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cGMP-induced differentiation of the promyelocytic cell line HL-60

(human leukemic cells/nitroprusside/$NaNO_2$/8-bromoguanosine 3′,5′-cyclic monophosphate)

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ABSTRACT **cGMP is a second messenger that mediates numerous metabolic events; in the present work a role in myeloid cell differentiation was demonstrated. Nitroprusside and $NaNO_2$, which activate cytosolic guanylate cyclase and increase the intracellular cGMP concentration, induced granulocytic differentiation of the human promyelocytic cell line HL-60; differentiation was measured by acquisition of the OKM1 antigen, morphological changes, and nitroblue tetrazolium reduction. When theophylline, a phosphodiesterase inhibitor, which by itself induced modest differentiation, was added to nitroprusside or $NaNO_2$, differentiation increased in an additive fashion. The degree of differentiation correlated with the increase in the intracellular cGMP concentration. 8-Bromoguanosine 3′,5′-cyclic monophosphate, a membrane-permeable cGMP analogue, also induced differentiation of HL-60 cells but was much more effective in the presence of theophylline, with the two agents interacting synergistically. The effect of theophylline in these studies could not be attributed to increasing the intracellular cAMP concentration. Dimethyl sulfoxide, an established inducer of differentiation of HL-60 cells, markedly enhanced the differentiation induced by nitroprusside and $NaNO_2$.**

The study of hematopoietic cell differentiation was aided by the establishment of cell lines, derived from leukemia patients, that can be induced to differentiate *in vitro* (1). Probably the best studied of these systems is the promyelocytic cell line HL-60, which can be induced to differentiate along either the granulocytic or the monocytic lineage, depending on the inducing agent (1, 2). The two most widely used inducers of granulocytic differentiation are dimethyl sulfoxide (DMSO) and retinoic acid (1, 2); agents that increase the intracellular cAMP concentration also induce granulocytic differentiation of HL-60 cells, albeit less effectively (3–5).

The importance of cGMP and its varied intracellular role has been underlined by the following findings: (*i*) a cGMP-gated conductance channel conveys light transduction in the eye; (*ii*) the major atrial natriuretic factor receptor is a membrane-bound guanylate cyclase; (*iii*) nitroglycerin, nitroprusside, and other nitric oxide-generating compounds induce vasodilatation by activating cytosolic guanylate cyclase; and (*iv*) *Escherichia coli* heat-stable toxin induces diarrhea by activating membrane-bound guanylate cyclase (6–9). We found that nitroprusside and $NaNO_2$, which increased the cGMP concentration in HL-60 cells, and 8-bromoguanosine 3′,5′-cyclic monophosphate (8-Br-cGMP), a membrane-permeable cGMP derivative, induced HL-60 cells to differentiate along the granulocytic pathway; the degree of differentiation was markedly enhanced by theophylline, a phosphodiesterase inhibitor, and correlated with the increase in the intracellular cGMP concentration.

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MATERIALS AND METHODS

Materials. Sodium nitroprusside (sodium nitroferricyanide), $NaNO_2$, 3-isobutyl-1-methylxanthine, and theophylline (1,3-dimethylxanthine) were from Sigma; the nitroprusside and $NaNO_2$ were frozen as 100× stock solutions at −70°C under N_2 and shielded from light. 8-Br-cGMP was from Boehringer Mannheim; M & B 22,948 was a generous gift of May & Baker (Dogenham, U. K.). The OKM1 mouse monoclonal antibody was from Ortho Diagnostics; the fluorescein-conjugated $F(ab')_2$ fragment of goat anti-mouse IgG was from Organon Teknika–Cappel. cGMP and cAMP assay kits were from Amersham.

Origin of Cell Line and Culture Conditions. The human promyelocytic cell line HL-60 was obtained from the American Type Culture Collection. The cells have a doubling time of ≈24 hr when diluted twice a week to a density of 10^5 cells per ml in RPMI 1640 medium supplemented with 10% (vol/vol) fetal bovine serum (10); cultures were reinitiated from frozen stocks every 6 months.

