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Reduced DNA Synthesis and Cell Viability in Small Cell Lung Carcinoma by Treatment with Cyclic AMP Phosphodiesterase Inhibitors

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ABSTRACT. This study investigated the effects of the adenosine 3′,5′-cyclic monophosphate (cAMP) phosphodiesterase inhibitors caffeine, theophylline, and 3-isobutyl-1-methyl-xanthine (IBMX) on the proliferation and viability of the small cell lung carcinoma (SCLC) cell lines NCI-H345, NCI-H128, and SCC-9. These effects were correlated with the ability of the drugs to induce intracellular Ca^{2+} mobilization. Treatment of NCI-H345 cells with caffeine resulted in rapid mobilization of Ca^{2+}, as indicated by Fura-2 fluorescence. Incubation of NCI-H345 cells with 6.25 mM caffeine resulted in a 62% inhibition of [^{3}H]thymidine uptake after 2 hr, indicating reduced DNA synthesis. Incubation with 25 mM caffeine resulted in almost total inhibition of [^{3}H]thymidine uptake after 2 hr. Similar effects on [^{3}H]thymidine uptake were seen upon treatment of NCI-H128 and SCC-9 cells with caffeine; however, these cells did not exhibit caffeine-induced Ca^{2+} mobilization. Inhibition of DNA synthesis (66–93%) also occurred upon incubation of all cell lines with theophylline and IBMX, which did not mobilize Ca^{2+}. Treatment of NCI-H345, NCI-H128, and SCC-9 cells with caffeine, theophylline, or IBMX markedly reduced cell viability. Levels of cAMP increased in the cells following treatment with caffeine, theophylline, or IBMX, reflecting the ability of these drugs to inhibit cAMP phosphodiesterase. These results suggest that the decrease in DNA synthesis and the subsequent cell death induced by these drugs are due to reduced cAMP phosphodiesterase activity, rather than to changes in intracellular Ca^{2+}. These findings indicate that drugs that alter cAMP signaling pathways are potentially valuable agents to inhibit SCLC survival. BIOCHEM PHARMACOL **56**;9:1229–1236, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. small cell lung carcinoma; cyclic AMP; methylxanthines; phosphodiesterases; viability; cell death

Building upon the explosion of information in cell and molecular biology, novel therapeutic strategies for the treatment of many types of cancers have developed at a rapid rate. However, breakthroughs in the treatment of patients with lung cancer have not kept pace. Lung cancer, currently the leading cause of cancer mortality in the United States, results in over 140,000 deaths per year [1]. SCLC† accounts for 20–25% of all bronchogenic malignancies, and follows a most aggressive clinical course. Metastases develop even in the early stages of the disease, so that surgery has a limited role [2–4]. SCLC displays an initial sensitivity to radio- and chemotherapy; however, resistance is acquired rapidly, relapses are common, and current prognosis is poor [5]. Less than 5% of patients with SCLC currently survive 5 years past the initial diagnosis [6, 7]. The inadequacy of current treatment protocols reinforces the need for new therapeutic approaches for this disease.

A clearer understanding of the intricacies of signaling pathways that govern proliferation, migration, and invasion, and, conversely, ones that induce necrosis or apoptosis will lead to the development of new treatment modalities. cAMP is a common signal transduction element used by a wide range of cells. Changes in intracellular cAMP levels have been implicated as signals regulating synaptic plasticity and neuronal differentiation [8, 9], cell proliferation [10], and differentiation, hormonal responses, metabolic and secretory processes, muscle contraction, tumorigenesis, and cell death [reviewed in Ref. 11]. Intracellular cAMP levels are a result of a balance of actions of numerous forms of adenylate cyclases and phosphodiesterases [11]. cAMP levels can be manipulated pharmacologically using methylxanthines such as caffeine and theophylline, which inhibit cAMP phosphodiesterases.

In this study, we investigated the effects of cAMP phosphodiesterase inhibitors on DNA synthesis and survival by SCLC cells. We report that treatment of NCI-H345 SCLC cells with caffeine resulted in a profound reduction of DNA synthesis and rapid cell death. Caffeine

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† *Abbreviations:* cAMP, adenosine 3′,5′-cyclic monophosphate; IBMX, 3-isobutyl-1-methyl-xanthine; and SCLC, small cell lung carcinoma.

