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CELL CYCLE SPECIFIC FLUCTUATIONS OF ADENOSINE 3′,5′-MONOPHOSPHATE AND PROSTAGLANDIN BINDING IN SYNCHRONIZED MASTOCYTOMA P-815 CELLS

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Abstract—Endogenous adenosine 3′,5′-monophosphate (cAMP) levels in mastocytoma P-815 cells, synchronized either at the G_1/S transition by amethopterin- or double thymidine-block or in mitosis by colcemid block, were highest during late S and early G_2 phases and lowest during mitosis. These cell cycle-dependent changes in cAMP levels were largely accounted for by the changes in adenylate cyclase and phosphodiesterase activities. Similar fluctuations occurred simultaneously with specific prostaglandin E_1 (PGE_1) binding, histidine decarboxylase activity, histamine content, and $[^{35}S]SO_4^{2-}$ incorporation into glycosaminoglycans of the cells. In addition, endogenous levels of the E group of prostaglandins (PGEs) and [^{14}C]arachidonic acid incorporations into PGE, phosphatidylcholine and phosphatidylinositol also exhibited fluctuation patterns similar to that of cAMP levels. Since cAMP levels still fluctuated in a serum-depleted medium where DNA synthesis and cell division were inhibited, endogeneous levels of prostaglandin and cAMP appeared not to be regulated solely by serum factor(s). Exposure of cells at G_1/S transition to 1-methyl-3-isobutylxanthine (MIX) resulted in a 10-fold elevation of cAMP levels throughout the cell cycle without affecting DNA synthesis. On the other hand, PGE_1 and/or MIX added at late S phase elevated cAMP levels, prolonged G_2 phase and retarded the cell division, but these agents added at the beginning of mitosis elevated cAMP levels without affecting the cell division. These results suggest that prostaglandins newly synthesized by the increased metabolism of phospholipids promote the cAMP synthesis via their binding to the receptors and thereby control the division and phenotypic expression of mastocytoma P-815 cells.

Accumulated evidence indicates that adenosine 3′,5′-monophosphate (cAMP) functions in the mammalian cell cycle. The endogenous levels of cAMP fluctuate during the cell cycle of synchronously growing cultures of several lines of mammalian cells: HeLa cells [1], Chinese hamster ovary cells [2, 3], human lymphoid cells [4], mouse fibroblasts [5, 6] and mouse melanoma cells [7]. The cellular binding of hormone to elevate cAMP levels as in the case of the binding of the melanocyte stimulating hormone to mouse melanoma cells [8], and the phenotypic expression such as the increase of ornithine decarboxylase activity in Chinese hamster ovary cells induced by cAMP [3, 9] are also dependent upon the cell cycle.

We have investigated the fluctuations of cAMP levels, PGE_1-binding, and several cellular functions in synchronized mastocytoma P-815 cells because the cells respond to N^6,$O^{2'}$-dibutyryl cAMP (Bt_2cAMP) [10, 11] and prostaglandin E_1 (PGE_1) [11] with decreased growth rate and increased expression of more mature, differentiated mast cell phenotypes, and also because PGE_1 specifically binds to the cells and elevates cAMP levels [12, 13]. This report describes the cell cycle-dependent changes of cAMP levels, PGE_1-binding, and certain cellular functions in synchronous mastocytoma P-815 cells. It also describes the effect on the cell cycle of cAMP levels experimentally altered by PGE_1.

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EXPERIMENTAL PROCEDURES

Cell culture. Mouse mastocytoma P-815 cells [14] were maintained in suspension culture at 37° in Fischer's medium supplemented with 5% fetal calf serum (standard medium), as described in Ref. 15.

Cell synchronization. Synchronization at the beginning of S phase was achieved by the amethopterin block method [16]. Mastocytoma P-815 cells (2×10^5 cells/ml) were exposed to amethopterin (2×10^{-6} M) plus hypoxanthine (2×10^{-4} M) and glycine (1×10^{-1} M) for 6 hr at 37°, and then thymidine (4×10^{-6} M) was added to the culture medium to release the block. In addition, the cells were also synchronized using the double thymidine-block method [17] by successive exposure to 2 mM thymidine for 12 hr, a thymidine-free medium for 6 hr, and 2 mM thymidine for an additional 4 hr. The second thymidine block was removed by resuspending the cells in a thymidine-free medium at a density of approximately 2×10^5 cells/ml. In addition, a colcemid block was also employed to induce synchronization at mitosis. Cells were exposed to colcemid (0.06 μg/ml) for 5 hr and then the block was removed by resuspending the cells in a colcemid-free medium as described in Ref. 18.