Measurement of Differentiation of HL-60 Cells. Undifferentiated HL-60 cells grow predominantly as promyelocytes with a substantial number of blast cells (2–4). Granulocytic differentiation requires 5–7 days and is characterized by changes in membrane antigens, morphology, and biochemical functions (2–4, 11). We assessed changes in each of these differentiation parameters by using the OKM1 antigen, Wright–Giemsa staining, and nitroblue tetrazolium (NBT) reduction, respectively. The OKM1 antigen is a membrane glycoprotein present on mature myelomonocytic cells and is expressed by <10% of undifferentiated HL-60 cells (11). Wright–Giemsa staining of cells has been the traditional method to monitor differentiation of HL-60 cells (2). After granulocytic maturation, HL-60 cells, like normal granulocytes, respond to membrane perturbation by activating a membrane-bound NADPH oxidase, which generates O_2^-; this can be assessed by counting the number of cells that reduce NBT to insoluble blue–black formazan (2–4).

Cultures were initiated at 5×10^4 cells per ml in Iscove's modified Dulbecco's medium supplemented with extensively dialyzed, heat-inactivated (30 min at 56°C) 10% (vol/vol) horse serum and cultured for 6 days at 37°C in 95% air/5% CO_2. Only cultures with >85% viability as assessed by trypan blue exclusion were used for further analyses. Cells were incubated with the OKM1 antibody and fluorescein-conjugated goat antimouse IgG as described (11); they were then incubated with propidium iodide (0.5 μg/ml) and subjected to flow cytometry on an Ortho Cytofluorograph IIS. The red-fluorescing dead cells were excluded from analysis and 5×10^3 live cells were analyzed for 90° green fluorescence; cells having a relative fluorescence greater than that of 95% of the control cells were counted as positive. Wright–

Abbreviations: 8-Br-cGMP, 8-bromoguanosine 3′,5′-cyclic monophosphate; DMSO, dimethyl sulfoxide; NBT, nitroblue tetrazolium.

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Giemsa staining was performed as described (10). The criteria for morphologic stage were as follows: (*i*) blasts—large round centrally placed nuclei with finely dispersed chromatin, two to four nucleoli, and scant deeply basophilic cytoplasm; (*ii*) promyelocytes—similar to blasts except prominent azurophilic granules in the cytoplasm; (*iii*) myelocytes—smaller more eccentrically positioned nuclei than in blasts or promyelocytes with chromatin clumping and less distinct nucleoli, no azurophilic granules, and a pink hue to the cytoplasm; and (*iv*) metamyelocytes—nuclear indentation with coarse chromatin clumping and uniformly pink cytoplasm with neutrophilic granules. Assessment of NBT reduction was performed as described with cells containing more than three formazan particles counted as positive (10).

Cells were examined for possible monocytic differentiation by staining for nonspecific esterase activity with 1-naphthyl butyrate and pararosaniline; cells induced by phorbol 12-myristate 13-acetate served as positive controls (1, 12).

Measurement of cGMP and cAMP Concentrations. The intracellular concentrations of cGMP and cAMP were measured by a radioimmunoassay using ^{125}I-labeled cGMP and ^{125}I-labeled cAMP, respectively; the assays are sensitive to 2 fmol. Approximately 0.5×10^5 cells were incubated for 1 hr in Iscove's medium. The compound to be tested was added and after various time periods the cells were extracted *in situ* by adding ice-cold perchloric acid to a final concentration of 0.5 M; the extracts were neutralized with 2.2 M $KHCO_3$. Extracting the cells *in situ* was necessary to avoid cyclic nucleotide catabolism during centrifugation. In preliminary experiments we found that <10% of the cellular cGMP was excreted into the medium—even under conditions of stimulated synthesis; these results are similar to those reported in other cell systems (13).