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treatment is known to have other marked effects on cells, notably the release of Ca^{2+} stores regulated by ryanodine receptors [12]. Treatment of NCI-H345 SCLC cells with caffeine resulted in mobilization of Ca^{2+}, consistent with the possibility that SCLC cells have Ca^{2+} stores regulated by ryanodine receptors. Many investigators have noted that agents that both increase cAMP and cause elevations in intracellular Ca^{2+} levels trigger the death of numerous cell types [reviewed in Ref. 13]. We found that caffeine also inhibited DNA synthesis and reduced the viability of SCC-9 and NCI-H128 SCLC cells; interestingly, these two cell lines did not exhibit caffeine-induced Ca^{2+} mobilization. These results suggest that the caffeine-mediated inhibition of DNA synthesis and reduction of cell viability may be due to inhibition of cAMP phosphodiesterase and not to Ca^{2+} mobilization. This is supported by findings that other cAMP phosphodiesterase inhibitors (theophylline and IBMX), which do not induce Ca^{2+} mobilization, also inhibit DNA synthesis in all the SCLC cell lines studied. Treatment with caffeine, IBMX, or theophylline also reduced cell viability in NCI-H345, NCI-H128, and SCC-9 cell lines. These results suggest that alteration of cAMP signaling pathways has rapid and profound effects on SCLC cell viability. These findings may be exploitable in the search for novel therapeutic protocols to combat lung cancer.

MATERIALS AND METHODS

Reagents

The fluorescent dye Fura-2 AM and pluronic were purchased from Molecular Probes. [^{3}H]Thymidine (sp. act. 82 Ci/mmol) was purchased from Amersham. All other reagents were obtained from the Sigma Chemical Co. unless otherwise noted in the text. Stock solutions of caffeine (100 mM) and theophylline (100 mM) were made by dissolving the drug in RPMI-1640 (Cellgro Mediatech) at 37°. Stock solutions of IBMX (8 mM) were made by dissolving the drug in boiling PBS, followed by cooling to 25°. All stock solutions were made immediately before use, diluted with complete medium, and used at 37°.

Cell Culture

The SCLC cell line SCC-9, established from a biopsy specimen of an SCLC skin metastasis, has been characterized extensively [14–17]. The SCLC cell lines NCI-H345 and NCI-H128 were obtained from the American Type Culture Collection. The cells were cultured in complete medium consisting of RPMI-1640 medium containing 10% calf bovine serum (Hyclone Laboratories), glutamine (0.3 mg/mL), penicillin (20 U/mL) and streptomycin sulfate (20 μg/mL). Cells were maintained at 37° in a humidified atmosphere of 5% CO_2/95% air at densities that promoted exponential growth.

[^{3}H]Thymidine Uptake Studies

Uptake of [^{3}H]thymidine by SCLC cells was used as an indicator of DNA synthesis, as previously described [14]. Cells were plated in 96-well microtiter plates at a density of 2×10^4 cells/well in complete medium and incubated for 1–2 days at 37° in 5% CO_2/95% air before experimental treatment. The cells were exposed to drugs and [^{3}H]thymidine (74 nM, 82 Ci/mmol) for 2 hr. After incubating at 37° in a humidified atmosphere of 5% CO_2/95% air, the cells were washed and lysed with distilled water and collected on filters, using an automatic cell harvester (Skatron). The filters were placed in Ultima-Gold scintillation fluid (Packard) and counted with an LS-6000IC β-counter (Beckman Instruments).

Viability Studies

The viability of the cells after incubation with or without indicated drugs was assayed by measuring the uptake of the vital dye neutral red [18]. Cells were plated in 24-well plates at a density of 2×10^5 cells/mL of complete medium and incubated for 1–2 days at 37° in 5% CO_2/95% air. After a 2-hr exposure to drugs, neutral red vital dye was added to the complete medium at a final concentration of 0.033%. Cells were incubated at 37° in a humidified atmosphere of 5% CO_2/95% air for an additional 2 hr. The SCLC cells were collected and washed with PBS, and the cell pellet was solubilized in 1% acetic acid in 50% ethanol. The optical density at 540 nm ($O.D._{540}$) of each sample was determined spectrophotometrically using a DU-70 Spectrophotometer (Beckman Instruments).

Measurement of Intracellular Free Ca^{2+} Concentration

Cells were incubated in complete medium containing Fura-2 AM (4 μM) and pluronic (0.1%) for 30 min at 37°, diluted 1:1 with complete medium, incubated for another 30 min, and washed. The ratio of intracellular Fura-2 fluorescence at 340 and 380 nm was measured with a CMX Scanning Cation MicroIlluminator (SPEX Industries) as previously described [16].

