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Inhibition of PDE3, PDE4 and PDE7 potentiates glucocorticoid-induced apoptosis and overcomes glucocorticoid resistance in CEM T leukemic cells

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ABSTRACT

Stimulation of the cAMP signaling pathway has been shown to induce apoptosis and augment the effects of glucocorticoids in inducing apoptosis in leukemic cells. We recently reported that in primary B cell chronic lymphocytic leukemic (B-CLL) cells, apoptosis could be induced by stimulating the cAMP signaling pathway with a phosphodiesterase4 (PDE4) inhibitor alone; while in contrast, in the CEM T leukemic cell line, PDE4 inhibitors alone were ineffective, and concurrent stimulation of adenylyl cyclase was required to see effects [Tiwari et al. (2005) [3]]. We report here that in the CEM and Jurkat T leukemic cell lines, the most abundantly expressed PDEs are PDE3B, PDE4A, PDE4D, PDE7A, and PDE8A. Selective inhibition of PDE3, PDE4 or PDE7 alone produces little effect on cell viability, but inhibition of all three of these PDEs together dramatically enhances glucocorticoid-induced apoptosis in CEM cells, and overcomes glucocorticoid resistance in a glucocorticoid-resistant CEM cell line. These studies indicate that for some leukemic cell types, a desired therapeutic effect may be achieved by inhibiting more than one form of PDE.

1. Introduction

Therapeutic induction of apoptosis of leukemic cells through targeting expressed forms of PDE to stimulate the cAMP signaling pathway has been the subject of investigation for some years and shows promise as a treatment modality for several types of leukemias (for review see Ref. [1]). In addition to inducing apoptosis of leukemic cells directly, PDE inhibitors have also been shown to augment the effects of glucocorticoids on induction of apoptosis of leukemic cells [2], and to overcome glucocorticoid resistance [3], which frequently develops during treatment of leukemias, and poses a serious problem in the effective treatment of this disease [4]. Lymphoid cells have generally been shown to express PDE3 and PDE4 as the most abundant forms, with appreciable expression of PDE7 detected as well [5,6]. In studies of primary B-CLL cells, selective inhibitors of PDE4 induced apoptosis, whereas inhibitors of PDE3 did not [7,8], although PDE3 inhibitors augmented the effects of PDE4 inhibitors in cells from a subset of patients resistant to the effects of PDE4 inhibitors alone [9]. Only very recently have pharmacological inhibitors of PDE7 become available with which to assess a functional role for PDE7 in lymphoid cells, although it was argued earlier that PDE7 may not be a relevant target for methylxanthine-induced apoptosis of B-CLL cells, since the IC_{50} for inhibition of PDE7 from these cells by theophylline is higher than the concentrations at which it induces apoptosis [10]. In contrast to primary B-CLL cells, PDE4-selective inhibitors alone have little direct effect on the T acute lymphocytic leukemic (T-ALL) cell line, CEM, in the presence or absence of glucocorticoids, unless cAMP synthesis is concurrently stimulated by agents such as forskolin [3].

Assessment of the functional role(s) of PDE7 in lymphoid cells has been controversial. Based on studies with targeted antisense oligodeoxynucleotides, PDE7A1 was reported to be required for T lymphocyte activation by CD3/CD28 [11], but T cells from PDE7A-deficient mice were activated normally by CD3/CD28 [12], and recently developed PDE7-selective inhibitors did not impair CD3/CD28-dependent activation of naïve or memory $CD4^+$ T cells [13]. In another study, the PDE7-selective inhibitor, BRL 50481, was shown to have no effect by itself on T lymphocyte proliferation, and only a marginal effect on cytokine release, but it enhanced the inhibitory effect of the PDE4-selective inhibitor, rolipram, on these processes [14]. The PDE7 gene family is comprised of two genes, PDE7A and PDE7B. Following the initial cloning and expression analysis of these genes, PDE7A, specifically the PDE7A1 splice variant, was found to be widely expressed in cells of the immune system [15,16]; whereas PDE7B was abundantly expressed in pancreas, brain and heart, but was not reported to be expressed in immune cells [17,18]. Recently, however, PDE7B was shown to be expressed in lymphoid cells, and in much higher levels in B-CLL cells than in normal lymphocytes [19]. Moreover, BRL 50481, an

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additional PDE7-selective inhibitor, and a dual PDE4/7 inhibitor induced apoptosis of B-CLL cells, with little effect on normal B cells, indicating that PDE7B may be a useful target for therapeutic treatment of CLL [19].

In this paper we examined the expression of PDEs in glucocorticoid-sensitive and resistant T leukemic cell lines, and assessed inhibitors of the expressed PDE forms for their ability to induce apoptosis of these cells, both alone, and in combination with hydrocortisone. We find the most abundant expressed forms of PDE in these cells to be PDE3B, 4A, 4D, 7A, and 8A. While selective inhibitors of the PDE3, PDE4 and PDE7 gene families have little effect on these leukemic cells by themselves, in the presence or absence of hydrocortisone, when inhibitors of PDE3, PDE4 and PDE7 are added together, they dramatically enhance the ability of hydrocortisone to induce apoptosis of these cells and overcome the resistance of the glucocorticoid-resistant cell line. This suggests that all three of these PDEs may contribute to regulating a pool of cAMP linked to apoptosis of these cells, and that for some leukemias, it may be necessary to inhibit more than one form of PDE to achieve therapeutic benefit.

2. Materials and methods

2.1. Materials

Milrinone, rolipram, hydrocortisone and forskolin were obtained from Biomol (Plymouth Meeting, PA). Dipyridamole, phenazine methosulfate (PMS), calmodulin, and protease inhibitor cocktail for use with mammalian cell and tissue extracts were from Sigma–Aldrich, St. Louis, MO. 3-Isobutyl-1-methylxanthine (IBMX) was from Calbiochem, Gibbstown, NJ. [3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium], inner salt (MTS) was from Promega, Madison, WI. Motapizone, piclamilast, zardaverine, PDE2 inhibitor PDP [20] and PDE7 inhibitor spiroquinazolinone were synthesized and supplied by the chemistry division of Nycomed GmbH, Konstanz, Germany. Zardaverine is a dual selective PDE3/4 inhibitor with an IC_{50} for PDE3 = 315 nM, and an IC_{50} for PDE4 = 160 nM. The spiroquinazolinone PDE7 inhibitor, 8′-chloro-6′-{4-[(4-methylpiperazin-1-yl) carbonyl] phenyl}-1′H-spiro [cyclohexane-1,4′-quinazolin]-2′(3′H)-one [21], structure shown in Supplementary Fig. S1, was synthesized based on the patent published by Warner-Lambert (patent publication no. WO/2002/074754; US Patent no. 7429598). The spiroquinazolinone inhibits PDE7 with an IC_{50} = 17 nM, but also inhibits PDE4 with an IC_{50} = 204 nM. The IC_{50} of spiroquinazolinone for members of all other PDE gene families is >1 μM. Tritiated cAMP was from GE Health Care (Freiburg, Germany). Polyclonal antibodies against PDEs 3B (sc-11835), 4A (sc-25810), 4B (sc-25812 and sc-25087), 4D (sc-25100), 7A (sc-11131), 8A (sc-30059), and 11A (sc-98557) were from Santa Cruz Biotechnology Inc., Santa Cruz, CA. Polyclonal antibodies against PDEs 1B (PD1B-201AP) and 1C (PD1C-301AP) were from FabGennix Inc., Frisco, TX. Polyclonal antibody against GAPDH was from Cell Signaling Technology, Danvers, MA.

