0026-895X/99/010170-05$3.00/0

MOLECULAR PHARMACOLOGY, **56:**170–174 (1999).

Phosphodiesterase 4B2 Is the Predominant Phosphodiesterase Species and Undergoes Differential Regulation of Gene Expression in Human Monocytes and Neutrophils

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Received January 15, 1999; accepted March 26, 1999

This paper is available online at http://www.molpharm.org

ABSTRACT

The type 4 phosphodiesterase (PDE4) is the predominant PDE isozyme in various leukocytes and plays a key role in the regulation of inflammatory cell activation. There are four PDE4 subtypes (A, B, C, and D), and within each subtype, there are multiple variants. Very recently, we found in monocytes that PDE4B gene expression is selectively induced by lipopolysaccharide (LPS) and that the induction is inhibited by interleukin (IL)-10 and IL-4. In this study, we show that the PDE4B gene is constitutively expressed in neutrophils and that this expression remains unaffected by LPS or IL-10. PDE4B is the predominant subtype in neutrophils and in unstimulated or LPS-stimulated monocytes, and in these cells, the PDE4B2 variant is the only detectable molecular species of PDE4B. Therefore, PDE4B2 is the predominant PDE isoform in human neutrophils and monocytes, and its expression is regulated differently by these two cell types. Furthermore, leukocytes are the most dominant source of PDE4B2, suggesting that PDE4B2 is a relatively specific target for discovering anti-inflammatory drugs.

Phosphodiesterases (PDEs; E.C. 3.1.4.17) constitute a superfamily of enzymes catalyzing the hydrolysis of the cyclic nucleotides: cAMP and cGMP. PDEs are the only cellular pathways for the degradation of cAMP and cGMP, underscoring their critical role in regulation of the intracellular levels of these second messengers and, consequently, functional responses of cells to a variety of extracellular agents such as hormones and neurotransmitters. There are nine structurally, biochemically and pharmacologically distinct PDE families, PDE 1–9, which are differentially expressed among various tissues and cells (Beavo, 1995; Manganiello et al., 1995; Fisher et al., 1998a,b; Soderling et al., 1998a,b). It is well established in various leukocytes, including neutrophils and monocytes, that PDE4, a cAMP-specific PDE, is the predominant PDE isozyme and that it plays a key role in the activation of inflammatory cells (Dent et al., 1994; Nicholson and Shahid, 1994; Muller et al., 1995; Palfreyman and Souness, 1996; Torphy, 1998). Thus, there has been significant interest in PDE4-selective inhibitors as a potential therapy for inflammatory diseases such as asthma, allergy, and arthritis. Nevertheless, because PDE4 is also present in many other tissues and cells, including brain (Dent et al., 1994; Nicholson and Shahid, 1994; Beavo, 1995; Manganiello et al., 1995; Muller et al., 1995; Palfreyman and Souness, 1996; Torphy, 1998), PDE4 inhibitors may have significant side effects. Indeed, emesis has been a significant issue with known PDE4 inhibitors that is likely to be due primarily to an action of the drugs in the brain (Palfreyman and Souness, 1996; Torphy, 1998).

Recently, molecular biological studies have revealed that within the PDE4 family, there are four subtypes (A, B, C, and D), each of which is derived from a distinct gene (Livi et al., 1990; Bolger et al., 1993; McLaughlin et al., 1993; Obernolte et al., 1993; Baecker et al., 1994; Engels et al., 1995), and that within each subtype, there are multiple variants generated by alternative splicing among the 5′-end exons and/or the use of different transcription initiation sites (Bolger, 1994). There are three highly homologous regions among various PDE4 molecules: a catalytic domain located in the central region of the protein sequence and two upstream conserved regions (UCR1 and UCR2) (Bolger et al., 1993; Bolger, 1994). PDE4 enzymes can be roughly divided into two groups: "long forms," having both UCR1 and UCR2, and "short forms," lacking UCR1. UCR1 and UCR2 may be involved in the regulation of the enzyme activity (Huston et al., 1996; Bolger et al., 1997; Owens et al., 1997; Torphy, 1998). Although there have been some studies on expression profiles of the mRNAs in various tissues and cells including some leukocytes (Conti et al., 1992; Engels et al., 1994, 1995; Muller et al., 1995; Gantner et al., 1997), no PDE4 molecule has been identified as a predominant PDE4 species in particular leukocytes. It can be reasoned that if there is a PDE4

ABBREVIATIONS: PDE, phosphodiesterase; LPS, lipopolysaccharide; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA end; IL, interleukin; UCR, upstream conserved region.

species that is predominantly present in inflammatory cells but not in other tissues, including brain, targeting such a molecule could be an effective approach to discovering anti-inflammatory drugs with reduced side effect potential.