Cell cycle analysis. The cell number was counted in a Coulter model Z counter (Coulter Electronics, Hialeah, FL, U.S.A.). Assay of DNA synthesis by

pulse-labeling the cells with [methyl-^{3}H]thymidine and measurement of mitotic index by calculating the number of the cells in metaphase and anaphase with a hematocytometer were performed as previously described [18]. Cell viability was determined by staining the cells in 0.2% nigrosine in Earle's balanced salt solution [19].

Assay of cAMP and adenylate cyclase and phosphodiesterase activities. cAMP was measured by the radioimmunoassay method of Okabayashi *et al.* [20]. Adenylate cyclase activity was determined according to the method of Ramachandran [21]. Cells suspended in 1 ml of 15 mM Tris–HCl (pH 7.4) containing 8 mM $MgCl_2$ were incubated with [8-^{3}H]ATP (0.5 μCi/0.5 μmole ATP) for 20 min at 37°. After the addition of 25 μl of 5 mM nonradioactive cAMP, [8-^{3}H]cAMP formed was separated by successive column chromatographies on neutral aluminium oxide (1 g) and Dowex 1-×8 at pH 7.8 (bed volume 1.0 ml). [8-^{3}H]cAMP adsorbed was eluted with 0.1 N HCl (4.0 ml). The radioactivity in 2 ml eluate was determined by liquid scintillation spectrometry in 15 ml of scintillant [4 g of 2,5-diphenyloxazole (PPO) in 1 liter of toluene–Triton X-100 (2:1, v/v)].

Phosphodiesterase activity was measured using a modification of the procedure of Thompson and Appleman [22]. About 1×10^7 cells suspended in 1 ml of 10 mM Tris–HCl buffer (pH 8.5) were sonically disrupted for 30 sec (Branson Sonifier, model W-135, Branson Sonic Power Co., Danbury, CT, U.S.A.). The assay mixture (0.4 ml), containing 10 mM Tris–HCl (pH 8.5), 4 mM $MgCl_2$, and 0.4 μM [8-^{3}H]cAMP (0.1 μCi), and the enzyme were incubated at 37° for 15 min. Reaction was terminated by heating at 100° for 2 min. To the chilled incubation mixture, 50 μg of snake (*Crotalus atrox*) venom was added, and the mixture was incubated further for 10 min. After removing pellets precipitated with perchloric acid, the supernatant fraction was neutralized with 1 N KOH and then applied to a column of Dowex 1-×8 (bed volume, 2 ml) equilibrated with 10 mM Tris–HCl (pH 8.5) containing 4 mM $MgCl_2$. The radioactivity eluted from the column with 0.5 N formate (3.0 ml) was determined by liquid scintillation spectrometry in 10 ml of scintillant as described above.

PGE_1 binding assay. The specific binding of [^{3}H]PGE_1 to mastocytoma cells was measured as described previously [13].

Assays of histamine production, histidine decarboxylase activity and incorporation of $[^{35}S]SO_4^{2-}$ into acidic glycosaminoglycan. These were performed as previously described in Ref. 12.

Determination of PGE and PGF groups. E and F groups of prostaglandins were extracted from mastocytoma cells and separated according to the method of Jaffe *et al.* [23], slightly modified by Ohuchi *et al.* [24]. Recoveries of PGE_1 and $PGF_{2\alpha}$ throughout the entire procedure, estimated by adding 0.1 μCi of [^{3}H]PGE_1 and [^{3}H]$PGF_{2\alpha}$ to the original extract, were in the range of 70–80 per cent. Each prostaglandin was measured using a [^{3}H]PGE or [^{3}H]PGF radioimmunoassay kit (Boehringer).