Measurement of cAMP Content

Cells in exponential growth were exposed to drugs for 2 hr. After incubating at 37° in a humidified atmosphere of 5% CO_2/95% air, the SCLC cells were collected, washed twice with PBS, and centrifuged (500 *g*, 5 min). The cell pellets were extracted for 10 min with 2 mL of ethanol containing 1% 1 N HCl. Samples were centrifuged (500 *g*, 5 min) to remove any particulate matter, and the supernatants were stored at −70°. Aliquots of each sample were removed for protein determination. cAMP determinations were completed using an Amersham cAMP-assay kit following the manufacturer's instructions (Amersham Life Science).

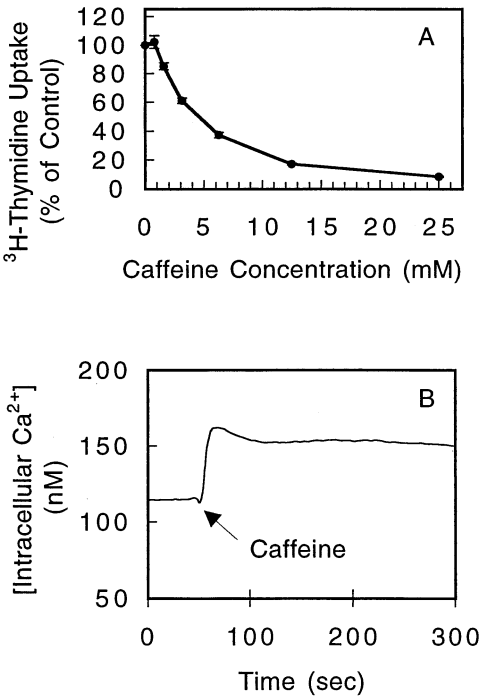

FIG. 1. Reduced DNA synthesis and increased Ca^{2+} mobilization in NCI-H345 cells by caffeine treatment. (A) [^{3}H]Thymidine uptake by NCI-H345 SCLC cells was measured after incubating the cells for 2 hr in the presence of caffeine. NCI-H345 cells incubated for 2 hr in the absence of caffeine served as controls. Control values of [^{3}H]thymidine uptake ranged from 1000 to 1200 cpm/2 × 10^4 cells. Results are the means (±1 SEM) of 9 determinations from at least three independent experiments. (B) Calcium mobilization in NCI-H345 cells loaded with Fura-2 was measured after exposing the cells to 33 mM caffeine at 60 sec. Higher concentrations of caffeine did not result in greater Ca^{2+} mobilization. Results are representative of at least three independent experiments.

RESULTS

Inhibition of [^{3}H]Thymidine Uptake and Mobilization of Ca^{2+} upon Treatment of NCI-H345 Cells with Caffeine

We investigated the effects of treatment with the phosphodiesterase inhibitor caffeine on [^{3}H]thymidine uptake by NCI-H345 SCLC cells. Incubation of NCI-H345 cells with caffeine for 2 hr profoundly decreased [^{3}H]thymidine uptake (Fig. 1A). Exposure of the cells to 6.25 mM caffeine led to a 62% inhibition of thymidine uptake, indicating a reduction in cell proliferation; incubation with 25 mM caffeine resulted in almost total inhibition of thymidine uptake (>90%), indicating almost complete stoppage of cell proliferation (Fig. 1A).

Caffeine has other effects on cells, in addition to that of inhibiting phosphodiesterase activity. The drug also induces the mobilization of Ca^{2+} from intracellular stores in cells that have Ca^{2+} stores regulated by ryanodine receptors [12]. Therefore, we investigated whether treatment of NCI-H345 cells with caffeine alters intracellular Ca^{2+} levels. We found that caffeine treatment mobilized Ca^{2+} in NCI-H345 cells (Fig. 1B), indicating that these cells have caffeine-sensitive Ca^{2+} stores.

Inhibition of [^{3}H]thymidine Uptake without Mobilization of Ca^{2+} upon Treatment of SCC-9 and NCI-H128 SCLC Cells with Caffeine

The inhibition of cell proliferation seen upon treatment of NCI-H345 cells with caffeine could be due to signaling through pathways utilizing cAMP, intracellular Ca^{2+}, or through interactions between the two signaling pathways. We investigated the effects of caffeine treatment on cell proliferation and Ca^{2+} mobilization using two other SCLC lines, SCC-9 and NCI-H128. Incubation of SCC-9 cells with 6.25 mM caffeine for 2 hr inhibited >70% of thymidine uptake; treatment with 25 mM caffeine resulted in >93% inhibition (Fig. 2A). The effects of caffeine on