In the present study, we find that PDE4B2 is the predominant molecular species of PDE in human monocytes and neutrophils, although its expression undergoes differential regulation in these two cell types. We further find that among various tissues and cells, leukocytes are the major source of PDE4B2. These results suggest that PDE4B2 may be a specific target for anti-inflammatory drug discovery.

Materials and Methods

Cell Preparation and Treatment. Human monocytes and neutrophils were prepared from fresh blood of healthy adult donors via elutriation (Wahl et al., 1994) and Ficoll-Paque centrifugation (Wang et al., 1994a), respectively. The purity of the cell preparations was greater than 95% as judged by Wright's staining and, for monocytes, by immunofluorescence assay using anti-CD14. Cells were suspended at a density of 1×10^6 cells/ml in RPMI 1640 medium, which was supplemented with 1% each of penicillin-streptomycin, nonessential amino acids, and L-glutamine and with 10% FBS (all from GIBCO, Grand Island, NY), and incubated for 1 h before each treatment at 37°C in a humidified atmosphere of 5% CO_2/95% air. The cells were treated with appropriate agents for various periods of time as indicated.

Competitive Polymerase Chain Reaction (PCR) for Quantifying PDE4 mRNAs. The primers for competitive PCR analysis (Becker-Andre and Hahlbrock, 1989; Wang et al., 1989; Gilliland et al., 1990) of PDE4 subtype mRNAs were designed as follows: PDE4A (GenBank accession no. L20965), 5′-TCGAGGAAGCTCTGGATGCAAC-3′ and 5′-TCTCAGGAGGGACAAGAGGACAAG-3′; PDE4B (accession no. L20966), 5′-TTGGAGTCAGAAAGCAAGACCAG-3′ (P1) (Fig. 1) and 5′-CAGGGGAAGGAAGTAAAATGTGG-3′ (P2); PDE4C (accession no. L20968), 5′-ACACTGAACTCCTGTCCCCTGAAG-3′ and 5′-GATGTGACTCAAGAGTGACCACTGG-3′, and PDE4D (accession no. L20970): 5′-TCGTTCTCCTGACACGTAACAGTG-3′ and 5′-TCCTCCTACTGGTAACAGATTCGTG-3′. All the PDE4-subtype PCR fragments corresponded to regions downstream of the catalytic domains and therefore were able to detect all known variants derived from the gene of each PDE4 subtype. The sizes of the PCR fragments were 546, 506, 410, and 479 bp for subtypes A, B, C, and D, respectively. PCR MIMICs of PDE4 subtypes for competitive PCR were prepared by using PCR MIMIC Construction Kit (Clontech, Palo Alto, CA), and had the sizes 450, 606, 606, and 606 bp, respectively. Total cellular RNA was prepared using TRIzol Reagent (GIBCO), treated with RNase-free DNase (Ambion, Austin, TX), and then reverse-transcribed into cDNA with the use of Advantage RT-for-PCR Kit (Clontech). PCR was performed by using Ex *Taq* DNA Polymerase (Takara, Madison, WI) in a total volume of 50 μl for 30 cycles using 2 μl of reverse-transcribed cDNA solution (for PDE4 subtypes, equivalent to 0.1 μg of total RNA) or of 10-fold-diluted cDNA solution (for β-actin or glyceraldehyde phosphate dehydrogenase) with the following cycle parameters: denaturation, 94°C for 30 s; annealing, 60°C for 30 s; and extension, 68°C for 1 min. For each target cDNA, a preliminary PCR was performed using 2 μl of each of 10-fold serial dilutions of the corresponding PCR MIMIC from 10^{-4} to 1 amol/μl. Then, based on the particular concentration range determined, in which equal amounts of the target and the MIMIC were produced, 2-fold serial dilutions of the MIMIC (total 7 points) were used to perform fine-tuned competitive PCR. After PCR amplification, 10 μl of each reaction solution was separated by electrophoresis on a 2% ethidium bromide-agarose gel. The PCR product bands were quantified by using ScanJet 3c (Hewlett-Packard, Palo Alto, CA) with the software Scan Analysis (BIOSOFT, Ferguson, MO). Competition equivalence point, representing the absolute amount of the target cDNA, was determined by interpolation on a plot of the logarithm of the calculated molar ratios of signals for the MIMIC-derived product over the signals for the target cDNA-derived product versus the logarithm of the amount of the added MIMIC.