2.2. Cell culture

Glucocorticoid-sensitive (CEM-S2) and glucocorticoid-resistant (CEM-R8) cell lines were isolated and subcloned from a parental CCRF-CEM T leukemic cell line as described previously [3]. Jurkat cells, originally obtained from ATCC, were generously provided by Dr. Maurice Feinstein of the University of Connecticut Health Center. CEM and Jurkat cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin, at 37 °C in a humidified atmosphere of 95% air and 5% CO_2.

2.3. MTS assay

CEM cells were plated in triplicate at a density of 5×10^3 cells/well in 96-well flat-bottom tissue culture plates in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, in a total volume of 0.1 ml of fresh medium containing the test reagents or vehicle as indicated. Following incubation at 37 °C for 72 h, 20 μl of a combined solution of MTS (2 mg/ml)/PMS (0.92 mg/ml) (20:1, mixed immediately before use) was added to each well, and the plates incubated for an additional 2 h at 37 °C, protected from light, following which the absorbency (O.D.) of the formazan product formed was determined at 492 nm using a microtiter plate reader (Titertek Multiskan Plus model MK II, Titertek, Huntsville, AL). With the exception of dexamethasone, all reagents tested were dissolved in DMSO and diluted into the cell culture medium such that the final concentration of DMSO in the assay was 0.1%. Percent cell viability is proportional to the amount of formazan product formed and was calculated as follows: (O.D. control sample − O.D. blank)/(O.D. test sample − O.D. blank) × 100, where blank refers to plate wells where media, vehicle and test reagents were added, as appropriate, but cells were omitted.

2.4. Quantitative real-time RT-PCR

Total RNA was isolated from cells as indicated, using RNeasy mini kits (Qiagen, Valencia, CA) according to the manufacturer's instructions. cDNA was synthesized using M-MLV reverse transcriptase (Promega, Madison, WI). Primers were designed using ABI Primer Express Software v3.0. and synthesized by Integrated DNA Technologies, Inc. (Coralville, IA). The primers used for the different mRNA expressions analyzed are presented in Supplementary Table S1. Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) was performed using an ABI 7500 fast system (Applied Biosystems Inc., Foster City, CA) and data analyzed using 7500 fast systems SDS software v3.0. Amplicon sizes were 100 bp.

2.5. Western immunoblot analysis

CEM and Jurkat cells were centrifuged, washed twice with ice-cold PBS, and lysed in 100 μl RIPA buffer (50 mM Tri–HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 0.25% Na-deoxycholate and 1:100 protease inhibitor cocktail). Protein concentration was determined using a Micro BCA Protein Assay Kit (Pierce, Rockford, IL). Equal amounts of protein (50 μg) were loaded and run on 12% SDS-PAGE gels. Molecular weight markers, also run on the gels, were Precision Plus All Blue Standards (Bio-Rad, Hercules, CA). Proteins were then transferred onto Immobilon-p Transfer Membrane (Millipore, Billerica, MA). Membranes were blocked with 5% nonfat dry milk in Tris-buffered saline overnight at 4 °C and probed with primary antibody (1:200) for 1 h at room temperature, washed three times with Tris-buffered saline–Tween 20 (TBS–T) buffer, and incubated with horseradish peroxidase-conjugated secondary antibody at a final dilution of 1:5000 for 1 h at room temperature and then washed three more times. Proteins were visualized with SuperSignal West Femto Maximum Sensitivity Substrate (Pierce, Rockford, IL) using a GENE-Snap BioImaging System (Syngene, Frederick, MD). Blots were stripped and reprobed with GAPDH antibody for normalization.

2.6. Determination of PDE isozyme activities

PDE activities were assayed as described previously [22], using homogenate from 5×10^4 CEM-S2 cells and 2×10^5 CEM-R8 cells

per assay in order to achieve comparable levels of total activity for both cell types. Substrate concentration was 0.5 μM cAMP for PDEs 1–4 and 0.1 μM cAMP for PDE7. Activities of individual PDE gene family isozymes were determined using PDE isozyme-specific activators and inhibitors as follows: PDE1: Ca^{2+} (1 mM)/calmodulin (0.1 μM)-stimulated activity in the presence of piclamilast (1 μM) and motapizone (10 μM); PDE2: activity inhibited by PDE2 inhibitor (0.1 μM) in the presence of cGMP (5 μM), piclamilast (1 μM) and motapizone (10 μM); PDE3: activity inhibited by motapizone (10 μM) in the presence of piclamilast (1 μM); PDE4: activity inhibited by piclamilast (1 μM); PDE7: activity inhibited by PDE7 inhibitor (1 μM) in the presence of piclamilast (1 μM), motapizone (10 μM), and PDE2 inhibitor (0.1 μM).

3. Results

3.1. Expression of PDE isoforms in CEM and Jurkat cells

3.1.1. qRT-PCR analysis of PDE mRNA expression

Using qRT-PCR, in two T leukemic cell lines, CEM and Jurkat, we examined and quantified the expression of members of the eleven known cAMP PDE gene families. For CEM, we had earlier isolated and subcloned variants of the parental cell line, one of which, CEM-S2, is extremely sensitive for the induction of apoptosis by glucocorticoids (IC_{50} for dexamethasone = 7 nM), and the other, CEM-R8, is resistant (viability unaltered by dexamethasone at least up to 10 μM) [3], and we examined this in both the CEM-S2 and CEM-R8 cell lines, as well as the Jurkat cell line. PDE mRNA expression in both of the CEM cell lines was similar, and similar to that in the Jurkat cell line as well (Fig. 1). There was relatively high expression of PDE3B, 4A, 4D, 7A and 8A in all three of these cell lines, as compared to expression of RPL19, the control gene. Some expression of PDE 1B mRNA was also noticeable in all three cell types, as well as a lesser amount of PDE1C, and some expression of PDEs 9, 10 and 11 was also seen, mostly in CEM cells. No expression of mRNA for PDEs 1A, 2A, 3A, 5A, 6B or 8B was seen in any of these cells. Of the four genes within the PDE4 gene family, expression of PDE4A was highest, with expression of PDE4D somewhat less, but expression of PDE4B and PDE4C in these cells was almost completely absent, representing only about 0.1–0.3% of the expression of PDE4A. Although PDE mRNA expression patterns were qualitatively quite similar in all three cell types, some quantitative differences were noted (Fig. 1 and Supplementary Table S2). PDE3B was the most abundantly expressed form in CEM cells, but showed lower expression in Jurkat cells. Expressions of PDE7A and PDE8A were quite high in all three cell types. Between the two CEM cell lines, with the exception of PDE4A, which showed equal expression in both, the degree of expression of the other major PDE forms was less in CEM-R8 cells than in CEM-S2 cells. For PDEs 3B, 7A and 8A genes the expression in CEM-S2 cells was about 2-fold greater than that in CEM-R8 cells, although for PDE4D, the expression in CEM-R8 cells was even lower, with PDE4D expression 5.3-fold greater in CEM-S2 cells. Of the two genes in the PDE7 family, expression of PDE7A was considerably greater than that of PDE7B, with PDE7B expressed only at about 0.07–0.1% of the level of PDE7A in all three cell types.