RESULTS

Effect of Nitroprusside, $NaNO_2$, and 8-Br-cGMP on HL-60 Cells. For maximal differentiation of HL-60 cells along the granulocytic pathway, the inducing agent must be present continuously for 72 hr (14). Since nitroprusside and $NaNO_2$ are unstable in solution and 8-Br-cGMP can be catabolized by intracellular enzymes (8, 15), relatively high initial concentrations of these compounds were necessary to induce differentiation of HL-60 cells; when cells were exposed to these agents for <72 hr, there was a corresponding decrease in the degree of differentiation.

When nitroprusside was added to HL-60 cells at an initial concentration of 1 mM, granulocytic differentiation resulted as identified by the following observations: (*i*) the percentage of cells bearing the OKM1 antigen increased >7-fold, from 4.3 ± 0.61% to 31.7 ± 5.5% (Table 1 and Fig. 1 *A* and *C*); (*ii*) the predominant cell type observed was at the myelocyte stage with occasional metamyelocytes also seen (Table 1); and (*iii*) the percentage of cells capable of reducing NBT increased >9-fold, from 1.4 ± 0.3% to 12.7 ± 2.2% (Table 1).

If nitroprusside induced differentiation by activating the cytosolic guanylate cyclase and increasing the intracellular cGMP concentration, then a phosphodiesterase inhibitor should potentiate its effect. Neither isobutylmethylxanthine, a potent phosphodiesterase inhibitor, nor M & B 22,948, a relatively selective cGMP phosphodiesterase inhibitor (16), could be used because when HL-60 cells were exposed to concentrations of 100 μM and 10 μM of these drugs, respectively, <80% of the cells were viable as measured by trypan blue exclusion. Instead, theophylline, a relatively weak but nontoxic phosphodiesterase inhibitor, was chosen. By itself, 1 mM theophylline induced a modest degree of differentiation (Table 1 and Fig. 1*B*); when it was combined with 1 mM nitroprusside, an approximate additive effect was observed in the percent of OKM1-positive cells (Table 1 and Fig. 1 *B–D*) and of NBT-positive cells (Table 1) as well as in morphological maturation (Table 1). It should be noted that differentiation beyond the metamyelocyte stage, even with 1.5% (vol/vol) DMSO or 1 μM retinoic acid, was unusual for the HL-60 cells used in these studies.

The dose–response curve for the induction of differentiation of HL-60 cells by nitroprusside was relatively linear between 100 μM and 1 mM nitroprusside (Fig. 2); these data are in the presence of 1 mM theophylline but a similar-shaped curve was obtained in its absence. Concentrations >1 mM nitroprusside could not be used because cell viability decreased to <85%.

$NaNO_2$, like nitroprusside, releases nitric oxide in solution but in most systems studied it does not increase the intracellular cGMP concentration as effectively as nitroprusside (13). At 4 mM, $NaNO_2$ induced less differentiation of HL-60 cells than 1 mM nitroprusside, increasing the percentage of cells that expressed the OKM1 antigen, and reduced NBT by ≈5-fold; morphological maturation was also less developed in the $NaNO_2$-treated cells than in the nitroprusside-treated cells (Table 1). When 4 mM $NaNO_2$ was combined with 1 mM theophylline an approximately additive effect on differentiation was again observed (Table 1).

The cGMP analogue 8-Br-cGMP is more lipophilic than the parent compound and crosses cell membranes (15). At concentrations as high as 3 mM, 8-Br-cGMP was a poor inducer of HL-60 differentiation but, when combined with 1 mM theophylline, marked synergism occurred resulting in pronounced induction of differentiation of HL-60 cells (Table 1). This synergism was presumably because the 8-Br-cGMP was rapidly catabolized to an ineffectual concentration in the absence of theophylline. Similar results were obtained with 1–3 mM N^6,$O^{2'}$-dibutyrylguanosine 3',5'-cyclic monophosphate (data not shown) but the results are more difficult to interpret because butyrate alone induces granulocytic differentiation of HL-60 cells (2).

