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The time-course of cyclic AMP signaling is critical for leukemia U-937 cell differentiation☆,☆☆

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Abstract

The regulation of the cAMP signaling is intimately involved in several cellular processes, including cell differentiation. Here, we provide strong evidence supporting that the time-course of cAMP signal is critical for leukemia U-937 cell differentiation. Three stimulating-cAMP agents were used to analyze the correlation between cAMP time-course and cell differentiation. All three agents denoted similar cAMP maximal responses in dose–response experiments. The kinetic of desensitization showed differential characteristics, while H2 receptor desensitized homologously without affecting PGE2 or forskolin effect, PGE2 response showed mixed desensitization characterized by a homologous initial phase followed by a heterologous phase. Regarding forskolin, long-term stimuli attenuated PGE2 and H2 agonist response without affecting adenylyl cyclase activity. In the absence of phosphodiesterase inhibitors, the three agents induced similar maximal cAMP levels after 5 min, but only that induced by the H2 agonist returned to basal levels. Consistent with this observation, H2 agonist was not able to induce U-937 cell maturation in contrast to PGE2 and forskolin, supporting the importance of time-course signaling in the determination of cell behavior.

Keywords: cAMP signaling; Cell differentiation; Leukemia

Most chemotherapeutic agents currently used in cancer therapies display significant toxicity and are often non-specific. A potential alternative to treat this prevalent disease includes agents that induce cell differentiation. The induction of differentiation as a therapeutic strategy has the greatest impact on hematopoietic malignancies, most notably on leukemia. Acute promyelocytic leukemia (APL), characterized by differentiation arrest of granulopoiesis at the promyelocytic stage, is the first human malignancy that can be efficiently treated with a cell differentiation inducer, all-*trans* retinoic acid [1].

Cyclic adenosine monophosphate (cAMP) plays an important role in the response to hormonal signals that induce cell proliferation, differentiation, and apoptosis in several systems. Several lines of evidence support its role in hematopoietic development. Cyclic AMP-elevating agents, like histamine H2 agonists, are able to induce granulocyte differentiation in the human promyelocytic cell line HL-60 [2,3]. In M1 mouse myeloid leukemia cells as well as in the human promonocytic U-937 cell line, dibutyryl cAMP (dbcAMP) induces monocyte maturation [4,5]. Cyclic AMP can also potentiate granulocytic differentiation of retinoid- or

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☆☆ *Abbreviations:* APL, acute promyelocytic leukemia; cAMP, cyclic adenosine monophosphate; dbcAMP, dibutyryl cAMP; PDE, phosphodiesterase; GRK, G protein-coupled receptor kinase; GPCR, G protein-coupled receptor; IBMX, isobutylmethylxanthine; PGE2, prostaglandin E_2; Fura 2-AM, Fura 2 acetoxymethyl ester; BSA, bovine serum albumin; rhC5a, recombinant human C5a; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide.

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arsenic trioxide-induced maturation of human APL cells [6,7].

Cyclic AMP is generated following the interaction of ligands with a receptor coupled to a transducer Gs protein. The occupied receptor promotes the exchange of GTP in the transducer, thus generating an activated α subunit, which in turn activates the effector adenylyl cyclase [8]. The activation of this membrane signal transduction machinery is transient because several mechanisms are activated to terminate the stimulation and to return the cell to a resting state. These include the phosphorylation of the receptor by different kinases and the recruitment of β-arrestins, or inactivation of Gs via hydrolysis of GTP at a rate controlled by the regulators of G protein signaling (RGS) protein [9]. The termination of the cAMP stimulus is critical for appropriate signaling, as several knockout models indicate that inactivation of desensitization mechanisms produces major impairment of cell function [10]. Activation of phosphodiesterases (PDEs), that are downstream of receptor/G protein/effector coupling, is an additional regulatory mechanism that induces the termination of the stimulus distal to the generation of cAMP [11].