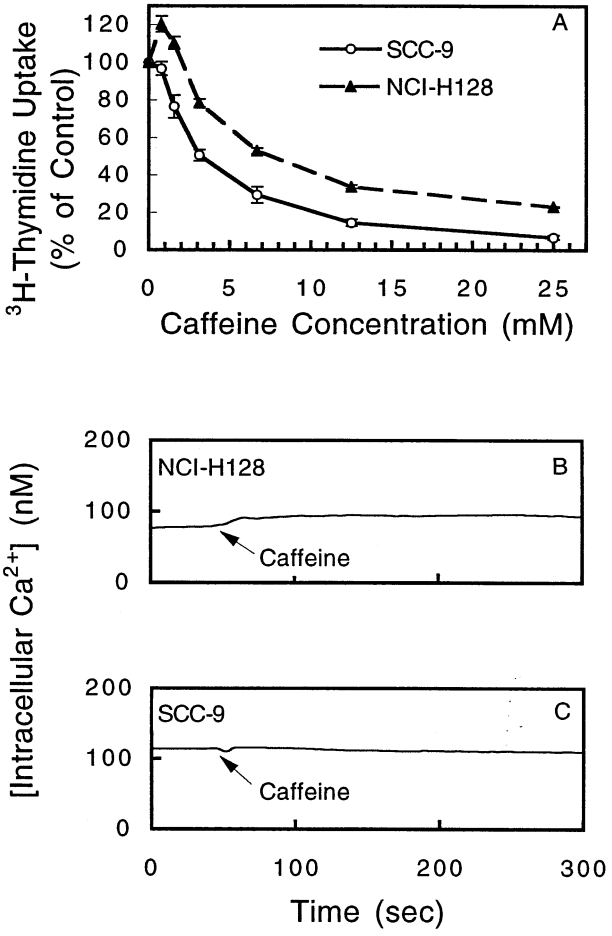

FIG. 2. Reduced DNA synthesis without Ca^{2+} mobilization in SCC-9 and NCI-H128 cells by caffeine treatment. (A) [^{3}H]Thymidine uptake by SCC-9 and NCI-H128 cells was measured after incubating the cells for 2 hr in the presence of caffeine. SCC-9 or NCI-H128 cells incubated for 2 hr in the absence of caffeine served as respective controls. Control values of [^{3}H]thymidine uptake ranged from 1600 to 2200 cpm/2 × 10^4 SCC-9 cells, and 300 to 400 cpm/2 × 10^4 NCI-H128 cells. Results are the means (±1 SEM) of 9 determinations from at least three independent experiments. Calcium mobilization in NCI-H128 (B) and SCC-9 (C) cells loaded with Fura-2 was measured after exposing the cells to 33 mM caffeine at 60 sec. Higher concentrations of caffeine did not result in greater Ca^{2+} mobilization. Results are representative of at least three independent experiments.

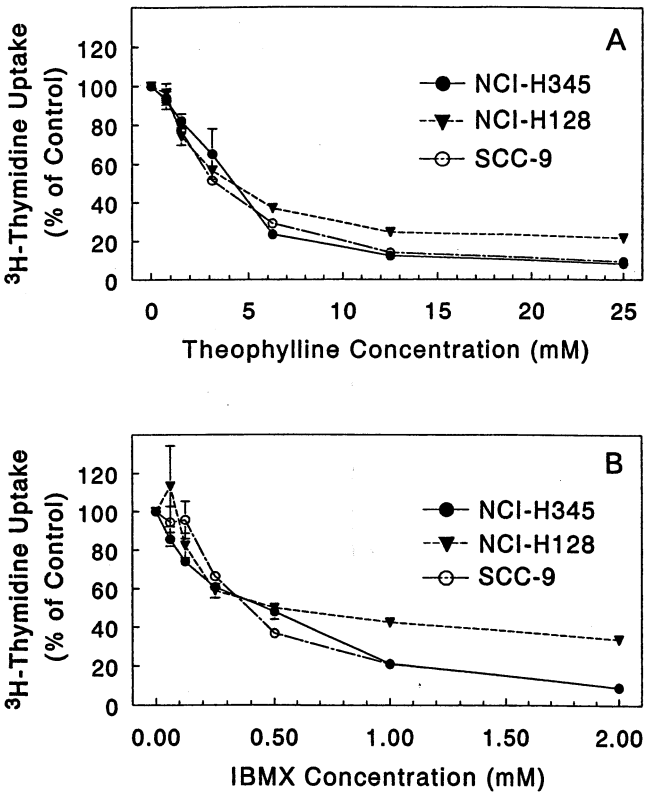

FIG. 3. Reduced DNA synthesis in SCLC cells by treatment with the cAMP phosphodiesterase inhibitors theophylline and IBMX. [^{3}H]Thymidine uptake by NCI-H345, SCC-9, and NCI-H128 cells was measured after the cells were incubated for 2 hr in the presence of theophylline (A) or IBMX (B). Cells incubated for 2 hr in the absence of drugs served as controls. Control values of [^{3}H]thymidine uptake ranged from 1200 to 2000 cpm/2 × 10^4 NCI-H345 cells, 1300 to 1700 cpm/2 × 10^4 SCC-9 cells, and 300 to 500 cpm/2 × 10^4 NCI-H128 cells. Results are the means (±1 SEM) of 9 determinations from at least three independent experiments.