Metabolism of [^{14}C]arachidonic acid in mastocytoma P-815 cells. Mastocytoma cells [2×10^7 cells in 5 ml of phosphate-buffered saline (PBS)] [25] were incubated with 5 μCi of [1-^{14}C]arachidonic acid and 5 μg of non-labeled arachidonic acid at 37° for 30 min. Then, after the addition of 1.8 ml of a mixture of $CHCl_3$ and methanol (1:2, v/v), the reaction mixture was partitioned by successive additions of 0.6 ml $CHCl_3$ and 0.6 ml water. After centrifugation at 200 *g* for 5 min, the lower phase was taken out and evaporated under a nitrogen stream. The dried lipid residues were dissolved in 0.1 ml $CHCl_3$ and subjected to thin-layer chromatography t.l.c. on Kieselgel $60F_{254}$ plates (Merck).

One-half aliquot (0.05 ml) was used for separation of phospholipids in $CHCl_3$–methanol–acetic acid–water (50:30:8:4, by vol.), and the residual 0.05 ml for separation of prostaglandins ($PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, PGE_2 + PGD_2, PGA_2 + PGB_2, arachidonic acid) in $CHCl_3$–methanol–acetic acid–water (90:9:1:0.65, by vol.). PGD_2 and PGE_2 were further identified by rechromatography of their mixture, which was extracted from the corresponding spot on the above t.l.c. plate, in the organic phase of ethyl acetate–isooctane–acetic acid–water (11:5:2:10, by vol.) [26]. The position of each prostaglandin was determined by the mobility of co-chromatographed standard compounds in the same solvent system. Radioactive products were separately scraped off the t.l.c. plate, extracted with a mixture of $CHCl_3$–methanol (1:1, v/v), and counted by a liquid scintillation counter as described above.

Chemicals and reagents. PGE_1 and $PGF_{2\alpha}$ were provided by the Ono Pharmaceutical Co., Osaka, Japan. 1-Methyl-3-isobutylxanthine (MIX) and *C. atrox* venom were obtained from the Sigma Chemical Co., St Louis, MO, U.S.A.; [5,6-^{3}H(N)]PGE_1 (74.8 Ci/mmole), [5, 6, 8, 11, 14, 15(N)-^{3}H]$PGF_{2\alpha}$ (120–170 Ci/mmole), [ring-2-^{14}C]L-histidine (50 mCi/mmole), [1-carboxy-^{14}C]L-histidine (160 mCi/mmole), [8-^{3}H]ATP (30 Ci/mmole), [8-^{3}H]cAMP (30 Ci/mmole), and [methyl-^{3}H]thymidine (40–60 Ci/mmole) from the Radiochemical Centre, Amersham, U.K.; carrier-free $[^{35}S]H_2SO_4$ from the Japan Atomic Energy Research Institute, Tokyo, Japan; Kieselgel $60F_{254}$ t.l.c. plates from E. Merck, Darmstadt, West Germany; and radioimmunoassay kits for PGE_1, PGE_2 and $PGF_{2\alpha}$ from Boehringer Mannheim GmbH, Biochemica, West Germany.

RESULTS

Concentration of cAMP and activities of adenylate cyclase and phosphodiesterase in synchronized mastocytoma P-815 cells. The mean duration of the S phase of mastocytoma P-815 cells was estimated to be 3.5 hr, of G_2 to be 2.0 hr, of M to be 1.5 hr, and of G_1 to be 2.0 hr for an average total generation time of 9 hr. As shown in Fig. 1A, the cAMP level in the cells synchronized by the amethopterin block method exhibited two distinct peaks during the late S phase and the early G_2 phase, a decline during the late G_2 to the lowest level in mitosis, and a slight rise during the G_1 phase. Similar fluctuation patterns of cAMP levels, with two peaks during the late S and G_2 phases, were obtained when the cells were synchronized either at the beginning of S phase by the double thymidine block method (Fig. 1B) or

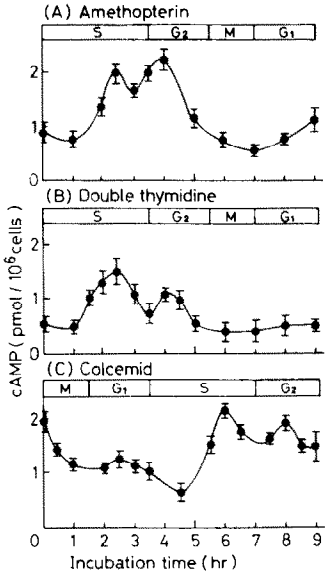

Fig. 1. cAMP content in mastocytoma P-815 cells synchronized by the amethopterin- (A), double thymidine- (B), and colcemid block method (C). The time lengths of S, G_2, M and G_1 were determined by the procedures as described in Experimental Procedures. Each point is the mean ± S.E. of three determinations.