The regulation of the desensitization process may be an important mechanism in the differentiation of hematopoietic cells. We have previously reported that in the promonocytic U-937 cell line, histamine H2 agonist transiently induces an important cAMP response, but fails to induce differentiation due to rapid receptor desensitization mediated by G protein-coupled receptor kinases (GRKs) [12,13]. Reduction in GRK2 levels by cDNA antisense construct determines a higher and prolonged cAMP response mediated by H2 receptor, due to lower receptor desensitization, allowing H2 agonist-stimulated cell differentiation [14].

We provide here, further evidence that strongly supports the important role of the kinetic of the cAMP signaling in the differentiation of U-937 cells.

Materials and methods

Materials. Cell culture medium, isobutylmethylxanthine (IBMX), cAMP, dbcAMP, forskolin, prostaglandin E_2 (PGE2), Fura 2 acetoxymethyl ester (Fura 2-AM), bovine serum albumin (BSA), and rhC5a were obtained from Sigma Chemical Company (St. Louis, MO). Fetal calf serum was purchased from Natocor (Argentina). Amthamine and 6[2-(4-imidazolyl)ethylamino]-*N*-(4-trifluoromethylphenyl)heptane-carboxamide (HTMT dimaleate) were from Tocris Cookson (Ballwin, MO). H-7 was obtained from Calbiochem (La Jolla, CA). [^{3}H]cAMP was purchased from Perkin–Elmer Life Sciences (Boston, MA). The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT)-based cell titer 96 kit, purchased from Promega (Madison, WI), was used as specified by the manufacturer. All other chemicals used were of analytical grade.

Cell culture. U-937 cells were cultured at 37 °C in a humidified atmosphere with 5% CO_2 in RPMI 1640 medium supplemented with 10% fetal calf serum and 50 μg/ml gentamicin.

cAMP assays. For dose–response assays, cells were incubated 3 min in RPMI 1640 medium supplemented with 1 mM IBMX at 37 °C, followed by 9 min exposure to different concentrations of the indicated agents.

For desensitization assays, pretreatment of cells with the different agents was performed in RPMI 1640 medium at 37 °C in a 5% CO_2 humidified atmosphere for periods ranging from 1 min to 4 h, in the absence of IBMX. Cells at a density of 10^6 cells/ml were then washed and resuspended in RPMI 1640 medium containing 1 mM IBMX, and exposed 9 min to the different agents, to evaluate whether the system was still able to generate cAMP.

For time-course cAMP accumulation studies, cells were resuspended in RPMI 1640 medium in the absence of IBMX, at a density of 10^6 cells/ml, and exposed to the specific agents at different periods of time.

In all experiments, the reaction was stopped by ethanol addition followed by 10 min centrifugation at 3000*g*. The ethanol phase was then dried and resuspended in 50 mM Tris–HCl, pH 7.4, 0.1% BSA. Cyclic AMP content was determined by competition of [^{3}H]cAMP for PKA, as previously described [15].

Proliferation assay. The MTT test was performed as described elsewhere [16]. Briefly, 10^3 cells/well in a final volume of 100 μl were seeded in a 96-well flat-bottomed-tissue culture plate. Each experiment was performed in quadruplicate. Blanks consisted of 100 μl/well of culture medium. After 3–4 days of culture, 15 μl MTT solution/well was added and cells were incubated for 4 h. The reaction was terminated by the addition of 100 μl solubilization-stop solution/well followed by overnight incubation. Optical density at 540 nm was recorded in a vertical ELISA Titertek Multiscan MCC/340 reader.

A linear relationship between optical density at 540 nm and U-937 cell number between 5×10^4 and 7×10^5/ml in the MTT test has been previously established.