3.1.2. Western immunoblot analysis of PDE protein expression

In order to examine PDE gene expression in these cells at the protein level, Western immunoblot analyses were performed using

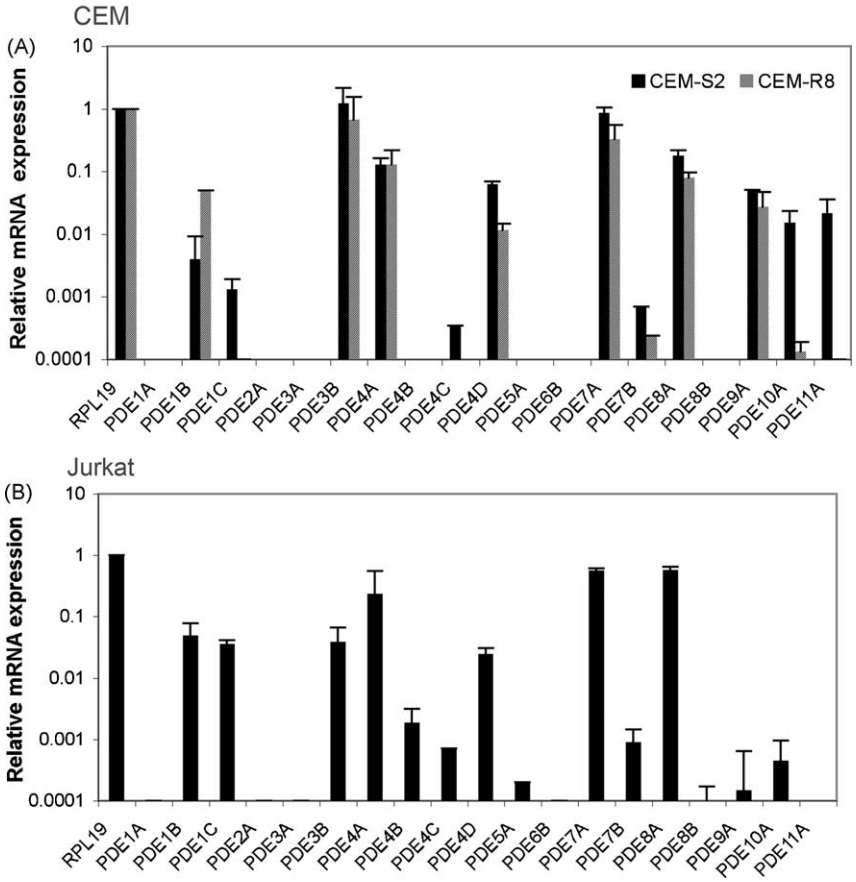

Fig. 1. PDE gene expression in CEM and Jurkat cells. mRNA expression of PDE isoforms in CEM and Jurkat cells was analyzed by qRT-PCR. (A) CEM-S2 and CEM-R8 cells; (B) Jurkat cells. Data show expression of target PDE mRNA relative to RPL19 control mRNA calculated by the $2^{-\Delta\Delta CT}$ method [44]. Data represent the mean ± S.D. of n = 2–3 experiments, each assayed in triplicate.

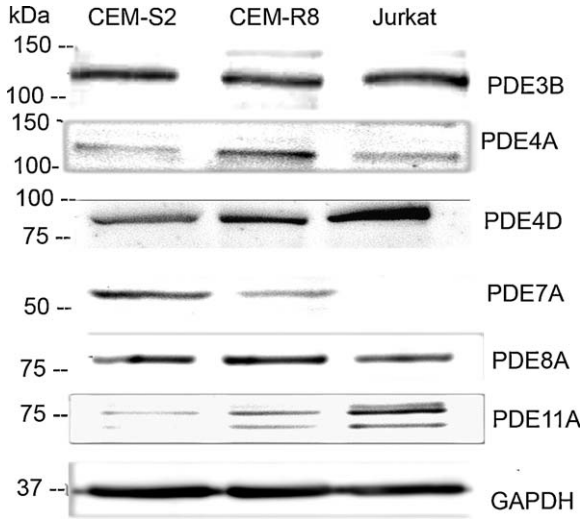

Fig. 2. Western immunoblots of cAMP PDEs in CEM and Jurkat cells. PDE protein expression was determined by immunoblots of whole cell lysates using polyclonal antibodies directed against specific cAMP PDE isoforms. Equal amounts of protein (50 μg) were loaded per lane. GAPDH expression was used for protein loading normalization. The position of molecular weight markers run on the gels is indicated on the left. Data represent one of two experiments for each PDE isoform with similar results.

PDE gene family-specific polyclonal antibodies directed against the cAMP PDE genes that showed mRNA expression by qRT-PCR analysis. As shown in Fig. 2, protein product was seen for PDEs 3B, 4A, 4D, 7A, 8A and 11A. Using two different antibodies, no protein product was seen at all for PDE4B, and no protein product was detected using antibodies directed against PDE1B or PDE1C either (not shown). Antibody directed against PDE3B recognizes a major band of Mr ≈ 137 kDa, consistent with the known molecular weight of this form. Antibody against PDE4A recognizes a major band of Mr ≈ 128 kDa consistent with the PDE4 long forms, PDE4A4 and/or PDE4A10 [22]. Antibody to PDE4D recognizes a major band of Mr ≈ 93 kDa, consistent with the PDE4D3 long form. Antibody to PDE7A recognizes a major band at Mr ≈ 57 kDa in both CEM cell lines, consistent with the splice variant PDE7A1, although surprisingly, only very little of this band was seen in Jurkat cells. Antibody to PDE8A recognized a major band of Mr ≈ 85 kDa, consistent with the PDE8A1 splice variant. Antibody to PDE11A recognizes bands of Mr ≈ 78 kDa and 66 kDa, consistent with PDE11A3 and PDE11A2 splice variants respectively.

3.1.3. Quantitative analysis of the activities of individual cAMP PDE isozymes

In order to quantitate the degree of protein expression of the cAMP PDE genes, we measured the activities of cAMP PDE gene family-specific isozymes in the two CEM cell lines. As shown in Fig. 3, consistent with the mRNA expression data and Western immunoblot analyses, appreciable activity is seen in these CEM cells for PDEs 3, 4, and 7, with PDE3 showing the highest level of activity amounting to about 56% of total activity in CEM-S2 cells and about 62% of total activity in CEM-R8 cells. Little or no PDE activity is seen for PDEs 1 and 2. Residual PDE activity unaccounted for by the PDE1, 2, 3, 4, and 7 PDE isoforms, amounting to 12% of total activity in CEM-S2 cells and 14% of total activity in CEM-R8 cells, was also seen. Based on the mRNA expression and Western immunoblot analyses, this residual PDE activity most likely represents PDE8 activity, although some of the residual activity could be from PDE11 as well, since expression of PDE11A protein was also detected by Western immunoblot analysis. Also, consistent with the PDE mRNA expression for these cell types, the activities of PDEs 3, 4 and 7 in CEM-R8 cells were lower than

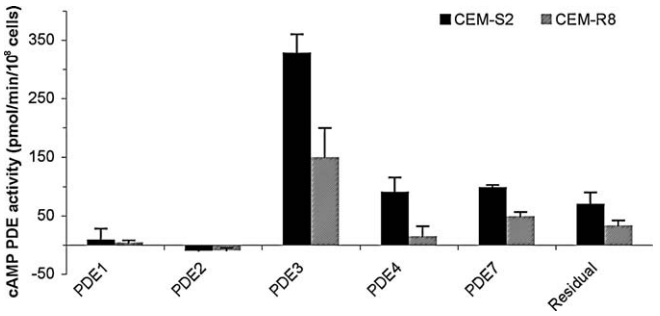

Fig. 3. cAMP PDE activities of individual gene family-specific PDE isoforms in CEM cells. Activities of individual PDE gene family isozymes were determined in CEM-S2 and CEM-R8 cells using PDE isozyme-specific activators and inhibitors as described in Section 2. Results represent the mean ± S.D. of $n = 3$–4 individual experiments assayed in quadruplicate.

that for CEM-S2 cells; about 2-fold lower for PDEs 3 and 7, comparable to the difference seen in their mRNA expressions, and about 6-fold lower for PDE4. The 6-fold lower PDE4 activity in CEM-R8 cells, as compared to CEM-S2 cells, is comparable to the degree of difference of the PDE4D mRNA expression between these two cell types.