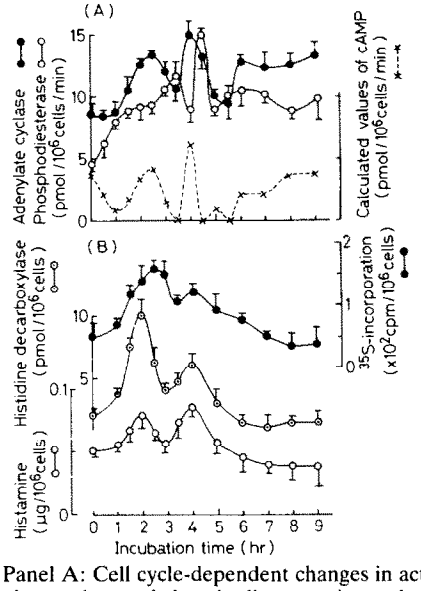

Fig. 2. Panel A: Cell cycle-dependent changes in activities of adenylate cyclase and phosphodiesterase in synchronized mastocytoma P-815 cells. Adenylate cyclase (●—●) and phosphodiesterase (○—○) were determined by the procedure described in Experimental Procedures. The cells used for each assay were collected by centrifugation of about 50 ml of the suspension culture (approximately 2.5×10^5 cells/ml) and washed once with PBS. The dotted line (×---×) represents the value of cAMP calculated by subtracting cAMP degraded by phosphodiesterase from cAMP formed by adenylate cyclase. Panel B: Cell cycle-dependent changes of histidine decarboxylase activity (⊙—⊙), [^{35}S]SO_4^{2-} incorporation into acidic glycosaminoglycan (●—●) and histamine content (○—○). The cells were collected by centrifugation of the suspension culture (30 ml, $2\text{–}2.5 \times 10^5$ cells/ml), washed once with PBS, and used for each assay described in Experimental Procedures. Each point in panels A and B is the mean ± S.E. of three determinations.

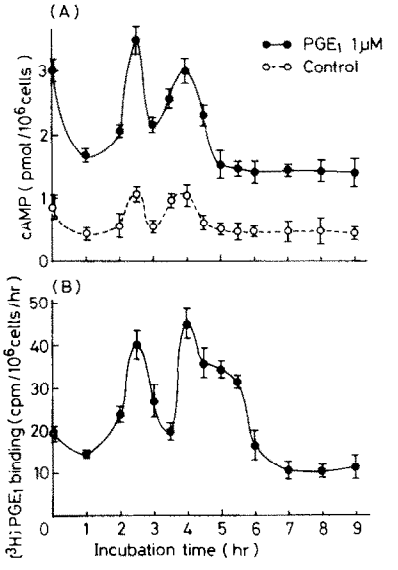

Fig. 3. Cell cycle-dependent changes of [^{3}H]PGE_1 binding and cAMP content. Cells harvested from about 30 ml of cell suspension ($2\text{–}2.5 \times 10^5$ cells/ml) were used for assays of cAMP content (A) and [^{3}H]PGE_1 binding (B). Panel A: (○---○) cAMP content in the control cells; and (●—●) cAMP content in the cells preincubated with 1 μM PGE_1. Panel B: (●—●) specific binding of [^{3}H]PGE_1. Each point in panels A and B is the mean ± S.E. of three determinations.

during mitosis by the colcemid block method (Fig. 1C). However, in most of the experiments, the amethopterin block method was employed because of its speed and reproducibility.

The activity of adenylate cyclase also fluctuated during the cell cycle, being elevated during the late S and early G_2 phases and during most of the M and G_1 phases. In contrast, the activity of phosphodiesterase was elevated during the inter S-G_2 and the mid G_2 phases (Fig. 2A). The difference between the specific activities of the two enzymes throughout the cell cycle (dotted line in Fig. 2A) coincided with the fluctuation of endogenous cAMP levels (Fig. 1A).