the NCI-H128 cells were similar; a >50% inhibition of thymidine uptake occurred upon treatment with 6.25 mM caffeine, whereas treatment with 25 mM caffeine inhibited 80% of thymidine uptake (Fig. 2A). Interestingly, treatment of these cell lines with caffeine did not mobilize Ca^{2+} (Fig. 2, B and C). These findings suggest that the reduction of thymidine uptake seen upon treatment of SCLC cells with caffeine does not depend upon Ca^{2+} mobilization.

Inhibition of [^{3}H]Thymidine Uptake upon Treatment of SCLC Cells with the Phosphodiesterase Inhibitors Theophylline or IBMX

To confirm the results that inhibition of SCLC proliferation is due to phosphodiesterase inhibition, we investigated the anti-proliferative effects of two other phosphodiesterase inhibitors, theophylline and IBMX. Incubation of NCI-H345, SCC-9, or SCI-H128 cells for 2 hr with 6.25 mM theophylline resulted in a 60–75% reduction in thymidine uptake (Fig. 3A). At higher doses, the effect of the drug was even more marked. Incubation of all cell lines with 25 mM theophylline reduced the uptake of thymidine to 20% of control (Fig. 3A). Treatment with the phosphodiesterase inhibitor IBMX produced similar results. Incubation of the cells with 0.5 mM IBMX for 2 hr resulted in a 36–50% inhibition of thymidine uptake (Fig. 3B). Treatment of the cells with 2 mM IBMX resulted in a reduction of thymidine uptake to that of 10–30% of control levels (Fig. 3B).

Incubation of NCI-H345, SCC-9, or NCI-H128 cells with theophylline (25 mM) or IBMX (2 mM) did not mobilize Ca^{2+} in these cells (Fig. 4). These results suggest that the inhibition of [^{3}H]thymidine uptake induced by these drugs is due to decreased cAMP phosphodiesterase activity, rather than Ca^{2+} mobilization. This probability is supported by our finding that incubation with caffeine, theophylline, or IBMX increased cAMP levels in these cells (Fig. 5). Further evidence that increases in cAMP affect

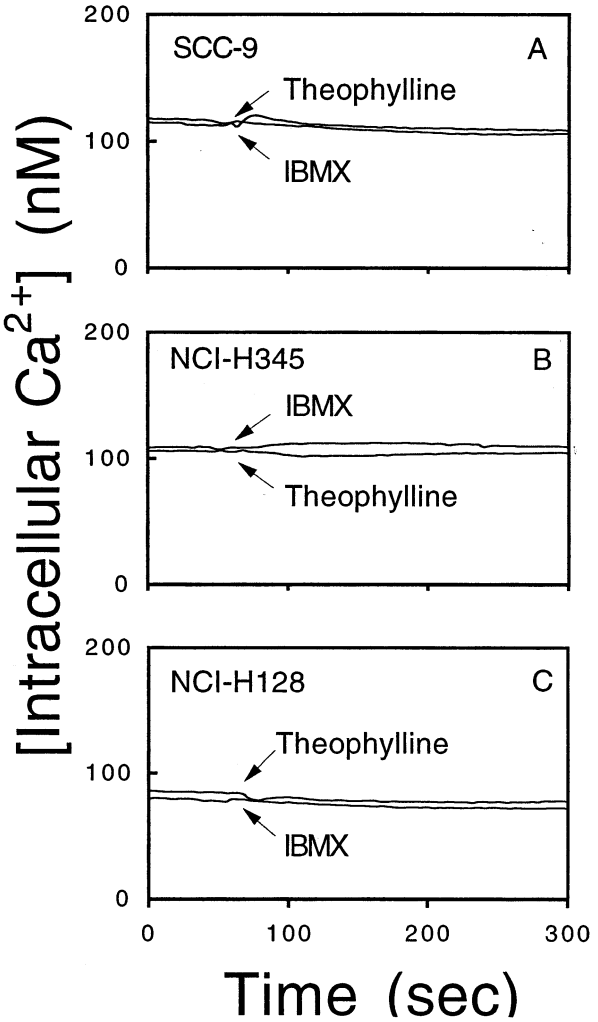

FIG. 4. Absence of Ca^{2+} mobilization upon treatment of cells with theophylline and IBMX. Calcium mobilization in SCC-9 (A), NCI-H345 (B), and NCI-H128 (C) cells loaded with Fura-2 was measured after exposing the cells to 25 mM theophylline or 2 mM IBMX at 60 sec. Results are representative of at least three independent experiments.