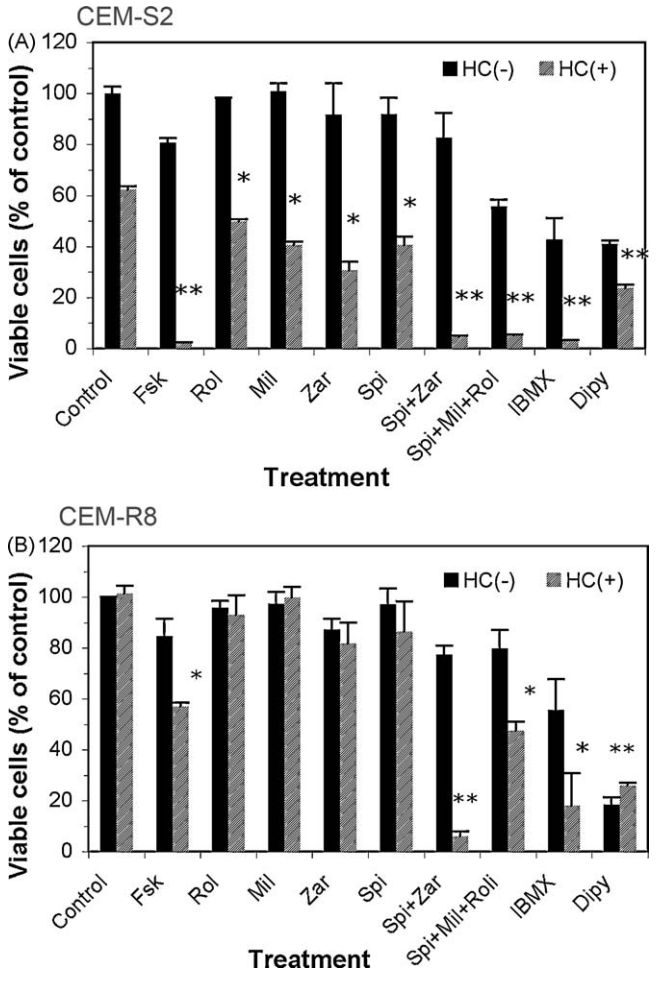

Fig. 4. Effect of forskolin and PDE inhibitors on the viability of CEM cells in the presence and absence of hydrocortisone. CEM cells were incubated for 72 h at 37 °C with hydrocortisone (HC 1 μM), forskolin (Fsk 10 μM), rolipram (Rol 10 μM), milrinone (Mil 10 μM), zardaverine (Zar 10 μM), spiroquinazolinone (Spi 1 μM), IBMX (500 μM), dipyridamole (Dipy 100 μM), or combinations thereof, as indicated. Cell viability was determined by MTS assay. (A) CEM-S2 ($n = 4$) and (B) CEM-R8 ($n = 6$). Data represent the mean ± S.D. $^{**}P < 0.01$ and $^{*}P < 0.05$ for agents added with HC compared to HC alone, as determined by Student's t test.

3.2. Effect of stimulation of the cAMP and glucocorticoid signaling pathways on viability of T leukemic cells

We examined the effects of stimulation of the cAMP signaling pathway alone and in combination with stimulation of the glucocorticoid signaling pathway, on viability of the glucocorticoid-sensitive and resistant CEM cell lines. The cAMP signaling pathway was stimulated by activation of adenylyl cyclase with forskolin, or by inhibition of PDEs, using inhibitors of the PDE3, PDE4 and PDE7 gene families, to determine the contribution of these PDE families to this process. A selective inhibitor of PDE8 was not available for this study. As shown in Fig. 4A, using CEM-S2 cells, the glucocorticoid, hydrocortisone (1 μM), reduces viability of these cells by 38%. Forskolin (10 μM) by itself reduces viability by only 19%, but stimulation of both the cAMP and glucocorticoid signaling pathways with forskolin and hydrocortisone together produces a synergistic effect, reducing viability by 98%. This synergistic effect is strikingly evident when dose responses of