Histamine concentration, histidine decarboxylase activity and the incorporation of[^{35}S]SO_4^{2-} into acidic glycosaminoglycans (Fig. 2B) also varied throughout the cell cycle in close synchrony with the fluctuation of cAMP levels (Fig. 2A).

Effect of PGE_1 binding. As shown in Fig. 3A, exposure of synchronized cells to PGE_1 for 10 min at various phases of the cell cycle resulted in an increase in the cAMP concentration above the basal level (Fig. 3A). PGE_1 was most effective in elevating cAMP levels when it was added to the cells at the late S and the early G_2 phases. The specific binding of [^{3}H]PGE_1 to the cells was also elevated during the late S phase and the G_2 phase (Fig. 3B).

Contents of PGE and PGF in synchronized mastocytoma cells. Since mastocytoma P-815 cells [27] as well as mast cells [28] were known to produce various types of prostaglandins, the cell cycle-dependent changes of prostaglandins were examined. As shown in Fig. 4A, the content of the E group of

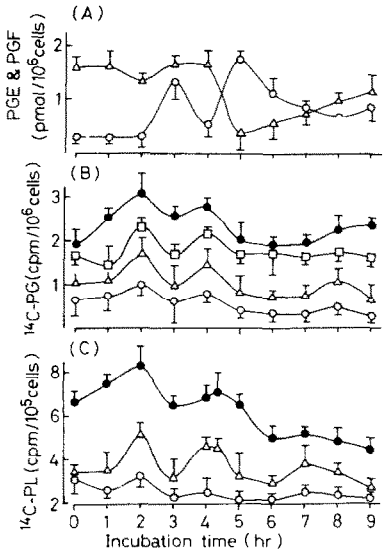

Fig. 4. Cell cycle-dependent changes of PGE and PGF contents and [^{14}C]prostaglandin and [^{14}C]phospholipid synthesis in synchronized mastocytoma P-815 cells. Each point is the mean ± S.E. of three determinations. Panel A: Contents of PGE and PGF were measured by the radioimmunoassay method using about 1×10^7 cells harvested from about 40–50 ml of the suspension cultures ($2–2.5 \times 10^5$ cells/ml). Key: (○—○) PGE; and (△—△) PGF. Panel B: [^{14}C]Prostaglandins formed were measured by the procedure as described in Experimental Procedures. Approximately $2–3 \times 10^7$ cells harvested at any given time were suspended in 5 ml of PBS containing 5 μCi of [1-^{14}C]arachidonic acid and incubated for 30 min at 37°. Each [^{14}C]prostaglandin formed was separated by t.l.c. and counted in 10 ml of toluene scintillant. Key: (●—●) [^{14}C]PGE_2; (○—○) 6-keto $PGF_{1\alpha}$; (□—□) [^{14}C]$PGF_{2\alpha}$; and (△—△) [^{14}C]PGD_2 + [^{14}C]PGB_2. Panel C: [^{14}C]Phospholipids formed from [^{14}C]arachidonic acid were measured by the procedure described in Experimental Procedures. Key: (○—○) [^{14}C]phosphatidylethanolamine; (●—●) [^{14}C]phosphatidylcholine; and (△—△) [^{14}C]phosphatidylinositol.

prostaglandins was significantly elevated during the late S phase and the G_2 phase. In contrast, the content of the F group of prostaglandins was high during the S phase and the early G_2 phase and then sharply declined to the lowest level during the late G_2 phase and mitosis.

Incorporation of [^{14}C]*arachidonic acid into prostaglandins and phospholipids in synchronized cells.* Incorporation of radioactivity into PGE_2, 6-keto $PGF_{1\alpha}$ and $PGD_{2\alpha}$ and PGD_2 from [^{14}C]arachidonic acid (Fig. 4B) was also related to the cell cycle phase, exhibiting two peaks at the late S phase and the early G_2 phase. The incorporation of [^{14}C]arachidonic acid into phosphatidylethanolamine, -inositol and -choline (Fig. 4C) also showed similar fluctuation during the cell cycle, exhibiting two peaks at the mid-S and the early G_2 phases, with another peak during G_1 phase.

Effect of cAMP on DNA synthesis. MIX added at 0 hr of the removal of the amethopterin block elevated the intracellular cAMP level approximately 10-fold over the control level throughout the cell cycle of synchronized cells (Fig. 5B), but it did not inhibit the DNA synthesis (Fig. 5A). However, cycloheximide and actinomycin D added to the cells 2 hr before the removal of the amethopterin block almost completely abolished the DNA synthesis although these drugs had little effect on the cell cycle-specific changes of the basal cAMP levels.