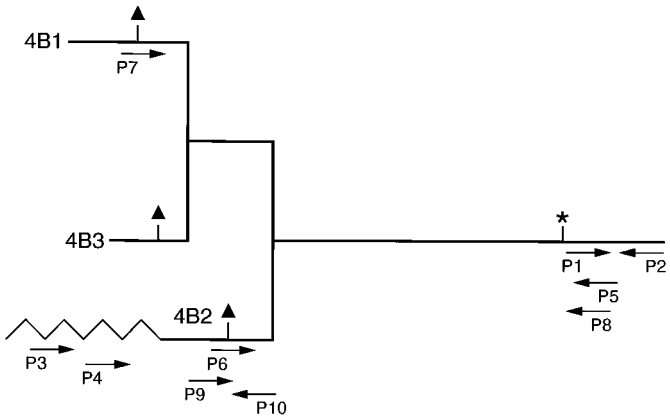

Fig. 1. Human PDE4B variants and locations of the PCR primers used. For more details on the structures of the various human PDE4B variants, see Bolger et al. (1993) and Huston et al. (1997). P1 and P2 were used in the competitive PCR for quantifying PDE4B mRNA. P3 and P4 were the adaptor primer and the nested adaptor primer used in the first and second PCRs, respectively, and P5 was the nested reverse primer for the second PCR in the 5′-RACE (P2 was used as the reverse primer for the first PCR). P6 and P7 were the forward primers used for detecting PDE4B2 and 4B1, respectively, in screening colonies in the 5′-RACE, whereas P8 was the common reverse primer in the screening. P9 and P10 were used in the determination of tissue distribution of PDE4B2. The arrows and asterisk indicate the initiation and termination codons, respectively.

Northern Blot Analysis of PDE4B. Northern blotting using total RNA was performed as described previously (Wang et al., 1994b). Total cellular RNA was prepared using TRIzol Reagent. The probes used corresponded to the same DNA regions as used for quantitative PCR and therefore were able to detect all known variants derived from the gene of each PDE4 subtype. The probes were generated using cDNAs (Clontech) from human testis (for PDE4A and PDE4C) or leukocytes (PDE4B and PDE4D) as templates.

Molecular Cloning of PDE4B Species by 5′-Rapid Amplification of cDNA Ends (RACE) PCR. Total cellular RNA was prepared using TRIzol Reagent. mRNA was prepared from total RNA by using BIOMAG mRNA Purification Kit (PerSeptive Biosystems, Framingham, MA). One microgram of mRNA was used to perform 5′-RACE PCR (Frohman et al., 1988) to clone PDE4B cDNAs by using the Marathon cDNA Amplification Kit (Clontech). After double-stranded cDNA synthesis and adaptor ligation, a PCR was performed using an adaptor primer (P3) and the PDE4B gene-specific primer 5′-CAGGGGAAGGAAGTAAAATGTGG-3′ (P2) corresponding to a region downstream of the stop codon and therefore able to detect all known PDE4B variants. The PCR was performed by using Ex *Taq* DNA Polymerase (Takara) in a total volume of 50 μl for 30 cycles using 5 μl of 10-fold-diluted double-stranded cDNA solution with the following cycle parameters: denaturation, 94°C for 1 min; annealing, 60°C for 30 s; and extension, 68°C for 5 min. After the first PCR, a second PCR was performed using a nested adaptor primer (P4) and the PDE4B gene-specific primer 5′-CTGGTCTTGCTTTCTGACTCCAA-3′ (P5) corresponding to a region downstream of the stop codon and, therefore, able to detect all known PDE4B variants. The PCR was performed by using Ex *Taq* DNA Polymerase (Takara) in a total volume of 50 μl for 30 cycles using 5 μl of the first PCR solution with the following cycle parameters: denaturation, 94°C for 1 min; annealing, 60°C for 30 s; and extension, 68°C for 3 min. The PCR product was cloned into the vector pNoTA/T7 from 5 Prime-3 Prime (Boulder, CO) according to the manufacturer's instructions. After transformation, colonies were screened by PCR using the upper primer 5′-CATGAAGGAGCACGGGGGC-3′ (P6) (specific for