Western blots. Cells were lysed in sample buffer (50 mM Tris–HCl, pH 6.8, 2% SDS, 100 mM 2-mercaptoethanol, 10% glycerol, and 0.05% bromphenol blue) and sonicated to shear DNA. Samples were boiled 5 min, and aliquots were subjected to electrophoresis in 12% SDS–polyacrylamide gels and transferred to nitrocellulose membranes. The residual binding sites were blocked with 5% non-fat powdered milk in PBST (PBS containing 0.05% Tween 20) and membranes were incubated with 1 μg/ml of anti c-Fos or anti c-Myc rabbit antibody (Santa Cruz Biotechnology, CA), in PBST. All subsequent washes were performed with the same buffer. Reactivity was developed using an anti-rabbit polyclonal antibody linked to horseradish peroxidase and enhanced chemiluminescence reagents, according to manufacturer's instructions (Amersham, Buckinghamshire, England).

Intracellular Ca^{2+} Measurements. Fura 2-AM was used as a fluorescent indicator. Cells from each experimental group were washed, resuspended, and incubated in a buffered saline solution (BSS; 140 mM NaCl, 3.9 mM KCl, 0.7 mM KH_2PO_4, 0.5 mM Na_2HPO_4, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, and 20 mM Hepes, pH 7.5, 10 mM glucose, and 0.1% BSA) in the presence of 2 μM Fura 2-AM). Cells were incubated for 30 min at 37 °C in an atmosphere of 5% CO_2, to allow Fura 2 to be intracellularly trapped by esterase cleavage. Cells were then washed twice in BSS without Fura 2-AM and brought to a density of 2×10^6 cells/ml BSS. Fluorescence was measured in a spectrofluorometer (Jasco, Tokyo, Japan) provided with the CA-261 accessory to measure Ca^{2+} under continuous stirring, with the thermostat adjusted to 37 °C and an injection chamber. Intracellular Ca^{2+} ($[Ca^{2+}]_i$) levels were registered every second by exposure to alternating 340- and 380-nm light beams, and the intensity of light emission at 505 nm was measured. Under these conditions, light intensities and the F340/F380 ratio were tracked. Different agents were injected (5 μl) into the chamber as a 100-fold concentrated solution without interrupting recording. The preparation was calibrated by determining maximal fluorescence induced by 0.1% Triton X-100 and minimal fluorescence in the presence of 6 mM EGTA (pH 8.3). $[Ca^{2+}]_i$ was calculated according to Grynkiewicz et al. [17].

Results

Cyclic AMP elevation in response to different agents in U-937 cells

Cyclic AMP levels were induced in U-937 cells by amthamine and PGE2, two agonists that stimulate G protein-coupled receptors (GPCR), histamine H2 receptor, and PGE2 receptors, respectively. Forskolin, a direct adenylyl cyclase-activating agent, was also used to increase cAMP levels. All agents elevated cAMP levels in a concentration-dependent manner, with EC_{50} values of 465 ± 95 nM ($n = 5$), 39 ± 6 nM ($n = 3$), and 40 ± 7 μM ($n = 3$) for amthamine, PGE2, and forskolin, respectively. These agents evoked similar maximal responses (Fig. 1).

Desensitization of the H2, PGE2, and forskolin responses

We had previously reported that in U-937 cells, H2 receptors desensitize homologously at a $t_{1/2}$ of ~20 min, by a mechanism involving GRKs [12]. In an attempt to study the mechanism underlying the desensitization of the PGE2-induced cAMP response, cells were exposed to 1 μM PGE2 (maximal response) for periods ranging from 1 min to 4 h. Cells were subsequently washed and exposed to PGE2 to determine whether the system was still able to generate cAMP. Furthermore, we examined the effect of PGE2 pretreatment on the response evoked by amthamine (H2 agonist) and forskolin. Fig. 2A illustrates the desensitization curve, where two phases can be observed. The first phase showed a rapid desensitization of the PGE2 receptor, without affecting the H2 agonist response whereas in the second phase (after 40 min), a parallel decrease in both responses was observed. However, there was no change in forskolin response, suggesting that the desensitization of the PGE2 and amthamine responses was due to an event upstream of the adenylyl cyclase activation. These results suggest the existence of mixed desensitization characterized by a homologous initial phase and a heterologous second phase. To investigate the participation of PKA/PKC in the heterologous phase, assays were carried out in the presence of H7, a PKA/PKC inhibitor. Cells were pretreated (120 min) with H7 and 1 μM PGE2. Results showed that H7 completely abolished PGE2-induced H2r desensitization but it partially inhibited PGE2r desensitization (Fig. 2B).