Effect of PGE_1 and/or MIX on cell cycle phase. PGE_1 and/or MIX, added at the late S phase of synchronized mastocytoma cells, significantly prolonged the G_2 phase, retarded the cell division, and elevated the cAMP levels (Fig. 6A). The order of potency for these agents to inhibit cell division and to elevate cAMP levels was PGE_1 + MIX > MIX > PGE_1. Removal of MIX at 5 hr (1.5 hr after its addition, corresponding to the late S phase of control cells) resulted in the decline of elevated cAMP levels to the basal level, shortening of prolonged G_2 phase, and resumption of the cell division. In contrast, these agents added at the beginning of mitosis (5 hr after removal of amethopterin block) did not inhibit the cell division, yet they still significantly elevated the cAMP levels (Fig. 6B).

Effect of serum deprivation on cAMP levels, DNA synthesis and cell division. Removal of fetal calf serum from the culture medium at the beginning of the S phase resulted in the inhibition of DNA synthesis and cell division. However, cAMP levels still fluctuated with two peaks during the S and G_2 phases even in the absence of the serum (Fig. 7).

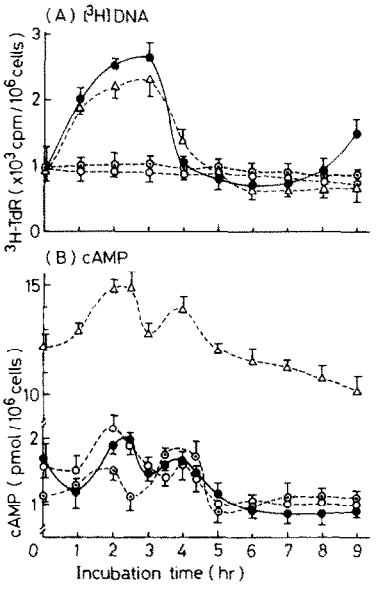

Fig. 5. Effects of MIX, actinomycin D and cycloheximide on synthesis of DNA and cAMP. Cells synchronized by the amethopterin block method were treated either with 1 mM MIX at the same time of reversal, or with actinomycin D (0.5 μg/ml) or cycloheximide (0.5 μg/ml) at 3 hr prior to the reversal. Panel A: About 6×10^6 cells, harvested at any given time after synchronization, were pulse-labeled with 0.2 μCi of [^{3}H]thymidine for the measurement of [^{3}H]DNA synthesis. Panel B: About 6×10^6 cells harvested at the same time indicated in panel A were used for the determination of cAMP. Key: (●—●) control; (△---△) + MIX; (⊙---⊙) + actinomycin D; and (○---○) cycloheximide. Each point in panels A and B is the mean ± S.E. of three determinations.