PDE4B2) or 5′-ATGAAGAAAAGCAGGAGTGTGATGACG-3′ (P7) (specific for PDE4B1) and the lower primer 5′-GGGCCCTCTAGATTATGTATCCACGGG-3′ (P8). Finally, DNA inserts of positive colonies were sequenced for confirmation.

Semiquantitative PCR for Determining Tissue Distribution of PDE4B2. The primers for determining tissue distribution of PDE4B2 mRNA were designed as follows (GenBank accession no. M97515): 5′-GAGACCGTTCCCTCCGCCTTC-3′ (P9) and 5′-GCGGCTGCAGGCTGTCCATAG-3′ (P10). This PCR fragment, which is 254 bp long, corresponded to a region in the N terminus of PDE4B2 (McLaughlin et al., 1993), which is not present in PDE4B1 (Bolger et al., 1993) or PDE4B3 (Huston et al., 1997). Human multiple tissue cDNA panels, PCR ready for mRNA tissue distribution studies, were obtained from Clontech. PCR was performed using Ex *Taq* DNA Polymerase (Takara) and 1 (for PDE4B2) or 0.4 (for β-actin) ng of cDNA in a total volume of 50 μl by the "touchdown PCR technique" (Don et al., 1991; Roux, 1995). After predenaturation at 94°C for 1 min, the PCR was performed first for 10 cycles with the following parameters: denaturation, 94°C for 1 min; annealing, 64°C for 30 s; and extension, 68°C for 1 min, then for 21, 23, and 25 (for PDE4B2) or 14, 16, and 18 (for β-actin) cycles with the same parameters except that the annealing temperature was 60°C, followed by final extension at 72°C for 7 min. After PCR amplification at each of the cycle numbers, 10 μl of each reaction solution was separated by electrophoresis on a 2% ethidium bromide-agarose gel. The PCR product bands were quantified as described above.

Other Reagents. Lipopolysaccharide (LPS) and actinomycin D were purchased from Sigma Chemical Co. (St. Louis, MO). Recombinant human interleukin (IL)-10, IL-4, and transforming growth factor-β1 were from R&D Systems (Minneapolis, MN). Cyclosporin A, rapamycin, and dexamethasone were from Calbiochem (San Diego, CA).

Results

PDE4B Undergoes Differential Regulation of Gene Expression in Monocytes and Neutrophils. Very recently, we identified PDE4B as a typical inducible gene in human monocytes. Like the cytokines tumor necrosis factor-α, IL-1, IL-6, and IL-8 and the enzyme cyclooxygenase-2, PDE4B (but not A, C, or D) gene expression was induced by LPS and the induction was inhibited by IL-10 or IL-4 (Ma et al., 1999). In the present study, we found that human neutrophils, in contrast to monocytes, constitutively expressed PDE4B mRNA at high levels (Fig. 2). The expression of PDE4B mRNA in neutrophils was not further enhanced by LPS (Fig. 2A) or other neutrophil stimuli (e.g., granulocyte/macrophage colony-stimulating factor, IL-1, tumor necrosis factor-α, interferon-γ, platelet-activating factor, and *N*-formyl-Met-Leu-Phe) at concentrations known to cause optimal gene induction in neutrophils (data not shown). Even prolonged incubation (14–16 h) of neutrophils with LPS did not elevate the mRNA level (data not shown). None of the other subtype (A, C, and D) mRNAs were detectable by Northern blot analysis in resting neutrophils or in cells stimulated with any of the above agents (data not shown).