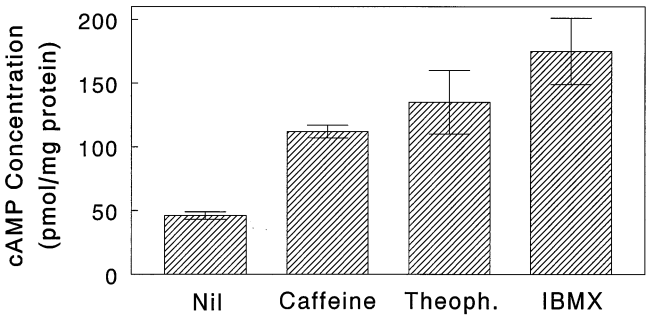

FIG. 5. Increases in cAMP levels upon treatment of cells with phosphodiesterase inhibitors. cAMP levels were measured after incubation of NCI-H345 cells with caffeine (25 mM), theophylline (25 mM), IBMX (2 mM), or no drug for 2 hr. Results shown are the means (±1 SEM) of triplicate samples from one experiment. A duplicate experiment produced similar results. Comparable findings were obtained using SCC-9 and NCI-H128 cells.

thymidine uptake was provided by our finding that incubation of NCI-H345, SCC-9, or NCI-H128 cells with 10 mM dibutyryl cAMP reduced thymidine uptake to 41.8 ± 5.3, 34.0 ± 1.1, and 40.7 ± 3.0% of control values, respectively (data not shown).

Reduction of the Viability of SCLC Cells upon Treatment with Phosphodiesterase Inhibitors

It is possible that treatment of SCLC cells with phosphodiesterase inhibitors results in the arrest of cell cycle progression without killing the cells. To determine the effects of these drugs on cell viability, we measured the uptake of the vital dye neutral red by the cells. Neutral red passes through the intact plasma membrane and becomes concentrated in the lysosomes of viable cells [19].

Treatment of SCLC cells with all of the phosphodiesterase inhibitors studied resulted in a profound reduction in cell viability (Fig. 6). Incubation with 3 mM caffeine reduced the viability to 60% of control in SCC-9 and NCI-H345 cells, and to 30% of control in NCI-H128 cells (Fig. 6A). At 25 mM caffeine, the effect on cell viability was much greater; cell viability was reduced to <15% of control in all cell lines studied (Fig. 6A). Similar results were seen upon treatment of cells with theophylline (Fig. 6B) and IBMX (Fig. 6C). The involvement of cAMP in these effects is indicated by our finding that treatment of SCC-9, NCI-H345, and NCI-H128 cells with 10 mM dibutyryl cAMP for 2 hr reduced the cell viability to 51.9 ± 6.9, 46.8 ± 5.4, and 58.6 ± 7.6% of control values, respectively (data not shown). These findings suggest that treatment of SCLC cells with phosphodiesterase inhibitors, which elevate intracellular concentrations of the second messenger cAMP, results not only in a marked reduction in the uptake of [^{3}H]thymidine, but also in a profound decrease in cell viability.

DISCUSSION

This study demonstrated that treatment of SCLC cells with caffeine rapidly decreased DNA synthesis by these cells. Caffeine inhibited DNA synthesis in SCLC cells that have Ca^{2+} stores mobilized by caffeine (NCI-H345 cells), and in lines that do not exhibit caffeine-induced Ca^{2+} mobilization (SCC-9 and NCI-H128 cells). The inhibition of DNA synthesis, therefore, occurs independently of increased intracellular Ca^{2+} concentrations. This suggests that the decrease in DNA synthesis is due to other effects of caffeine, such as inhibition of cAMP phosphodiesterase, resulting in elevation of intracellular levels of cAMP. Supporting this probability are our findings that [^{3}H]thymidine incorporation was also potently inhibited by theophylline and IBMX, which are phosphodiesterase inhibi-