To investigate whether cAMP response to forskolin in this cell line was susceptible to desensitization, U-937 cells were exposed for different periods of time to 75 μM forskolin and restimulated with the same compound. In addition, we also evaluated the response of PGE2 and amthamine after forskolin pretreatment. PGE2 and amthamine responses were desensitized by forskolin pretreatment, but no changes in cAMP production were observed after forskolin stimulation, indicating that the sustained increase in cAMP levels attenuated the response of the receptors coupled to adenylyl cyclase without affecting the activity of the effector (Fig. 3A).

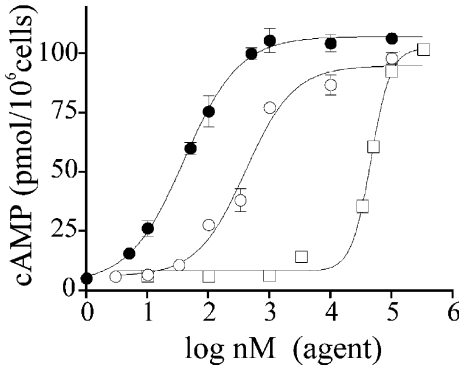

Fig. 1. Concentration–response curves to cAMP production. U-937 cells were incubated for 9 min with increasing concentrations of amthamine (○), PGE2 (●), and forskolin (□) at 37 °C in RPMI medium supplemented with 1 mM IBMX. Cyclic AMP levels were determined as described in Materials and methods. Data are calculated as means ± SD of assay triplicates. Similar results were obtained in at least three independent experiments.

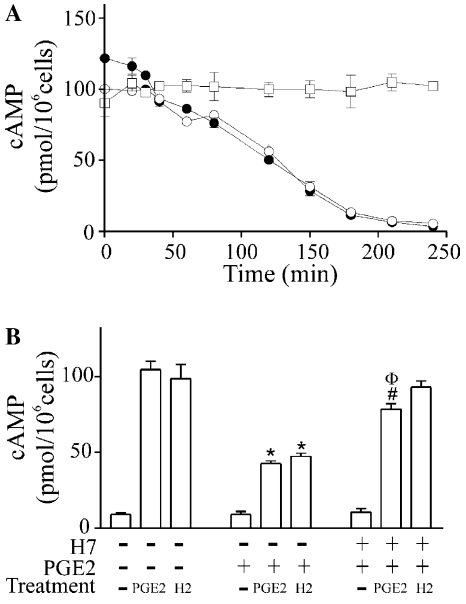

Fig. 2. Desensitization of PGE2- and amthamine-induced cAMP response by PGE2. (A) U-937 cells were preincubated at different time intervals with 1 μM PGE2, washed, and restimulated with 1 μM PGE2 (●), 10 μM amthamine (○), or 75 μM forskolin (□) in the presence of 1 mM IBMX. Cyclic AMP production was determined as described in Materials and methods. Data are calculated as means ± SD of assay triplicates. Similar results were obtained in at least three independent experiments. (B) U-937 cells were pretreated for 20 min or not, in the presence of 20 μM H7 and exposed 120 min or not to 1 μM PGE2. Cells were then washed and stimulated with 1 μM PGE2 or 10 μM amthamine (H2) in the presence of 1 mM IBMX. Data are calculated as means ± SEM of three experiments. $^{*}p < 0.001$ vs. non-pretreated cells stimulated with PGE2 and H2, respectively; $^{\#}p < 0.01$ vs. non-pretreated cells stimulated with PGE2; $^{\Phi}p < 0.05$ vs. PGE2 pretreated cells stimulated with PGE2.