Table 1. Induction of differentiation of HL-60 cells by nitroprusside, $NaNO_2$, or 8-Br-cGMP in the absence or presence of theophylline

Addition(s) to culture	OKM1-positive cells, %	Wright–Giemsa stain, %				NBT-positive cells, %
		Blasts	Promyelocytes	Myelocytes	Metamyelocytes	
None	4.3 ± 0.61	18	78 ± 5	4 ± 2	<1	1.4 ± 0.3
Theophylline (1 mM)	13.9 ± 2.4	<1	85 ± 9	15 ± 3	<1	13.7 ± 2.5
Nitroprusside (1 mM)	31.7 ± 5.5	<1	43 ± 5	52 ± 7	5 ± 3	12.7 ± 2.2
Nitroprusside (1 mM) + theophylline (1 mM)	40.1 ± 6.3	<1	10 ± 5	76 ± 10	14 ± 5	25.3 ± 2.6
$NaNO_2$ (4 mM)	19.4 ± 2.5	<1	59 ± 6	39 ± 5	2 ± 1	10.1 ± 1.9
$NaNO_2$ (4 mM) + theophylline (1 mM)	29.6 ± 3.7	<1	33 ± 5	58 ± 6	9 ± 2	22.6 ± 2.1
8-Br-cGMP (3 mM)	6.9 ± 1.8	<1	84 ± 9	16 ± 4	<1	9.3 ± 4.4
8-Br-cGMP (3 mM) + theophylline (1 mM)	28.0 ± 5.2	<1	5 ± 3	78 ± 8	17 ± 5	36.3 ± 5.9

Cells cultured for 6 days were assessed for their degree of differentiation. Antibody OKM1 measures a membrane antigen; Wright–Giemsa stain measures the cell's morphological development; NBT reduction measures the cell's ability to generate O_2^- in response to membrane perturbation. The data are expressed as percentages (means ± SD) of at least four independent experiments performed in duplicate.