PDE4B mRNAs from neutrophils or monocytes (both resting and LPS stimulated), analyzed on a gel, exhibited very similar molecular sizes of about 4 kilobase (kb; Fig. 2B). Subsequent analysis of mRNA species by 5′-RACE PCR confirmed that there was only one molecular species of PDE4B in these cells (see below). The level of PDE4B mRNA in unstimulated monocytes was much lower than that in neutrophils, but LPS stimulation elevated the mRNA to a comparable level (Fig. 2B).

Treatment of neutrophils with the RNA synthesis inhibitor actinomycin D largely eliminated the PDE4B mRNA within 3 h (Fig. 3), indicating that the PDE4B mRNA undergoes a rapid turnover. However, the constitutive expression of PDE4B mRNA in neutrophils was not inhibited by any of the major gene induction inhibitors IL-10, IL-4, transforming growth factor-β, cyclosporin A, rapamycin, or dexamethasone, even though they all inhibit expression of other specific genes in neutrophils (Fig. 3).

PDE4B Is the Predominant Subtype in Neutrophils and Monocytes. To obtain a clearer picture of PDE4 subtype profile in these cells, competitive PCR was used to quantify PDE4-subtype mRNAs (Table 1). In neutrophils, subtype B accounted for essentially all of the total PDE4

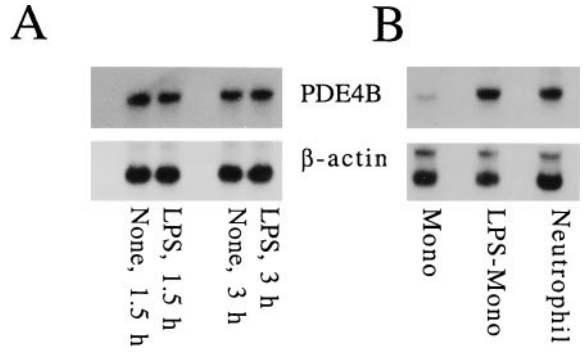

Fig. 2. Effects of LPS on PDE4B mRNA production in human neutrophils and monocytes. A, neutrophils (45 million cells per reaction) were incubated in the absence or presence of 100 ng/ml LPS for 1.5 or 3 h. Total RNA was extracted, and 10 μg of each sample was subjected to Northern blot analysis. B, monocytes (30 million cells per reaction) were incubated in the absence or presence of 100 ng/ml LPS for 1.5 h. Total RNA was extracted, and 10 μg of each sample was subjected to Northern blot analysis probed for PDE4B, along with 10 μg of neutrophil total RNA. After probing for PDE4B, the membranes were stripped and reprobed for β-actin as an internal control. Experimental details are described in *Materials and Methods*.

TABLE 1

Distribution of PDE4 subtypes in human neutrophils and monocytes

Numbers are mean ± S.E.M. in amol/fmol β-actin (neutrophil, $n = 6$) or glyceraldehyde phosphate dehydrogenase (monocytes, $n = 3$) from separate RNA preparations. Percentages in parentheses are those of each subtype in total PDE4 (A + B + C + D). Because the numbers were obtained by using an identical amount of RNA per reaction tube, the absolute levels of PDE4 subtypes can be compared between the two different cell types. Experimental details are described in *Materials and Methods*.

Cell	PDE4 Subtype mRNA			
	A	B	C	D
Neutrophil	<0.004 (<0.1%)	70.174 ± 4.336 (99.7%)	<0.002 (<0.1%)	0.192 ± 0.069 (0.3%)
Monocyte	0.970 ± 0.252 (14.4%)	5.393 ± 0.290 (80.2%)	0.008 ± 0.002 (0.1%)	0.352 ± 0.091 (5.2%)
LPS-monocyte	1.322 ± 0.483 (3.5%)	35.382 ± 1.432 (95.5%)	0.005 ± 0.002 (<0.1%)	0.322 ± 0.038 (0.9%)

mRNA. In unstimulated monocytes, subtype B (5.4 amol/fmol glyceraldehyde phosphate dehydrogenase) accounted for about 80% of the total cellular PDE4 mRNA (6.7 amol), less than that in neutrophils. In LPS-stimulated monocytes, subtype B mRNA was selectively elevated by about 7-fold to a level (35.4 amol) comparable to that of neutrophils, accounting for about 96% of the total PDE4 mRNA (37.0 amol). These results are consistent with the observations by Northern blot analysis.