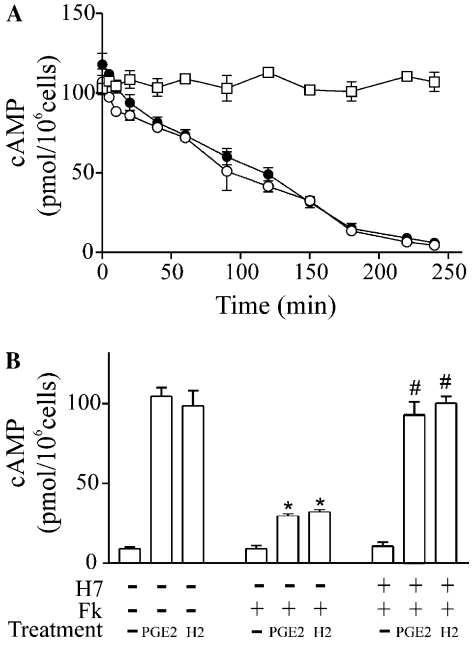

Fig. 3. Desensitization of PGE2r and H2r induced by forskolin. (A) U-937 cells were preincubated for different periods of time with 75 μM forskolin, washed, and restimulated with 1 μM PGE2 (●), 10 μM amthamine (○), or 75 μM forskolin (□) in the presence of 1 mM IBMX. Cyclic AMP production was determined as described in Materials and methods. Data are calculated as means ± SD of assay triplicates. Similar results were obtained in at least three independent experiments. (B) U-937 cells were pretreated for 20 min or not in the presence of 20 μM H7 and exposed 120 min or not to 75 μM forskolin (Fk). Cells were then washed and stimulated with 1 μM PGE2 or 10 μM amthamine (H2) in the presence of 1 mM IBMX. Data are calculated as means ± SEM of three experiments. $^{*}p < 0.001$ vs. non-pretreated cells stimulated with PGE2 and H2, respectively, $^{\#}p < 0.001$ vs. pretreated cells stimulated with PGE2 and H2, respectively.

This desensitization was mediated by second messenger-dependent kinases, since it was inhibited in the presence of H7 (Fig. 3B).

Time-course of cAMP levels in the absence of IBMX

Due to the existence of different patterns of cAMP production after a long-term exposure to the different agents (amthamine, PGE2, and forskolin) and in an attempt to determine cAMP levels resulting from the production–degradation balance, the time-course of cAMP accumulation was determined in the absence of IBMX (a phosphodiesterase inhibitor). Results showed that the three agents induced similar maximal cAMP responses at 5 min, but only PGE2 and forskolin showed a higher residual response significantly different from basal levels that was sustained until 4 h (Fig. 4).

Effects of amthamine, PGE2, and forskolin on cell proliferation and differentiation

We then evaluated whether the different kinetics of cAMP evoked by the three agents assayed influenced cell

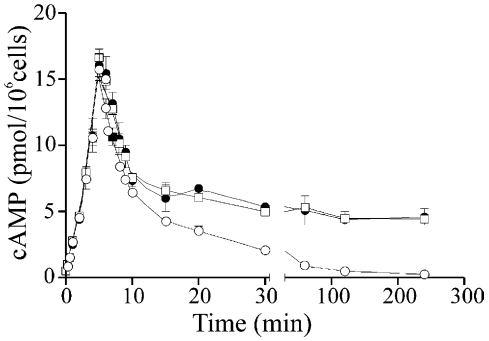

Fig. 4. Time-course of cAMP levels in the absence of IBMX. U-937 cells were incubated for different periods of time with 10 μM amthamine (○), 1 μM PGE2 (●), or 75 μM forskolin (□) at 37 °C and cAMP levels were determined as stated in Materials and methods. Data are calculated as means ± SD of assay triplicates. Similar results were obtained in at least three independent experiments.