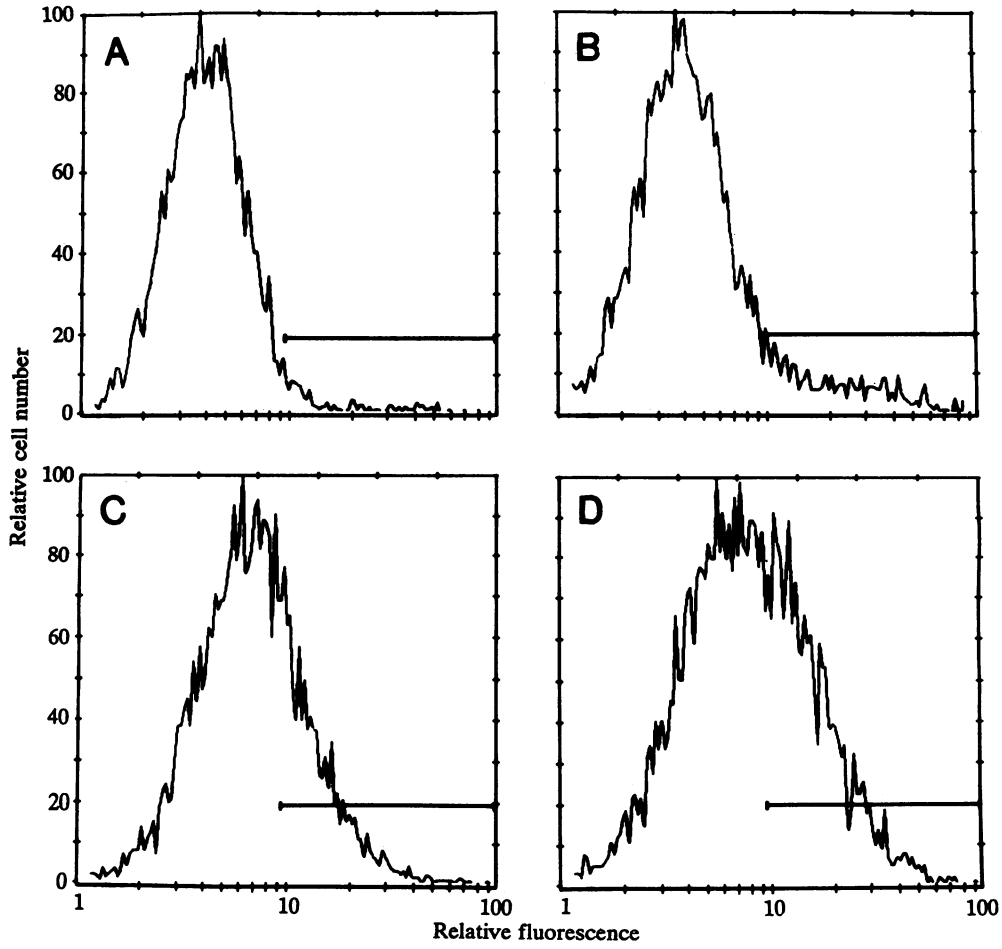

FIG. 1. Flow cytometry analysis of OKM1 antigen present on HL-60 cells treated with theophylline, nitroprusside, or both. Cells were cultured for 6 days under the indicated condition and prepared for analysis. The percentage of cells in the region delineated by the marker is 4.8% in the control state (*A*), 12.5% in the presence of 1 mM theophylline (*B*), 28.9% in the presence of 1 mM nitroprusside (*C*), and 40.7% in the presence of both 1 mM theophylline and 1 mM nitroprusside (*D*).

As described for other inducing agents (2–4, 14), nitroprusside, $NaNO_2$, and 8-Br-cGMP induced terminal differentiation with a loss of cell proliferation and the cells became committed to differentiation because removing the agent at 72 hr did not prevent differentiation. The differentiation induced by nitroprusside, $NaNO_2$, and 8-Br-cGMP was granulocytic: monocytic morphology was not observed on the Wright–Giemsa-stained slides, the cells were not surface adherent, and <10% of the cells exhibited nonspecific esterase activity.

Effect of Nitroprusside and $NaNO_2$ on the Intracellular cGMP Concentration in HL-60 Cells. Within 1 min of adding 1 mM nitroprusside to undifferentiated HL-60 cells, the intracellular cGMP concentration increased significantly ($P < 0.05$; t test) reaching a plateau value by 20 min and remaining elevated for at least 72 hr. The addition of 1 mM theophylline to undifferentiated cells caused a small but consistent elevation in the intracellular cGMP concentration; when it was added to cells treated with nitroprusside, the cGMP concentration increased to approximately twice that observed in the control cells (Table 2).

For the first 8 hr after addition, $NaNO_2$ increased the cGMP concentration by a similar amount as nitroprusside (Table 2), but unlike nitroprusside, $NaNO_2$ did not maintain the cGMP concentration at a consistently elevated level much beyond 12 hr (in the presence or absence of theophylline). The cGMP concentration could not be measured in the experiments with the 8-Br-cGMP because the anti-cGMP antibody cross-reacted with the 8-Br-cGMP.

Effect of Theophylline on the Intracellular cAMP Concentration in HL-60 Cells. As a nonspecific phosphodiesterase inhibitor, theophylline would be expected to increase the intracellular cAMP concentration. Since an increased intracellular cAMP concentration of severalfold induces HL-60 cells to differentiate, it was possible that the potentiating effect of theophylline on the differentiation induced by nitroprusside, $NaNO_2$, and 8-Br-cGMP was secondary to increasing the intracellular cAMP concentration. In the absence of any other agent, 1 mM theophylline increased the intracellular cAMP concentration by 70% (Table 2). This is somewhat greater than the 15–20% increase in the intracellular cAMP concentration found by Chaplinski and Niedel (17) when they treated HL-60 cells with 500 μM theophylline. The intracellular cAMP concentration was not changed by 1 mM nitroprusside, 4 mM $NaNO_2$, or 3 mM 8-Br-cGMP (Table 2). Interestingly, the addition of theophylline to nitroprusside or $NaNO_2$ increased the cAMP concentration by $\approx$30% and did not increase the cAMP concentration at all when added to 8-Br-cGMP (Table 2); this lesser effect of theophylline on the cAMP concentration in the presence of nitroprusside, $NaNO_2$, or 8-Br-cGMP may be secondary to activation of the cGMP-stimulated phosphodiesterase by these latter compounds (16).