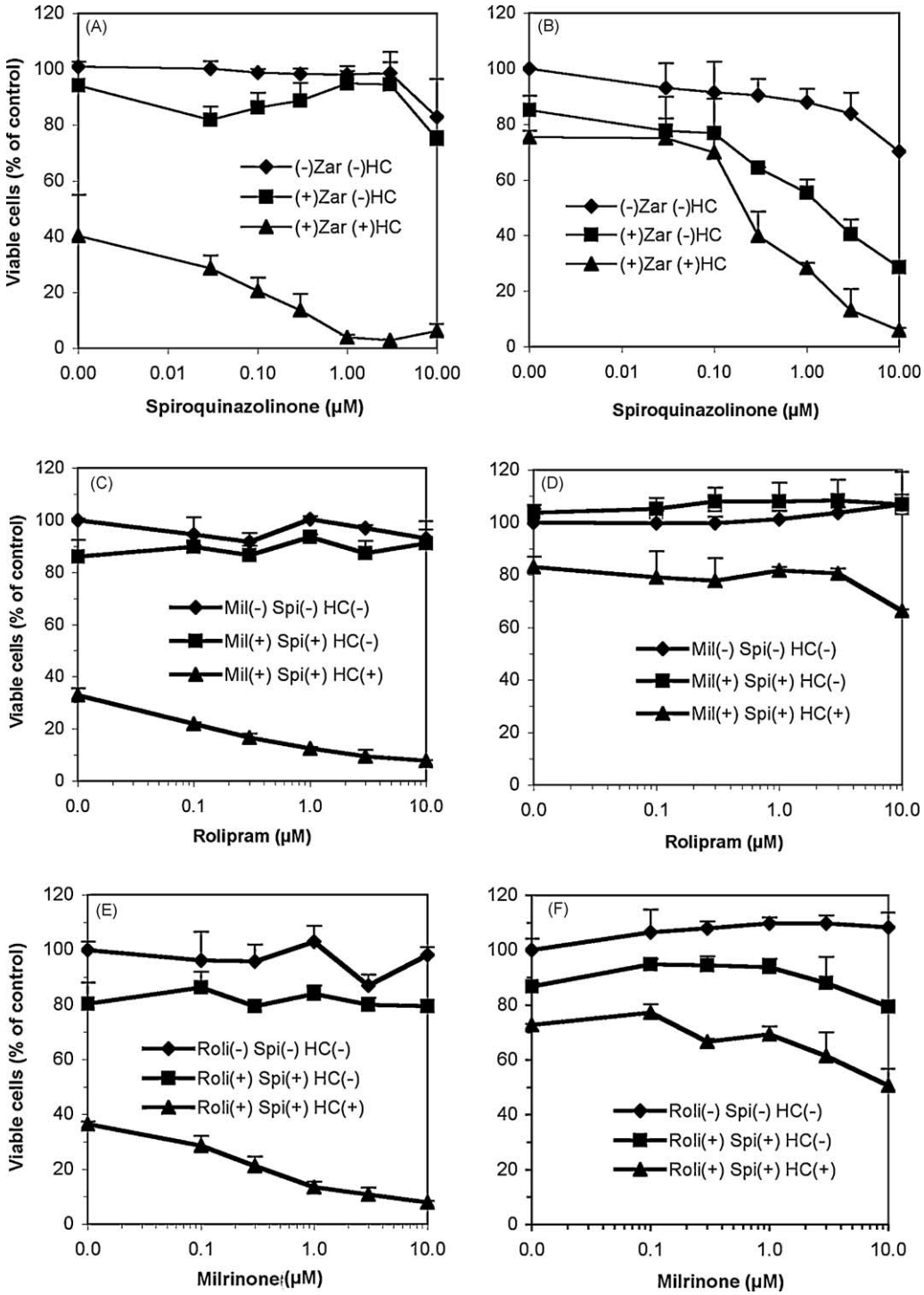

Fig. 5. Dose response of PDE inhibitors on the viability of CEM cells in the presence and absence of hydrocortisone. CEM cells were treated with different concentrations of spiroquinazolinone (A and B), rolipram (C and D) or milrinone (E and F) with or without zardaverine (10 μM), milrinone (10 μM), spiroquinazolinone (1 μM), rolipram (10 μM) and hydrocortisone (1 μM), as indicated, for 72 h and cell viability determined by the MTS assay. Panels A, C and E: CEM-S2 cells; panels B, D and F: CEM-R8 cells. Data represent the mean ± S.D. of $n = 3$ experiments for panels A and B and $n = 2$ experiments for panels C–F, each assayed in triplicate.

forskolin are performed in the presence of hydrocortisone, where it can be seen that forskolin reduces cell viability by >90% at concentrations of >3 μM (Supplementary Fig. S2). Neither the PDE4-selective inhibitor, rolipram, nor the PDE3-selective inhibitor, milrinone, nor the spiroquinazolinone PDE7 inhibitor, produced any effect on viability of CEM-S2 cells by themselves, but they did potentiate the reduction of cell viability in the presence of hydrocortisone to 50–60%. Zardaverine, a dual PDE3/4 selective inhibitor, which also had no effect by itself, also reduced cell viability further in the presence of hydrocortisone to 70%. Of particular note, when PDEs 3, 4 and 7 were all inhibited by the combination of spiroquinazolinone with zardaverine or spiroquinazolinone with milrinone and rolipram, some effect on the viability of the CEM-S cells is now manifested in the absence of hydrocortisone, and in the presence of hydrocortisone, viability is reduced by 95%, an effect comparable to that seen with forskolin and hydrocortisone.

Results with the CEM-R8 cell line (Fig. 4B) were somewhat similar. In this case, since these cells are glucocorticoid-resistant, hydrocortisone (1 μM) had no effect on viability of the cells by itself, but when forskolin (10 μM) and hydrocortisone (1 μM) were added together, viability was reduced by 43%. When dose responses of forskolin on cell viability were examined, however, even with CEM-R8 cells, viability was reduced by 93% at 100 μM forskolin in the presence of hydrocortisone (Supplementary Fig. S2). Rolipram, milrinone, spiroquinazolinone and zardaverine had little or no effect on viability of CEM-R8 cells by themselves, in the absence or presence of hydrocortisone, but when PDEs 3, 4 and 7 were all inhibited by the addition of spiroquinazolinone with zardaverine, cell viability was reduced by 94% in the presence of hydrocortisone. Inhibition of these PDEs with spiroquinazolinone, milrinone and rolipram in the presence of hydrocortisone also appreciably reduced viability of these glucocorticoid-resistant cells, although with this combination, only by 53%.

Although a PDE8-selective inhibitor was not available for these studies, dipyridamole, which inhibits PDE8 with an $IC_{50} \approx 4–9$ μM [23–25] was tested and found to reduce viability of both CEM-S2 and CEM-R8 cells both in the absence and presence of hydrocortisone. Dipyridamole (100 μM) reduced viability of CEM-S2 cells by 59% in the absence and 76% in the presence of hydrocortisone; viability of CEM-R8 cells was reduced by 81% in the absence and 74% in the presence of hydrocortisone (Fig. 4). Although dipyridamole inhibits PDE8, it should be noted that it is a nonselective PDE inhibitor, capable of inhibiting most of the PDE gene families, including PDE4 and PDE7, with IC_{50}s in the micromolar range [1,17,18,26]. Similarly, the nonselective PDE inhibitor, IBMX, which inhibits all known PDE gene families with the exception of PDE8 and 9, also reduced viability of CEM-S2 and CEM-R8 cells. IBMX (500 μM) reduced viability of CEM-S2 cells by 57% in the absence and 97% in the presence of hydrocortisone; viability of CEM-R8 cells was reduced by 44% in the absence and 82% in the presence of hydrocortisone (Fig. 4).

That the reduction of viability of these cells, as measured by the MTS assay, represents apoptosis, was verified by observing characteristic ladder patterns of endonuclease-digested DNA of these cells in the presence of these reagents on agarose gels, as shown in Supplementary Fig. S3.

3.3. Dose response of PDE inhibitors on viability of CEM cells

Dose responses of PDE inhibitors on viability of CEM cells were examined in the presence and absence of hydrocortisone (Fig. 5). As shown in Fig. 5A, the spiroquinazolinone PDE7 inhibitor has only very minimal effect on viability of CEM-S2 cells in the absence of hydrocortisone, even when zardaverine is added to also inhibit PDEs 3 and 4. However, when hydrocortisone is added along with zardaverine, viability is reduced by 60% and spiroquinazolinone further reduces the viability of the CEM-S2 cells by 97%, with an $IC_{50} \approx 0.1$ μM for this effect. In CEM-R8 cells (Fig. 5B) zardaverine plus hydrocortisone reduce viability by 25%. Addition of spiroquinazolinone further reduces viability by 94%, with an IC_{50} for this effect of spiroquinazolinone ≈ 0.4 μM. Interestingly, the CEM-R8 cells are more sensitive to the effects of zardaverine and spiroquinazolinone, the combination of which reduces the viability by 71%, even in the absence of hydrocortisone. As shown in Fig. 5C, rolipram has little effect on viability of CEM-S2 cells in the absence of hydrocortisone, even when milrinone and spiroquinazolinone are added to inhibit PDEs 3 and 7. However, when hydrocortisone is added along with milrinone and spiroquinazolinone, viability is reduced by 67% and rolipram further reduces the viability of the CEM-S2 cells by 92%, with an $IC_{50} \approx 0.3$ μM for this effect. Rolipram also reduced viability of CEM-R8 cells in the presence of milrinone, spiroquinazolinone, and hydrocortisone, but in this case by only 34% (Fig. 5D). As shown in Fig. 5E, milrinone has little effect on viability of CEM-S2 cells in the absence of hydrocortisone, even when rolipram and spiroquinazolinone are added to inhibit PDEs 4 and 7. However, when hydrocortisone is added along with rolipram and spiroquinazolinone, viability is reduced by 64% and milrinone further reduces the viability of the CEM-S2 cells by 92%, with an $IC_{50} \approx 0.5$ μM for this effect. Milrinone also reduced viability of CEM-R8 cells in the presence of rolipram, spiroquinazolinone, and hydrocortisone, but in this case by only 50% (Fig. 5F).