proliferation and/or differentiation. Cell proliferation was evaluated in U-937 cells treated for 3 days with amthamine, PGE2, and forskolin, at maximal cAMP response concentration. Amthamine was not able to inhibit cell division as it was previously reported for another H2 agonist, dimaprit [5]. In contrast, PGE2 and forskolin inhibited U-937 cell proliferation (Fig. 5). This effect was dose-dependent, with an EC_{50} value of 43 ± 5 nM for PGE2 (data not shown) and 22 μM for forskolin [18]. As we had reported induction of the PDE4 family activity when cells were treated with amthamine [12], we assayed the effect of amthamine plus rolipram (PDE4 family phosphodiesterase inhibitor) on cell proliferation. Results showed that under these conditions, amthamine was able to reduce U-937 cell proliferation.

To evaluate the ability of U-937 cells to differentiate in the presence of amthamine, PGE2, and forskolin, we first analyzed the pattern of c-Fos and c-Myc expression. The sustained c-Fos expression and the

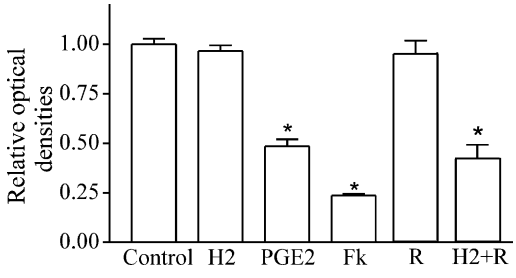

Fig. 5. Effects of amthamine, PGE2, and forskolin on U-937 cell proliferation. U-937 cells were seeded in tissue culture plates and incubated for 3 days with 10 μM amthamine (H2), 1 μM PGE2, 75 μM forskolin (Fk), 10 μM rolipram (R) or 10 μM amthamine + 10 μM rolipram (H2 + R). Control values correspond to non-treated cells. The MTT test was performed as described in Materials and methods. Relative optical densities are the recorded optical densities expressed as proportions of the mean control density. Results are expressed as means ± SEM of triplicate assays. $^{*}p < 0.01$ vs. control.

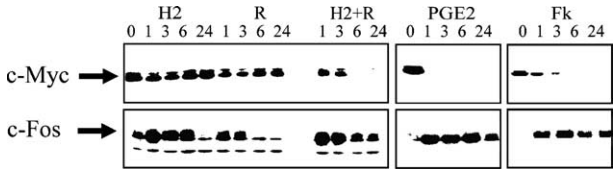

Fig. 6. Effect of amthamine, PGE2, and forskolin treatment on c-Fos and c-Myc protein levels. U-937 cells were incubated with 10 μM amthamine (H2), 1 μM PGE2, 75 μM forskolin (Fk), 10 μM rolipram (R) or 10 μM amthamine + 10 μM rolipram (H2 + R) for the indicated periods of time before harvest and lysis as described in Materials and methods. Samples were electrophoresed in 12% SDS–polyacrylamide gels, transferred to nitrocellulose, and immunoblotted with polyclonal purified rabbit serum against Fos and Myc. Data are representative of at least three independent experiments.

down-regulation of c-Myc were associated with U-937 cell differentiation. Amthamine produced a transient increase in c-Fos levels with maximal response after 1 h, which remained higher than controls for 6 h and decreased to basal levels after 24 h. However, treatment with PGE2, forskolin or amthamine plus rolipram induced a sustained pattern of c-Fos expression detectable even evident after 24 h stimulation. Concerning the pattern of c-Myc expression, treatment with PGE2, amthamine plus rolipram, and forskolin, but not amthamine alone induced the down-regulation of this proto-oncogen (Fig. 6).