Effect of DMSO on Nitroprusside- or $NaNO_2$-Induced Differentiation of HL-60 Cells and on cGMP Content of HL-60 Cells. DMSO is an effective inducer of differentiation of HL-60 cells with maximal differentiation occurring at concentrations between 1.25% and 1.5% (2, 14). When either

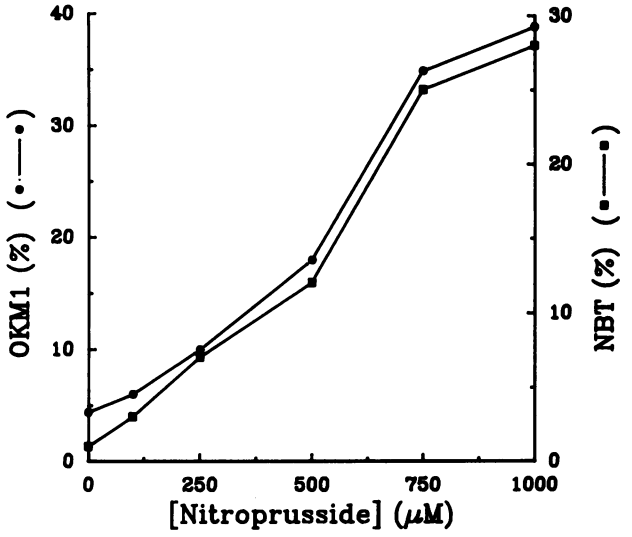

FIG. 2. Acquisition of the OKM1 antigen and reduction of NBT in HL-60 cells treated with increasing concentrations of nitroprusside. Cells were cultured for 6 days in the presence of 1 mM theophylline and the indicated concentration of nitroprusside and assessed for their degree of differentiation. The data are expressed as percentages and are the means of two independent experiments performed in duplicate.

nitroprusside or $NaNO_2$ were added to these concentrations of DMSO, no further increase in differentiation of HL-60 cells was observed (apparently the cells were already maximally differentiated). However, when DMSO at concentrations between 0.5% and 1% was combined with either nitroprusside or $NaNO_2$ marked augmentation of differentiation of HL-60 cells occurred. Thus, in the presence of 0.75% DMSO, 19.3 ± 1.7% of the cells expressed the OKM1 antigen; when this concentration of DMSO was added to cells treated with 1 mM nitroprusside or 4 mM $NaNO_2$, the percentage of cells that expressed the OKM1 antigen increased from 32.3 ± 4.9% to 72.6 ± 8.2% and from 19.1 ± 2.3% to 44.3 ± 3.6%, respectively (means ± SD of four experiments performed in duplicate).

At concentrations up to 1%, DMSO had no effect on the intracellular cGMP concentration; 1 mM nitroprusside increased the intracellular cGMP concentration in the DMSO-treated cells by a similar amount as in the cultures treated with nitroprusside alone (Table 2).