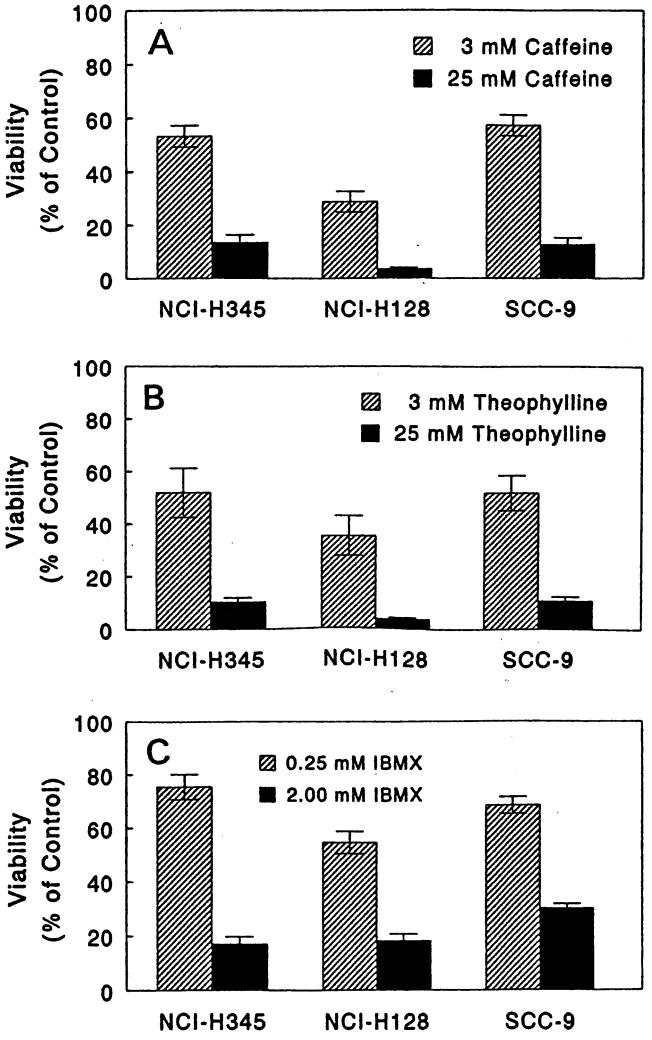

FIG. 6. Reduction in viability of SCLC cells by phosphodiesterase inhibitors. Cells (2×10^5 cells/mL) were incubated for 2 hr in the presence or absence of caffeine (A), theophylline (B), or IBMX (C). Neutral red dye was added, the cells were incubated for an additional 2 hr, and cell viability was determined. Cells incubated in the absence of drugs served as controls. Results are the means (±1 SEM) of four determinations from at least three independent experiments.

tors that do not mobilize Ca^{2+}. Treatment with caffeine, theophylline, or IBMX increased cAMP levels, and rapidly and markedly reduced cell viability in all of the SCLC cell lines studied. Our findings indicate that decreased cAMP phosphodiesterase activity in SCLC cells results in rapid inhibition of DNA synthesis and leads to subsequent cell death.

Our study is the first to provide evidence for the existence of caffeine-sensitive Ca^{2+} stores in SCLC cells. Ca^{2+} stores regulated by ryanodine receptors in lung epithelial cells have been described previously [20]. Only one of the SCLC lines demonstrated caffeine-sensitive Ca^{2+} mobilization; this, therefore, may not be a common characteristic of SCLC. It is possible that caffeine releases Ca^{2+} in NCI-H345 cells from stores other than those regulated by ryanodine receptors. Definitive identification of the source of the Ca^{2+} mobilized upon treatment of NCI-H345 cells with caffeine will require further investigation. Our findings indicate that Ca^{2+} mobilization is not implicated in cell death caused by phosphodiesterase inhibition in SCLC cells. Supporting this probability is our observation that NCI-H345 cells are not any more sensitive to caffeine treatment than are the SCC-9 or the NCI-H128 cells (Figs. 1A and 2A).

Increases in intracellular cAMP levels have been shown to both induce and inhibit cell death, depending on the cell system and conditions being investigated. Elevation of cAMP causes cell death in normal B cells [21] and malignant B cells isolated from patients with B-chronic lymphocytic leukemia [22], IPC-81 rat myeloid leukemia cells [23], thymocytes [24, 25], a human mammary carcinoma cell line [26], and in cultured primary granulosa cells [27]. In contrast, elevation of cAMP levels inhibits cell death in neutrophils [28, 29] and a bone-marrow-derived IL-3-dependent cell line [30]. Increasing levels of cAMP protect hepatocytes [31], MCF-7 cells [32], cerebellar neurons [33, 34] and hypothalamic cells [35] from cell death induced by various experimental manipulations. Cholera toxin, which increases cAMP levels by stimulating adenylate cyclase activity, inhibits *in vitro* growth of SCLC cells [36, 37] and induces the death of SCLC cells [37, 38]. However, cAMP agonists do not consistently mimic the growth inhibitory effects of cholera toxin on SCLC cells [38].