The expression of C5a receptor (CD88) was assayed as a differentiation marker. The C5a receptor is a GPCR associated with Ca^{2+} release from intracellular stores. No response was observed either in control cells or in cells treated for 2 days with amthamine. When these cells were stimulated with 6[2-(4-imidazolyl)ethylamino]-*N*-(4-trifluoromethylphenyl)heptane-carboxamide (H1 agonist), known to elevate $[Ca^{2+}]_i$ levels, the typical spike was observed, indicating that these cells were able of evoking a Ca^{2+} response. In the presence of PGE2, forskolin or amthamine plus rolipram, U-937 cells acquired the CD88 receptor as it can be deduced from the Ca^{2+} response evoked by rhC5a (Fig. 7).

Discussion

The formation of the different blood cells is essential for the development of a normal individual. New blood cells belonging to different cell lineages are formed from stem cells not only during embryogenesis but also during the adult lifetime. Abnormalities in the normal developmental program of blood cell formation result in various types of hematological diseases, including leukemia which involves an uncoupling of the regulatory mechanisms that normally integrate cell proliferation with maturation. Such an alteration may produce an asynchrony of behavior so that the cells maintain proliferation but without maturation. Endogenous regulators or foreign agents can control the abnormal growth of certain types of leukemic cells and suppress malignancy by inducing differentiation to mature non-dividing cells, thus providing new therapeutical approaches [1].

Present findings demonstrate that by regulating cAMP levels, biological responses such as cellular differentiation can be triggered in U-937 promonocytic cells. Transient activation of cAMP-related pathways has different effects compared to long-term activation.

The importance of a signaling pathway time-course has also been shown for other systems. In cultured PC12 cells, epidermal growth factor induces transient activation of the MAP kinase pathway, causing the cells to multiply slowly. Nerve growth factor, in contrast, induces sustained activation of the MAP kinase cascade, and the cells become specialized to resemble nerve cells [19].

In U-937 cells, cAMP is associated with cell differentiation [5,18]. However, histamine, which increases cAMP levels by activating H2 receptors, fails to promote cell maturation [5]. This failure was bypassed by introducing an antisense GRK2 cDNA, which reduced the H2 receptor desensitization and allowed the H2 agonist to induce markers of cell maturation [14].

In the present work, we comparatively evaluated the effect of different cAMP-inducing agents on cell

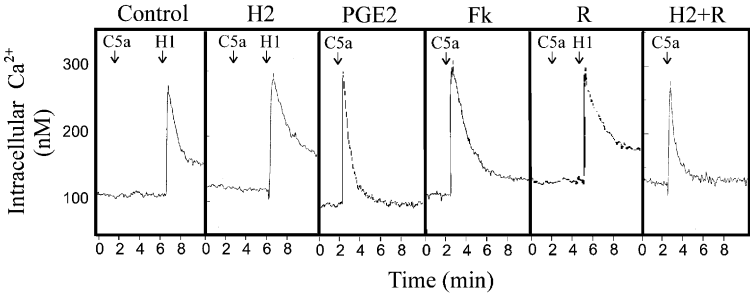

Fig. 7. Effect of rhC5a on $[Ca^{2+}]_i$ in amthamine-, PGE2-, or forskolin-treated cells. U-937 cells were cultured for 48 h in the presence of 10 μM amthamine (H2), 1 μM PGE2, 75 μM forskolin (Fk), and 10 μM rolipram (R) or 10 μM amthamine + 10 μM rolipram (H2 + R). Control represents non-treated cells. $[Ca^{2+}]_i$ was determined as described under Materials and methods. Arrows indicate the addition of 50 nM rhC5a or 10 μM H1 agonist. Similar results were obtained in at least three independent experiments.

differentiation, without modifying the system genetically. We used two agents that act via G-protein coupled receptors, amthamine (H2 receptor agonist), and PGE2. The other mediator, forskolin, directly activates adenylyl cyclase. PGE_2 binds to four different cell surface receptor subtypes, EP1, -2, -3, and -4, that are coupled to G proteins and mediate PGE2 biological responses. Three of these receptors modulate cAMP intracellular levels. Activation of the EP2 and -4 receptor subtypes results in intracellular cAMP elevation, whereas activation of the EP3 receptor induces cAMP reduction. The fourth receptor, EP1, is involved in the regulation of intracellular calcium [20]. In U-937 cells, EP4 receptors seem to play a preponderant role for PGE2-mediated cAMP formation [21].