DISCUSSION

In the present work I found that nitroprusside and $NaNO_2$ induced granulocytic differentiation of HL-60 cells. The differentiation appeared secondary to an increase in the intracellular cGMP concentration because (*i*) the degree of differentiation correlated with the quantitative increase in the cGMP concentration, (*ii*) nitroprusside stimulated a more sustained increase in the cGMP concentration and was a more effective inducer of differentiation than $NaNO_2$, and (*iii*) the combination of theophylline with nitroprusside or $NaNO_2$ increased differentiation in an additive fashion. The effect of theophylline in these studies could not be attributed to an increase in the intracellular cAMP concentration because in the presence of nitroprusside, $NaNO_2$, or 8-Br-cGMP, theophylline caused only a marginal increase in the cAMP concentration and, as discussed below, a 2- to 4-fold increase in the cAMP concentration is necessary for the induction of differentiation of HL-60 cells. Because 8-Br-cGMP mimics many of the effects of cGMP (18), the induction of differentiation of HL-60 cells by 8-Br-cGMP provides additional support for cGMP as a mediator of granulocytic differentiation of HL-60 cells. Whether the 8-Br-cGMP was converted intracellularly to cGMP could not be determined from these studies because the antibody in the radioimmunoassay reacted with both cyclic nucleotides. It is unlikely that 8-Br-cGMP was acting by conversion to an 8-bromoguanylate because hydrolysis of the phosphodiester bond would be the required first step and theophylline did not prevent the action of 8-Br-cGMP but actually caused a synergistic increase in differentiation.

The maximum increase in the cGMP concentration occurred with the combination of nitroprusside and theophylline and was ≈2-fold greater than that found in control cells (Table 2). This is in contrast to larger increases in the cGMP concentration observed in mouse hepatic slices treated with nitroprusside or in several cultured cell systems treated with atrial natriuretic peptide (13, 19). A major difference between these investigations and the present work is that in these former studies phosphodiesterase activity was inhibited by either 0.5 mM M & B 22,948 or 0.5 mM isobutylmethylxanthine; both of these agents are considerably more potent inhibitors of the three known phosphodiesterases than is theophylline (16). That a sustained 2-fold increase in the cGMP concentration was sufficient to induce differentiation of HL-60 cells is in agreement with the data reported for cAMP-induced differentiation: the combination of prostaglandin E_2 and theophylline, which induces differentiation of HL-60 cells, increases the cAMP concentration by 2- to 4-fold 24 and 48 hr after addition (17). It is interesting to note that in HL-60 cells the cGMP concentration was found to be slightly greater than the cAMP concentration; this is unusual and suggests that in myeloid cells cGMP plays at least as important a role as cAMP (20).

The mechanism of induction of mammalian cell differentiation by DMSO is unknown (2, 21). DMSO enhanced

Table 2. Intracellular cGMP and cAMP concentrations in HL-60 cells treated with theophylline, nitroprusside, $NaNO_2$, 8-Br-cGMP, DMSO, or combinations of these agents

Addition(s) to culture	cGMP, pmol per 10^6 cells	cAMP, pmol per 10^6 cells
None	11.8 ± 1.5	7.2 ± 1.2
Theophylline (1 mM)	13.5 ± 1.8	12.3 ± 1.6
Nitroprusside (1 mM)	15.5 ± 1.9	8.5 ± 1.3
Nitroprusside (1 mM) + theophylline (1 mM)	21.8 ± 2.3	11.1 ± 1.9
$NaNO_2$ (4 mM)	15.2 ± 1.4	7.9 ± 1.1
$NaNO_2$ (4 mM) + theophylline (1 mM)	20.7 ± 1.9	10.4 ± 1.3
8-Br-cGMP (3 mM)	ND	7.1 ± 1.2
8-Br-cGMP (3 mM) + theophylline (1 mM)	ND	7.2 ± 1.3
DMSO (1%)	12.0 ± 1.6	ND
DMSO (1%) + nitroprusside (1 mM)	15.1 ± 1.9	ND

Cells were incubated for 1 hr prior to adding the indicated compound(s); 4 hr later they were extracted and the cGMP and cAMP concentrations were measured by a radioimmunoassay. The data (means ± SD) are from at least four experiments performed in duplicate. ND, not determined.

nitroprusside- and $NaNO_2$-induced differentiation of HL-60 cells with an apparent synergism between the DMSO and the nitroprusside or $NaNO_2$. The synergism may be because I have found (unpublished results) that DMSO significantly increases cGMP-dependent protein kinase activity in HL-60 cells.