4. Discussion

PDEs are actively being investigated as targets for developing novel therapeutic treatments for leukemias [1]. Most studies have pointed to PDE4 as being the most promising target in this regard [2,7,8], but a recent study with primary B-CLL cells revealed a large upregulation of PDE7B in these cells and showed induction of apoptosis with PDE7-selective inhibitors, indicating that PDE7 may also provide a good target for the treatment of CLL [19]. Inasmuch as PDE7B expression and the effects of PDE7 inhibitors have not been examined in T leukemic cells, we examined PDE expression in two T leukemic cell lines, Jurkat and CEM, and also examined the effects of PDE inhibitors on the viability of glucocorticoid-sensitive and resistant CEM cell lines.

Earlier studies had shown PDE3, PDE4 and PDE7 to be the predominant PDEs expressed in isolated $CD4^+$ and $CD8^+$ T lymphocytes [5,6]. Isolated $CD19^+$ B lymphocytes also showed major expression of PDE4 and PDE7, but little or no expression of PDE3, and no expression of PDE1, PDE2 or PDE5 [27]. Following the discovery of PDE8, PDE8A1 was shown to be expressed in activated human T lymphocytes [28] and activated mouse splenocytes [29], but absent [28] or expressed at low levels [29] in the resting state. PDE8A1 was, however, shown to be constitutively expressed in the T lymphoblastoid cell line, Hut78 [28]. In the recent study by Zhang et al. [19], the four most abundant forms of PDE expressed in isolated human peripheral blood mononuclear cells (PBMC) were PDE3B, 4B, 7A and 8A. Much smaller or trace amounts of expression of PDE1B, 1C, 2A, 3A, 4A, 4C, 4D, 5A, 7B, 8B, and 9A were also detected. Of particular note, although expressed at low levels in normal PBMC, PDE7B expression was ≈ 23-fold higher on average in B-CLL cells [19]. That isolated normal B cells show only ≈3-fold increase in PDE7B expression relative to PBMC indicates that this large increase in PDE7B expression in B-CLL cells is a result of the transformed state of these cells, and not a function of their B cell lineage. We also showed recently that PDE3B, 4B, 7A and 8A represent major expressed forms of PDE in isolated activated murine $CD4^+$ T lymphocytes, both in vivo and in vitro (Vang et al., PDE8 regulates Teff cell adhesion and proliferation independent of ICER, submitted for publication).

In our study reported here, by qRT-PCR, we find CEM and Jurkat T leukemic cell lines to express PDE 3B, 4A, 4D, 7A and 8A as their major forms. Western immunoblot analysis and assay of individual cAMP PDE isozymes largely reflected the expression seen for PDE mRNA by qRT-PCR in that appreciable PDE activity was observed for PDEs 3, 4, and 7, and protein product was detected for PDEs 3B, 4A, 4D, 7A and 8A. However, protein product for PDE11A was also detected by Western immunoblot, even though mRNA expression for PDE11A was quite low. Additionally, whereas some mRNA expression for PDE1B and PDE1C was detected in these cells, no protein product was detected by Western immunoblot and activity for PDE1 by isozyme-specific activity measurement was negligible. Also, whereas PDE7A mRNA was relatively highly expressed in all three cell types, protein expression for PDE7A, as determined by Western immunoblot analysis, was readily detectible in the two CEM cell types, but not in Jurkat cells. Similar observations of expression of PDE1B and 7A mRNA without concomitant expression of protein for these genes have been observed in lymphoid cells before. For example, primary B-CLL cells readily express mRNA for PDE1B, but express no PDE1B protein, as evidenced by a lack of any Ca^{2+}/calmodulin-stimulated PDE1 activity [7]. Similarly, human B lymphoid cell lines express high levels of PDE7A mRNA, but no detectible PDE7A protein or activity [15]. It has been suggested that in these cases, either much of the mRNA is not translated, or that much of the translated protein is inactive or unstable [15].

Normal T and B cells were earlier shown by regular RT-PCR to readily express PDE4B [19,27,30,31], and by qRT-PCR PDE4B was found to be the most abundant PDE4 isozyme expressed in normal human PBMC [19], and the second most abundant PDE4 isozyme, behind PDE4D, in purified human $CD4^+$ T cells [32]. In this study we find expression of PDE4B mRNA to be extremely low in the CEM and Jurkat T leukemic cells, representing only about 0.1–0.3% of the expression levels of PDE4A mRNA in these cells. Further, using two different polyclonal antibodies, no protein product for PDE4B was detected in these cells by Western immunoblot. By regular RT-PCR, we had previously shown an absence of expression of PDE4B mRNA in Molt 4 [30] and an absence of expression of PDE4B protein in parental CEM [3] T leukemic cell lines, and others reported an absence of any PDE4B expression in Jurkat cells as well [33,34], suggesting that there is a general loss of expression of PDE4B common to T leukemic cells. The reason for the loss of PDE4B expression in T leukemic cells relative to normal T cells is unclear. It may represent a selective loss of expression of this gene as a result of long term culturing of these cells, or it may represent a loss of expression that occurs as a result of their transformed state. Further work will be needed to distinguish between these possibilities. Also, although B-CLL cells exhibited a large upregulation of PDE7B relative to normal PBMC and isolated B cells, yielding an expression of PDE7B in B-CLL cells comparable to that of the expression of PDE7A [19], in contrast, we find expression of PDE7B in T leukemic cells to be extremely low, amounting to only about 0.07–0.1% of the expression of PDE7A in these cells. This indicates that in contrast to B leukemic cells, PDE7B is not readily upregulated in its expression in T leukemic cells. This difference in PDE7B expression could, however, represent a difference between primary vs. cultured cells, and studies examining PDE7B expression in T-CLL cells would be needed to definitively conclude that this represents a true difference between the different cell lineages of these different types of leukemias.

The potential of PDE7 as a therapeutic target to treat inflammatory illnesses has been investigated in a number of laboratories, and it still remains controversial [35]. Although based on antisense targeted against PDE7A1, PDE7 was reported to be required for T lymphocyte activation [11], T cells from PDE7A-deficient mice were activated normally by CD3/CD28 [12], and PDE7 inhibitors did not impair CD3/CD28-dependent activation of T cells [13]. However, there is some suggestion that PDE7 may play a role as a target for treating inflammation in conjunction with inhibition of PDE4, since the PDE7 inhibitor, BRL 50481, enhanced the effects of the PDE4 inhibitor, rolipram, on inhibition of lymphocyte proliferation and cytokine release [14]. Additionally, T-2585, a potent PDE4 inhibitor (IC_{50} = 0.013 nM), which also inhibits PDE7 with an IC_{50} = 1.7 μM, inhibited proliferation and cytokine release from T cells under conditions in which the highly selective PDE4 inhibitor, piclamilast had no effect [36]. Similarly, the PDE inhibitor, ASB16165, which inhibits PDE7A with an IC_{50} = 15 nM and PDE4 with an IC_{50} = 2.1 μM, also inhibited CD3/CD28-stimulated T cell proliferation and cytokine release [37]. Additionally, in an in vivo mouse model of smoke-induced lung inflammation, combined antisense inhibition of the expression of PDEs 4B, 4D and 7A produced a much greater anti-inflammatory effect than use of the PDE4-selective inhibitor, roflumilast, alone [38]. These and similar observations whereby PDE3 inhibitors, though inactive by themselves, potentiated the anti-inflammatory actions of PDE4 inhibitors, have led to the suggestions that PDE inhibitors with broader PDE selectivity, targeting multiple PDE isozymes, such as PDE3/4, PDE 4/7, or PDE 3/4/7 inhibitors, may prove to have greater clinical efficacy as anti-inflammatory agents than highly selective PDE4 inhibitors alone [39,40].