In concentration–response curves, all three agents elicited similar maximal cAMP responses. These experiments were performed in the presence of IBMX and showed the maximal cAMP accumulation in the absence of phosphodiesterase activity. However, as homologous and heterologous desensitization may occur, further studies were performed. The desensitization exhibited by the three agents showed distinctive characteristics. Histamine H2 receptor desensitized homologously by a GRK dependent mechanism, without affecting the response of other GPCRs up to 3 h of stimulus [12]. However, the PGE2 cAMP-response evoked a mixed desensitization process: a first short phase involving only the PGE2 response, and a second, PKA/PKC dependent phase, affecting the response of other GPCRs like the H2 receptor. In turn, cAMP induced by forskolin did not affect the ability of adenylyl cyclase to generate cAMP, but it interfered with other GPCRs in an H7 sensitive manner.

Previous studies have shown that other cAMP-regulating mechanisms operate in U-937 cells. Agents that elevate cAMP content increase the activity of cyclic nucleotide PDE4, including H2 agonists, PGE2, and forskolin, constituting a feedback regulation [12,22]. Regulation of PDE4 appears to play critical roles in different processes as the differentiation in granulosa cells of the ovarian follicle and the induction of myogenesis [23,24].

Present findings support the existence of various mechanisms that may be responsible for the different time-courses of cAMP levels induced by the different agents. Thus, after 5 min of stimulation, cAMP levels induced by the three agents were similar, but only those induced by amthamine (H2 agonist) returned to basal levels, whereas forskolin- and PGE2-induced cAMP were stabilized at higher levels, reinforcing the importance of such mechanism in the regulation of cAMP levels.

Finally, we analyzed the effect of amthamine, PGE2, and forskolin on cell proliferation and differentiation, concluding that the amplitude and the kinetic of cAMP were responsible for the evoked response. In the present study, the pattern of c-Fos and c-Myc expression, and the induction of C5a receptor were evaluated as markers of cell maturation. The regulation of the proto-oncogenes c-Fos and c-Myc is an early event in the differentiation pathway whereas the expression of C5a receptor in cell membrane denotes a characteristic terminal maturation. Monocytic maturation in U-937 cells was associated with the sustained induction of c-fos gene expression and down-regulation of c-myc, by several differentiation agents including PMA [25] and dbcAMP [5]. The chemoattractant C5a receptor is up-regulated when cells are stimulated with differentiation agents [26]. Agents that maintained a residual response of cAMP induced inhibition of cell proliferation concomitantly with cell maturation. In the same way, the combined treatment of cells with amthamine and rolipram (PDE4 inhibitor) produced a similar effect.

Overall, these results establish a correlation between the time-course of cAMP signaling induced by different agents and the evoked cellular responses like proliferation and differentiation.

Although not addressed here, the spatial dimension of the cAMP signaling may play an additional role in the differential regulation of transcription. Several studies report cAMP signaling compartmentalization where cAMP increases in discrete regions of the cells, and little diffusion from these microdomains is detected [27,28]. Moreover, several findings indicate that PDEs play an important role in preventing diffusion of cAMP and defining the signaling in a membrane cAMP microdomain [29].

Compartmentalization in addition to response intensity and duration provides more complexity to the system to evoke a specific response following stimulation with agents that share common second messengers in the signal transduction pathway.

This complexity is essential if we considered that cells continually receive multiple stimuli from the environment and have to interpret them to give rise to a specific response. In the present work, we show that different agents, that modulate the same transduction pathway, induce cell differentiation in U-937 cells, depending on the amplitude and duration of the cAMP response. The present study provides important keys that may be used to design differentiation treatment protocols for leukemia therapies.

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