There is a precedent for cGMP to modulate cell differentiation since it plays a key role in differentiation of the slime mold *Dictyostelium discoideum* (22). A role for cGMP in myeloid cell differentiation was suggested when it was reported that 10–100 nM cGMP increased the formation of myeloid colony-forming units in cultures of human and mouse bone marrow progenitor cells; however, no clear dose–response relationships were established with higher cGMP concentrations inhibiting differentiation (23, 24). The present work provides evidence that cGMP is important to mammalian cell differentiation; future work should determine whether cGMP mediates its effect through the cGMP-dependent protein kinase, changes in membrane conductance, or some other mechanism.

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1. Koeffler, H. P. (1986) *Semin. Hematol.* **23**, 223–236.
2. Collins, S. J., Ruscetti, F. W., Gallagher, R. E. & Gallo, R. C. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2458–2462.
3. Chaplinski, T. J. & Niedel, J. E. (1982) *J. Clin. Invest.* **70**, 953–964.
4. Chaplinski, T. J., Sloan, G. J. & Niedel, J. E. (1985) *Leukemia Res.* **9**, 897–903.
5. Fontana, J., Munoz, M. & Durham, J. (1985) *Leukemia Res.* **9**, 1127–1132.
6. Stryer, L. (1986) *Annu. Rev. Neurosci.* **9**, 87–119.
7. Kuno, T., Andresen, J. W., Kamisaki, Y., Waldman, S. A., Chang, L. Y., Saheki, S., Leitman, D. C., Nakane, M. & Murad, F. (1986) *J. Biol. Chem.* **261**, 5817–5823.
8. Katsuki, S., Arnold, W. P. & Murad, F. (1977) *J. Cyclic Nucleotide Res.* **3**, 239–247.
9. Eldeib, M. M. R., Parker, C. D., Veum, T. L., Zinn, G. M. & White, A. A. (1986) *Arch. Biochem. Biophys.* **245**, 51–65.
10. Pilz, R. B., Van den Berghe, G. & Boss, G. R. (1987) *J. Clin. Invest.* **79**, 1006–1009.
11. Ferrero, D., Pessano, S., Pagliardi, G. L. & Rovera, G. (1983) *Blood* **61**, 171–179.
12. Tucker, S. B., Pierre, R. V. & Jordon, R. E. (1977) *J. Immunol. Methods* **14**, 267–269.
13. Wood, K. S. & Ignarro, L. J. (1987) *J. Biol. Chem.* **262**, 5020–5027.
14. Fibach, E., Peled, T. & Rachmilewitz, E. A. (1982) *J. Cell. Physiol.* **113**, 152–158.
15. Ignarro, L. J., Lippton, H., Edwards, J. C., Baricos, W. H., Hyman, A. L., Kadowitz, P. J. & Gruetter, C. A. (1981) *J. Pharmacol. Exp. Ther.* **218**, 739–749.
16. Weishaar, R. E., Cain, M. H. & Bristol, J. A. (1985) *J. Med. Chem.* **28**, 537–545.
17. Chaplinski, T. J. & Niedel, J. E. (1986) *J. Leukocyte Biol.* **39**, 323–331.
18. Miller, J. P., Boswell, K. H., Muneyama, K., Simon, L. N., Robins, R. K. & Shuman, D. A. (1973) *Biochemistry* **12**, 5310–5319.
19. Leitman, D. C., Andresen, J. W., Catalano, R. M., Waldman, S. A., Tuan, J. J. & Murad, F. (1988) *J. Biol. Chem.* **263**, 3720–3728.
20. Pastan, I. H., Johnson, G. S. & Anderson, W. B. (1975) *Annu. Rev. Biochem.* **44**, 492–522.
21. Friend, C. & Freedman, H. A. (1978) *Biochem. Pharmacol.* **27**, 1309–1313.
22. Mato, J. M., Krens, F. A., van Haastert, P. J. M. & Konijn, T. M. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2348–2351.
23. Taetle, R. & Koessler, A. (1980) *Cancer Res.* **40**, 1223–1229.
24. Oshita, A. K., Rothstein, G. & Lonngi, G. (1977) *Blood* **49**, 585–591.