As with development of PDE inhibitors as anti-inflammatory agents, development of PDE inhibitors as anti-leukemic agents capable of inducing apoptosis of leukemic cells had also pointed to PDE4 as a primary PDE gene family to target [7,8]. Although, B-CLL cells isolated from a subset of patients resistant to this effect of PDE4 inhibitors could be induced to undergo apoptosis by the co-administration of a PDE3 inhibitor along with a PDE4 inhibitor, indicating that in some circumstances the concurrent inhibition of PDE3 along with PDE4 may be important [9]. Also, it had been shown that cultured B lymphoblastoid cells derived from a patient with ALL could be induced to undergo apoptosis both by pharmacological inhibition of PDE1 and PDE4, as well as by antisense targeting of PDE1B1, which is expressed in these cells [41]. In each of these cases in which these different PDEs were targeted, induction of apoptosis was selective for leukemic cells, with little or no apoptosis seen in normal lymphocytes in response to these same treatments [7,42,43]. Similarly, the recent discovery that PDE7B is highly upregulated in B-CLL, and that PDE7-selective and dual PDE4/7 inhibitors can induce apoptosis of these cells with little effect on normal lymphocytes has led to the suggestion that PDE7B may also be a good therapeutic target for treatment of B-CLL [19]. Inasmuch as the expression of PDE7A was still somewhat greater than that of PDE7B in these B-CLL cells, and inasmuch as the inhibitors used do not distinguish between the two PDE7 subtypes, it cannot really be ascertained yet if PDE7A, PDE7B or both of these are the targets linked to apoptosis of these cells. In our study of T-ALL cells presented here, in contrast to B-CLL cells, in which PDEs 4B and 7B are major expressed forms, we find almost no expression of PDE4B or PDE7B in these cells. Rather, the major expressed forms in T-ALL cells are PDEs 3B, 4A, 4D, 7A and 8A. We also find that although selective inhibitors of PDE3, PDE4 and PDE7 have no effect on the viability of these cells, in the presence or absence of glucocorticoids, inhibition of PDEs 3, 4 and 7 together greatly potentiates the effects of glucocorticoids on these cells and reduces their viability almost completely, even in a strain of cells completely resistant to the effects of glucocorticoids. It is unclear why inhibition of PDEs 3 and 4 with the dual PDE3/4 inhibitor, zardaverine, is more effective than inhibition of these PDEs with their individual selective inhibitors, rolipram and milrinone, but it may be a reflection of the greater potency of zardaverine for inhibition of these PDE isoforms. These results suggest that in CEM cells, PDEs 3, 4 and 7 may all contribute to regulating a pool of

cAMP linked to apoptosis of these cells, and that PDE7 may thus have a functional role in this process. Inasmuch as there is almost no expression of PDE7B in these cells, it suggests, that at least for these T-ALL cells, PDE7A is the relevant target, rather than PDE7B. Our results support the notion that PDE inhibitors may be therapeutically useful for treating leukemias and for overcoming the glucocorticoid resistance that develops during treatment, and suggest that for some leukemic cell types, it may be necessary to inhibit more than one PDE isoform to achieve a full therapeutic effect.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bcp.2009.09.001.