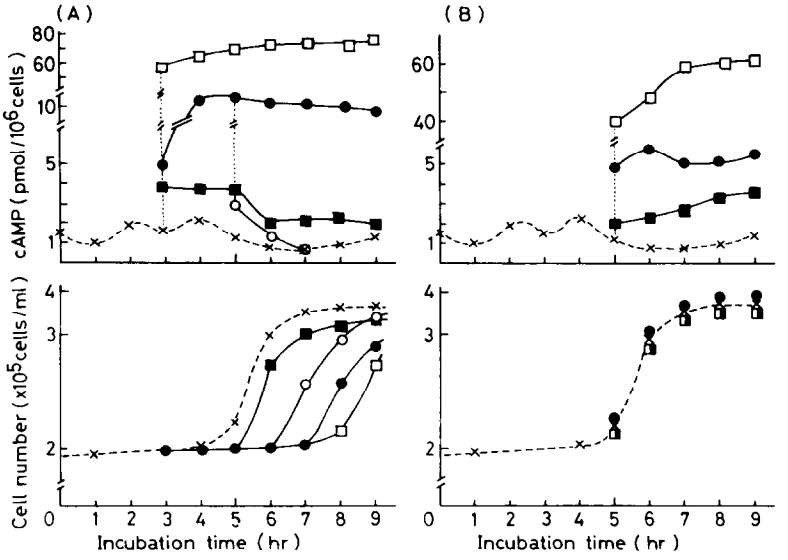

Fig. 6. Effects of PGE_1 and/or MIX on cAMP levels and cell division. Panel A: Cells at S–G_2 traverse (3.5 hr after removal of the block) were treated with 10 μM PGE_1 with (□—□) or without (■—■) 1 mM MIX, or with 1 mM MIX alone (●—●); (×---×) control. An aliquot of treated cells was washed 1.5 hr later with fresh medium to remove MIX (○—○). cAMP content in 4×10^6 cells harvested at each point (upper figure), and the cell numbers of growing cells in 1 ml of suspension (lower figure), were measured. Each point is the mean of three determinations. Panel B: Cells at G_2–M traverse (5 hr after removal of the block) were treated with 10 μM PGE_1 with (□—□) or without (■—■) 1 mM MIX, or with 1 mM MIX alone (●—●). Other conditions were similar to those described for panel A.

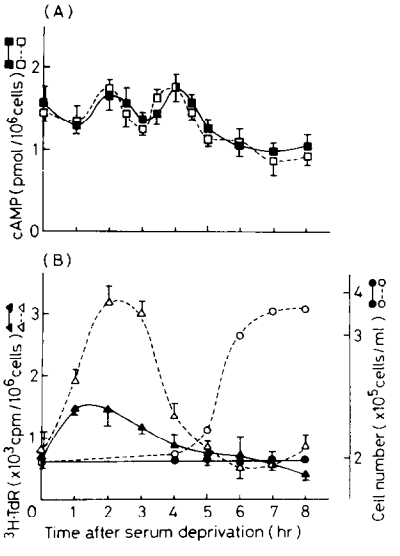

Fig. 7. Effect of serum deprivation on DNA synthesis and cAMP content. Synchronized cells were cultivated until the logarithmic growing phase in the medium supplemented with 5% fetal calf serum (FCS). One portion of the cells was deprived of FCS (—) by washing with the fresh medium without serum at 0 time of reversal of S-phase block. The other portion of cells was cultured in the medium containing 5% FCS (---). Panel A: (□, ■) cAMP contents (assayed using about 4×10^6 cells). Panel B: (△, ▲) incorporation of [^{3}H]thymidine into [^{3}H]DNA (determined using about 3×10^6 cells); and (○, ●) cell number. Each point in the cAMP content and [^{3}H]thymidine incorporation is the mean ± S.E. of three determinations. Each point in the cell number is the mean of two determinations.

DISCUSSION

The present results show that in mastocytoma P-815 cells, synchronized either at the G_1/S transition by amethopterin- or double thymidine-block or in mitosis by colcemid block, the intracellular cAMP levels were highest during the late S phase and the early G_2 phase and lowest during mitosis. The fluctuation pattern was similar to that observed in synchronous HeLa cells [1], except for the lack of cAMP peak during the G_1 phase (Fig. 1). Increased cAMP levels during the G_1 phase were also observed in synchronized Chinese hamster ovary cells [2, 3], human lymphoid cells, regenerating rat liver [29], and in mouse parotid gland stimulated by catecholamine *in vivo* [30]. These increases are perhaps related to the events leading up to DNA synthesis. Growth rate is known to be inversely correlated with cAMP levels in several types of cultured cells [31, 32] including mastocytoma P-815 cells [12], and the increased generation time is accounted for primarily by an expansion of the G_1 phase [33]. Therefore, low cAMP levels in the G_1 phase of mastocytoma P-815 cells might be due to their relatively short duration of G_1 phase (<2 hr) and relatively high growth rate (generation time = *ca.* 9 hr).

The cell cycle-dependent changes in cAMP levels can be largely accounted for by the activities of adenylate cyclase and phosphodiesterase in mastocytoma P-815 cells (Fig. 2). A similar relationship between cAMP levels and these two enzyme activities was reported for synchronous *Tetrahymena pyriformis* [34, 35]. As in the case of the response of synchronous mouse melanoma cells to the melanocyte stimulating hormone [9], the response of mas-

tocytoma P-815 cells to PGE_1 with increase in cAMP content was also cell cycle dependent (Fig. 3).