These disparate and contradictory effects of cAMP on cell proliferation and viability are not surprising in view of the findings that mammalian cells have multiple forms of adenylate cyclase. There are also many forms of cAMP phosphodiesterases. The heterodimeric enzyme protein kinase A can consist of various forms of regulatory and catalytic subunits [reviewed in Ref. 11]. The heterogeneity of proteins that govern cAMP levels in the cell could obviously lead to enormous variations in response to activation of cAMP signaling pathways [11].

All the SCLC lines examined in this study were killed by treatment with phosphodiesterase inhibitors, suggesting that this response may be a characteristic of all SCLC cells. Interestingly, we found no evidence of apoptotic death induced by these drugs. We have examined the SCLC cells using the TUNEL method, staining by ethidium bromide/acridine orange, and DNA fragmentation analysis, and have not detected morphological or biochemical signs of apoptosis induced by phosphodiesterase inhibitors (data not shown). This observation is consistent with the results of Allam *et al.* [38], who also did not observe increases in DNA fragmentation (an indication of apoptotic cell death) upon treatment of NCI-H345 cells with the phosphodiesterase inhibitor IBMX. These investigators found that treatment with IBMX does not alter cholera toxin-induced apoptosis of SCLC cells; the authors suggested that alterations in cAMP signals do not play a role in cholera toxin-induced apoptosis [38]. Interestingly, our treatment of NCI-H345 cells with concentrations of IBMX identical to those used by Allam *et al.* [38] resulted in a 50% decrease in DNA synthesis and cell viability within 2–4 hr. The effects of IBMX on necrotic cell death were not reported in the study of SCLC cells conducted by Allam *et al.* [38].

Other agents that disrupt signaling pathways in SCLC cells have been shown to induce apoptosis. Drugs that inhibit tyrosine kinase activity block the growth of SCLC cells, most likely by inducing apoptosis [39–41]. Substance P analogues, which are broad spectrum neuropeptide antagonists, act as potent growth inhibitors [42, 43]; evidence suggests that these compounds may also act by stimulating apoptosis in SCLC cells [42]. Fenretinide, a synthetic retinoid, has also been shown to inhibit the *in vitro* growth of several SCLC cell lines by the induction of apoptosis [44].

Most investigators consider apoptosis and necrosis as morphologically and physiologically distinct modes of cell death. However, there is increasing evidence that classical apoptosis and necrosis may represent extreme ends on a continuum of possible death modalities [45]. Both types of death can occur simultaneously in tissues or cell cultures exposed to the same stimulus. Different levels of oxidative stress may induce apoptosis or trigger necrosis in RINm5F pancreatic cells [46] and in neuronal cells [47, 48]. Serum and potassium withdrawal induces a mixture of apoptosis and necrosis in cerebellar granule cells in culture [34]. These cells in culture may exhibit populations undergoing both rapid necrosis and delayed apoptosis in response to glutamate toxicity [48, 49]. It is possible that many signals can induce both apoptosis and necrosis; some early events may be common to both types of cell death [45, 50].

The fact that in our study treatment with phosphodiesterase inhibitors reduced viability of SCLC cells so rapidly (in 2–4 hr) argues against a classically apoptotic mechanism. However, atypical types of apoptotic cell death triggered by cAMP signaling pathways have been reported [51]. In some developmental systems, programmed cell death occurs that does not exhibit the hallmark characteristics of apoptosis [52]. Intriguingly, we may be looking at necrosis resulting prior to the completion of an apoptotic pathway [45, 48]. Treatment of SCLC cells with lower

doses of the phosphodiesterase inhibitors for longer periods of time may induce apoptotic cell death.

cAMP phosphodiesterase inhibitors may be useful tools for dissecting the signal transduction pathways regulating tumor growth and proliferation. The rapid, marked effects of these drugs on SCLC viability indicate that they may be useful in combination with traditional chemotherapeutic agents. Such a combined approach incorporating cAMP phosphodiesterase inhibitors has been proposed for the treatment of other cancers [22]. An advantage of using cAMP phosphodiesterase inhibitors to kill SCLC cells is that the entry of these drugs into the cells does not depend upon the expression of specific cell surface receptors. This is in contrast to cholera toxin, which inhibits SCLC proliferation only if the cells express G_{M1} ganglioside [37]. The results of our study indicate that the alteration of cAMP signaling pathways by phosphodiesterase inhibitors warrants further investigation as an exploitable target for therapeutic intervention in SCLC.

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