 lymphocytes/tube, as described in "Materials and Methods." Concentrations of mitogenic agents used were: PHA, 330 µg/ml; Con A, 27 µg/ml; PWM, 1:30 dilution; SLO, 1:30 dilution. All results represent the average of duplicate determinations.

### Cyclic Nucleotide Phosphodiesterase Activity

<table>
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<tr>
<th>Agent</th>
<th>Protein (µg)</th>
<th>Cyclic AMP (0.25 µM)</th>
<th>Cyclic GMP (0.1 µM)</th>
<th>[3H]Thymidine Incorporation (cpm)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total units/mg protein</td>
<td>Units/mg protein</td>
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</tr>
<tr>
<td>Control</td>
<td>72</td>
<td>0.18</td>
<td>2</td>
<td>0.017</td>
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<td>PHA</td>
<td>145</td>
<td>2.6</td>
<td>17</td>
<td>0.26</td>
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<td>19</td>
<td>0.22</td>
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</table>

### Effect of Specific Enzyme Inhibitors on PHA Activation of Cyclic Nucleotide Phosphodiesterases

These results show that mitogenesis per se is not required for lectin-induced phosphodiesterase since hydroxyurea, IMBX, and actinomycin D all inhibited mitogenesis without completely inhibiting the activation of phosphodiesterase activity. Therefore, the 2 processes appear not to be interdependent. However, activation of phosphodiesterase could be a necessary step involved in the actions of plant lectins. Activation of phosphodiesterase was seen only at lectin concentrations which produced mitogenesis and which reached maximum levels at the time of maximum [3H]thymidine incorporation. Moreover, addition of the phosphodiesterase inhibitor, IMBX, completely inhibited PHA-induced mitogenesis. Although the addition of this inhibitor resulted in an even greater elevation of cyclic AMP phosphodiesterase activity in the cells, one possible explanation is that in vivo the enzyme is nonfunctional as long as IMBX is present, and the expression of more enzyme activity may be a compensatory response of the cell to this enzyme inhibition, as has been observed for cultured fibroblast (30) and lymphoma cells (3).

In serum-deprived, quiescent baby hamster kidney fibroblasts, we have found that serum and insulin produce a 10-fold activation of cyclic nucleotide phosphodiesterase, resulting in a 3-fold lowering of cellular cyclic AMP content and stimulation of DNA synthesis (24, 25). The activation of phosphodiesterase appears to be necessary for the cells to enter the S phase of the cell cycle, since inhibition of phosphodiesterase by IMBX...
inhibits this process (24). Lymphocyte activation requires interaction with lectin during at least 3 different stages in order for the cells to progress through the steps necessary for entrance into the S phase of the cell cycle and for commitment to mitogenesis (45). Therefore, it is possible that in lymphocytes increased phosphodiesterase activity is required during lectin activation for cells to traverse the steps related to movement through G1 and entrance into S. This correlates conceptually with observations that cyclic AMP and agents that raise intracellular levels of cyclic AMP inhibit lectin-induced mitogenesis (7, 16, 33, 42). However, we have found that, despite the activation of cyclic nucleotide phosphodiesterase activity by mitogens, there is no difference in basal cyclic AMP levels in control and in PHA-stimulated lymphocytes at 0, 1, 24, 48, and 72 hr in culture. Others who have investigated cyclic AMP levels in human lymphocytes in response to PHA have found that following a very early, transient increase in cyclic AMP, the levels decline to the original basal level, or slightly lower, by 24 hr, and remain at that level through 72 hr in culture (23, 32). Recently, however, in mouse splenic lymphocytes, it has been shown that stimulation by Con A produces a 6-fold increase in cyclic AMP and a subsequent decline to its original level just prior to the onset of DNA synthesis (40). Blockage of either the increase or the decline in cyclic AMP prevented mitogenesis.

A 10-fold induction of phosphodiesterase activity without any change in basal cyclic AMP content has also been observed during commitment to sporulation in the lower eukaryote, Blastocladiella emersonii (11). One possible explanation for this observation is that cyclic AMP may exist primarily in a bound state and thus be resistant to enzymatic hydrolysis (31). In this case, phosphodiesterase may be regulating a small, unbound pool and function primarily to return cyclic AMP levels back to basal following their elevation by stimulation of adenylyl cyclase without altering basal levels, as has been shown for cultured 349 lymphoma cells (3). Since lymphocytes have β-adrenergic (32), prostaglandin (32), histamine (29), and adenosine (47) receptors, all of which produce large increases in lymphocyte cyclic AMP content, it is possible that increased phosphodiesterase activity could serve as a protective mechanism to ensure that cyclic AMP levels are not elevated to the point of being inhibitory to activated lymphocytes and therefore ensure that the cells can traverse the stages necessary for commitment to mitogenesis.

Byus et al. (4) have shown that stimulation of human lymphocytes with concentrations of Con A optimal for mitogenesis leads to a specific and sustained activation of type I cyclic AMP-dependent protein kinase within 4 hr. Concentrations of dibutyryl adenosine cyclic 3':5'-monophosphate inhibitory to mitogenesis or high concentrations of Con A, also inhibitory to mitogenesis, produce activation of both type I and type II cyclic AMP-dependent protein kinase. Since cyclic AMP-dependent protein kinase appears to be involved in the induction of phosphodiesterase activity in mouse lymphoma cells (3), it is possible that the activation of type I protein kinase may be responsible not only for the induction of ornithine decarboxylase (4) but also for the induction of phosphodiesterase in mitogen-stimulated lymphocytes.

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