We previously demonstrated that exponentially growing mastocytoma P-815 cells responded to the E and I groups but not to the D group of prostaglandins with a rapid increase in cAMP content, and that the cells had specific receptors for the E group but not for the I group of prostaglandins on the cell membrane [13]. Therefore, the changes in cAMP levels during the cell cycle can be explained by the stimulation of adenylate cyclase by PGE_1 through its binding to the specific receptor on the cell membrane, which is available only during the late S phase and the G_2 phase. PGE_1 may also activate phosphodiesterase, because the increase of the low K_m phosphodiesterase activity was detected within 2 min of the addition of PGE_1 to mastocytoma P-815 cells [13]. High levels of the E group of prostaglandins (Fig. 4A) and their elevated synthesis from arachidonic acid (Fig. 4B) during the late S and G_2 phases also support the above assumption. However, it is not clear yet how the synthesis of prostaglandin is controlled during the cell cycle. The activation of fatty acid cyclooxygenase in mastocytoma P-815 cells by Na butyrate [27], which is known to induce hyperacetylation of histones [36], suggests that prostaglandin synthesis may be regulated somehow at the transcriptional level.

It is not certain whether the receptor for PGE_1 becomes available by *de novo* synthesis or by the unmasking of a silent receptor. It is interesting that the synthesis of phosphatidylcholine and phosphatidylinositol from arachidonic acid is also elevated at the S and G_2 stages (Fig. 4C). Hirata *et al.* [37] suggested the importance of phospholipid methylation for the control of membrane fluidity and β-adrenergic receptor–adenylate cyclase coupling in rat reticulocyte ghosts. It is also known that the changes of membrane phospholipid composition affect the adenylate cyclase activity in membranes of several types of mammalian cells [38–40], and that the activity of rat brain adenylate cyclase incorporated into artificial phospholipid vesicles is increased by raising the phosphatidylcholine content of the liposome [41]. These data are favorable for the assumed importance of phospholipid metabolism in regulation of receptor availability and adenylate cyclase activity. Furthermore, metabolism of membrane phospholipid is important in order to supply arachidonic acid for prostaglandin synthesis (Fig. 4).

Our results on the effect of cAMP levels on DNA synthesis are in general agreement with those reported for synchronous HeLa cells [1]. The exposure of the cells to 1 mM MIX immediately after reversal of an amethopterin block resulted in a 5- to 10-fold increase in cAMP levels throughout the cell cycle, without affecting the general fluctuation pattern of cAMP levels in the control cells (Fig. 5B). Despite the elevation of cAMP, the incorporation of [^{3}H]thymidine into DNA was practically not affected (Fig. 5A).

The elevation of cAMP levels at the late S phase of synchronized cells also resulted in the prolonged G_2 phase and the retarded mitosis (Fig. 6A). However, the inhibition was easily reversed by removing the reagents by washing. On the other hand, the elevation of cAMP levels at the beginning of mitosis had no effect on the cell division (Fig. 6B). These results suggest that cAMP acts as an important regulator during G_2 traverse and that the decline of cAMP levels at the late G_2 stage is necessary for the cells to initiate mitosis.

It is not certain whether factors other than prostaglandins are also involved in control of the basal cAMP concentration. Although the growth of mastocytoma P-815 cells was entirely dependent upon the presence of serum and the DNA synthesis was also significantly depressed in the absence of serum, the synchronous cells cultured in a serum-deprived medium still exhibited fluctuations of cAMP levels similar to those of the control cells (Fig. 7). These results suggest that no factor is present in serum which directly controls the cellular cAMP levels.

Various factors for control of growth and phenotypic expression of mammalian cells are present in serum and some of them have been identified [42]. However, the relationship between serum factors and cellular growth and phenotypic expression in mastocytoma P-815 cells is still only poorly understood. The histidine decarboxylase activity, histamine content and [^{35}S]SO_4^{2-} incorporation into acidic glycosaminoglycans also fluctuated in synchrony with cAMP levels in synchronized cells (Fig. 2B). It is still not determined whether these changes are caused solely by prostaglandins newly synthesized in mastocytoma cells exposed to serum. However, it is likely that prostaglandins newly synthesized by the increased phospholipid metabolism promote the cAMP synthesis through their bindings to the unmasked receptors, and thereby control cell division and phenotypic expression. Further investigations are certainly required to elucidate the exact mechanism of cAMP action in mastocytoma P-815 cells.

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