References

[1] Lerner A, Epstein PM. Cyclic nucleotide phosphodiesterases as targets for treatment of haematological malignancies. Biochem J 2006;393:21–41.
[2] Ogawa R, Streiff MB, Bugayenko A, Kato GJ. Inhibition of PDE4 phosphodiesterase activity induces growth suppression, apoptosis, glucocorticoid sensitivity, p53, and p21(WAF1/CIP1) proteins in human acute lymphoblastic leukemia cells. Blood 2002;99:3390–7.
[3] Tiwari S, Dong H, Kim EJ, Weintraub L, Epstein PM, Lerner A. Type 4 cAMP phosphodiesterase (PDE4) inhibitors augment glucocorticoid-mediated apoptosis in B cell chronic lymphocytic leukemia (B-CLL) in the absence of exogenous adenylyl cyclase stimulation. Biochem Pharmacol 2005;69:473–83.
[4] Ploner C, Schmidt S, Presul E, Renner K, Schrocksnadel K, Rainer J, et al. Glucocorticoid-induced apoptosis and glucocorticoid resistance in acute lymphoblastic leukemia. J Steroid Biochem Mol Biol 2005;93:153–60.
[5] Tenor H, Staniciu L, Schudt C, Hatzelmann A, Wendel A, Djukanovic R, et al. Cyclic nucleotide phosphodiesterases from purified human CD4+ and CD8+ T lymphocytes. Clin Exp Allergy 1995;25:616–24.
[6] Giembycz MA, Corrigan CJ, Seybold J, Newton R, Barnes PJ. Identification of cyclic AMP phosphodiesterases 3, 4 and 7 in human CD4+ and CD8+ T-lymphocytes: role in regulating proliferation and the biosynthesis of interleukin-2. Br J Pharmacol 1996;118:1945–58.
[7] Kim DH, Lerner A. Type 4 cyclic adenosine monophosphate phosphodiesterase as a therapeutic target in chronic lymphocytic leukemia. Blood 1998;92:2484–94.
[8] Siegmund B, Welsch J, Loher F, Meinhardt G, Emmerich B, Endres S, et al. Phosphodiesterase type 4 inhibitor suppresses expression of anti-apoptotic members of the Bcl-2 family in B-CLL cells and induces caspase-dependent apoptosis. Leukemia 2001;15:1564–71.
[9] Moon E, Lee R, Near R, Weintraub L, Wolda S, Lerner A. Inhibition of PDE3B augments PDE4 inhibitor-induced apoptosis in a subset of patients with chronic lymphocytic leukemia. Clin Cancer Res 2002;8:589–95.
[10] Lee R, Wolda S, Moon E, Esselstyn J, Hertel C, Lerner A. PDE7A is expressed in human B-lymphocytes and is up-regulated by elevation of intracellular cAMP. Cell Signal 2002;14:277–84.
[11] Li L, Yee C, Beavo JA. CD3- and CD28-dependent induction of PDE7 required for T cell activation. Science 1999;283:848–51.
[12] Yang G, McIntyre KW, Townsend RM, Shen HH, Pitts WJ, Dodd JH, et al. Phosphodiesterase 7A-deficient mice have functional T cells. J Immunol 2003;171:6414–20.
[13] Nueda A, Garcia-Roger N, Domenech T, Godessart N, Cardenas A, Santamaria-Babi LF, et al. Phosphodiesterase 7A1 is expressed in human CD4+ naive T cells at higher levels than in CD4+ memory cells and is not required during their CD3/CD28-dependent activation. Cell Immunol 2006;242:31–8.
[14] Smith SJ, Cieslinski LB, Newton R, Donnelly LE, Fenwick PS, Nicholson AG, et al. Discovery of BRL 50481 [3-(N,N-dimethylsulfonamido)-4-methyl-nitrobenzene], a selective inhibitor of phosphodiesterase 7: in vitro studies in human monocytes, lung macrophages, and CD8+ T-lymphocytes. Mol Pharmacol 2004;66:1679–89.
[15] Bloom TJ, Beavo JA. Identification and tissue-specific expression of PDE7 phosphodiesterase splice variants. Proc Natl Acad Sci USA 1996;93:14188–92.
[16] Smith SJ, Brookes-Fazakerley S, Donnelly LE, Barnes PJ, Barnette MS, Giembycz MA. Ubiquitous expression of phosphodiesterase 7A in human proinflammatory and immune cells. Am J Physiol Lung Cell Mol Physiol 2003;284:L279–89.
[17] Hetman JM, Soderling SH, Glavas NA, Beavo JA. Cloning and characterization of PDE7B, a cAMP-specific phosphodiesterase. Proc Natl Acad Sci USA 2000;97:472–6.
[18] Gardner C, Robas N, Cawkill D, Fidock M. Cloning and characterization of the human and mouse PDE7B, a novel cAMP-specific cyclic nucleotide phosphodiesterase. Biochem Biophys Res Commun 2000;272:186–92.
[19] Zhang L, Murray F, Zahno A, Kanter JR, Chou D, Suda R, et al. Cyclic nucleotide phosphodiesterase profiling reveals increased expression of phosphodiesterase 7B in chronic lymphocytic leukaemia. Proc Natl Acad Sci USA 2008;105:19532–7.
[20] Seybold J, Thomas D, Witzenrath M, Boral S, Hocke AC, Burger A, et al. Tumor necrosis factor-alpha-dependent expression of phosphodiesterase 2: role in endothelial hyperpermeability. Blood 2005;105:3569–76.
[21] Lorthiois E, Bernardelli P, Vergne F, Oliveira C, Mafroud AK, Proust E, et al. Spiroquinazolinones as novel, potent, and selective PDE7 inhibitors. Part 1. Bioorg Med Chem Lett 2004;14:4623–6.
[22] Tenor H, Hedbom E, Hauselmann HJ, Schudt C, Hatzelmann A. Phosphodiesterase isoenzyme families in human osteoarthritis chondrocytes—functional importance of phosphodiesterase 4. Br J Pharmacol 2002;135:609–18.
[23] Soderling SH, Bayuga SJ, Beavo JA. Cloning and characterization of a cAMP-specific cyclic nucleotide phosphodiesterase. Proc Natl Acad Sci USA 1998;95:8991–6.
[24] Fisher DA, Smith JF, Pillar JS, St Denis SH, Cheng JB. Isolation and characterization of PDE8A, a novel human cAMP-specific phosphodiesterase. Biochem Biophys Res Commun 1998;246:570–7.
[25] Gamanuma M, Yuasa K, Sasaki T, Sakurai N, Kotera J, Omori K. Comparison of enzymatic characterization and gene organization of cyclic nucleotide phosphodiesterase 8 family in humans. Cell Signal 2003;15:565–74.
[26] Hoffmann R, Wilkinson IR, McCallum JF, Engels P, Houslay MD. cAMP-specific phosphodiesterase HSPDE4D3 mutants which mimic activation and changes in rolipram inhibition triggered by protein kinase A phosphorylation of Ser-54: generation of a molecular model. Biochem J 1998;333(Pt 1):139–49.
[27] Gantner F, Gotz C, Gekeler V, Schudt C, Wendel A, Hatzelmann A. Phosphodiesterase profile of human B lymphocytes from normal and atopic donors and the effects of PDE inhibition on B cell proliferation. Br J Pharmacol 1998;123:1031–8.
[28] Glavas NA, Ostenson C, Schaefer JB, Vasta V, Beavo JA. T cell activation up-regulates cyclic nucleotide phosphodiesterases 8A1 and 7A3. Proc Natl Acad Sci USA 2001;98:6319–24.
[29] Dong H, Osmanova V, Epstein PM, Brocke S. Phosphodiesterase 8 (PDE8) regulates chemotaxis of activated lymphocytes. Biochem Biophys Res Commun 2006;345:713–9.
[30] Jiang X, Paskind M, Weltzien R, Epstein PM. Expression and regulation of mRNA for distinct isoforms of cAMP-specific PDE-4 in mitogen-stimulated and leukemic human lymphocytes. Cell Biochem Biophys 1998;28:135–60.
[31] Gantner F, Tenor H, Gekeler V, Schudt C, Wendel A, Hatzelmann A. Phosphodiesterase profiles of highly purified human peripheral blood leukocyte populations from normal and atopic individuals: a comparative study. J Allergy Clin Immunol 1997;100:527–35.
[32] Peter D, Jin SL, Conti M, Hatzelmann A, Zitt C. Differential expression and function of phosphodiesterase 4 (PDE4) subtypes in human primary CD4+ T cells: predominant role of PDE4D. J Immunol 2007;178:4820–31.
[33] Erdogan S, Houslay MD. Challenge of human Jurkat T-cells with the adenylate cyclase activator forskolin elicits major changes in cAMP phosphodiesterase (PDE) expression by up-regulating PDE3 and inducing PDE4D1 and PDE4D2 splice variants as well as down-regulating a novel PDE4A splice variant. Biochem J 1997;321(Pt 1):165–75.
[34] Seybold J, Newton R, Wright L, Finney PA, Suttorp N, Barnes PJ, et al. Induction of phosphodiesterases 3B, 4A4, 4D1, 4D2, and 4D3 in Jurkat T-cells and in human peripheral blood T-lymphocytes by 8-bromo-cAMP and Gs-coupled receptor agonists. Potential role in beta2-adrenoreceptor desensitization. J Biol Chem 1998;273:20575–88.
[35] Giembycz MA, Smith SJ. Phosphodiesterase 7 (PDE7) as a therapeutic target. Drugs Future 2006;31:207–29.
[36] Nakata A, Ogawa K, Sasaki T, Koyama N, Wada K, Kotera J, et al. Potential role of phosphodiesterase 7 in human T cell function: comparative effects of two phosphodiesterase inhibitors. Clin Exp Immunol 2002;128:460–6.

[37] Kadoshima-Yamaoka K, Murakawa M, Goto M, Tanaka Y, Inoue H, Murafuji H, et al. ASB16165, a novel inhibitor for phosphodiesterase 7A (PDE7A), suppresses IL-12-induced IFN-gamma production by mouse activated T lymphocytes. Immunol Lett 2009;122:193–7.
[38] Fortin M, D'Anjou H, Higgins ME, Gougeon J, Aube P, Moktefi K, et al. A multitarget antisense approach against PDE4 and PDE7 reduces smoke-induced lung inflammation in mice. Respir Res 2009;10:39.
[39] Giembycz MA. Can the anti-inflammatory potential of PDE4 inhibitors be realized: guarded optimism or wishful thinking? Br J Pharmacol 2008; 155:288–90.
[40] Spina D. PDE4 inhibitors: current status. Br J Pharmacol 2008;155:308–15.
[41] Jiang X, Li J, Paskind M, Epstein PM. Inhibition of calmodulin-dependent phosphodiesterase induces apoptosis in human leukemic cells. Proc Natl Acad Sci USA 1996;93:11236–41.
[42] Epstein PM. Antisense inhibition of phosphodiesterase expression. Methods 1998;14:21–33.
[43] Meyers JA, Su DW, Lerner A. Chronic lymphocytic leukemia and B and T cells differ in their response to cyclic nucleotide phosphodiesterase inhibitors. J Immunol 2009;182:5400–11.
[44] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001;25:402–8.