PDE4B2 Is the Predominant PDE4B Species in Neutrophils and Monocytes. To identify the molecular species of PDE4B in neutrophils and monocytes, we used 5′-RACE PCR to clone PDE4B cDNAs. Because all known variants within each PDE4 subtype differ only in the 5′-end sequences, this technique allowed for detection of all possible variants of the PDE4B subtype. All our PDE4B clones generated by 5′-RACE PCR were PDE4B2 in colony screening by PCR. Furthermore, several clones from each cell type (seven from neutrophils, six from resting monocytes, and seven from LPS-stimulated monocytes, with two or three different RNA preparations of each cell type) were sequenced for confirmation, and all of them were PDE4B2. PDE4B1-specific sequence was not detected in neutrophils or in monocytes (unstimulated or LPS stimulated).

Leukocytes Are the Predominant Source of PDE4B2. We used a semiquantitative PCR technique to determine the relative levels of PDE4B2 mRNA in various tissues. Each PCR was performed for various cycles, and after each selected cycle, a portion of the PCR product was quantified. Then the amounts of the PCR product at various cycle numbers were plotted to confirm the linearity of PCR product accumulation (Fig. 4A). The relative levels of PDE4B2 in various tissues were determined by normalizing PDE4B2 against β-actin in the linear ranges (31 or 33 cycles for PDE4B2 and 26 cycles for β-actin). Among the tissues tested, leukocytes expressed the highest level of PDE4B2 (Fig. 4, B and C).

Discussion

There is a striking difference in the regulation of PDE4B gene expression between human neutrophils and monocytes. In neutrophils, the gene is constitutively expressed at a high level, and it is neither stimulated by neutrophil activation nor suppressed by gene transcription inhibitors. On the other hand, in monocytes, PDE4B behaves as a typical inducible gene. The absolute level of mRNA is low in unstimulated cells, and expression is induced by LPS and inhibited by IL-10. Of three human PDE4B cDNA species, B1 (Bolger et al., 1993), B2 (McLaughlin et al., 1993; Bolger et al., 1993), and B3 (Huston et al., 1997), only B2 is detectable in neutrophils or monocytes. Hence, these two cell types may utilize a common PDE4B mRNA splicing mechanism and/or a common transcription start site, despite their differential regulation. Presently, nothing is known about the genomic structure or promoter of the PDE4B gene. Cloning and characterization of the genomic DNA would help elucidate the molecular mechanisms by which PDE4B gene expression is regulated differentially between neutrophils and monocytes.

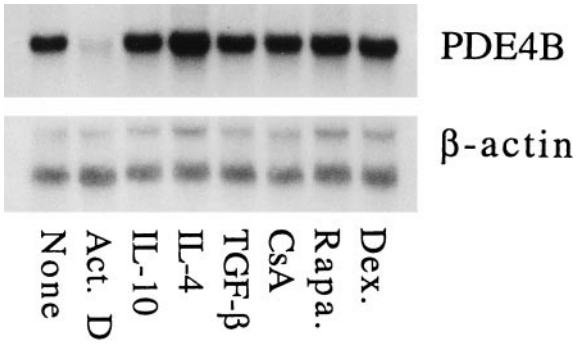

Fig. 3. Effects of various gene transcription inhibitors on PDE4B mRNA production in human neutrophils. Neutrophils (45 million cells per reaction) were incubated in the absence or presence of actinomycin D (Act. D, 5 μg/ml), IL-10 (10 ng/ml), IL-4 (10 ng/ml), transforming growth factor-β1 (TGF-β, 10 ng/ml), cyclosporin A (CsA, 5 μg/ml), rapamycin (Rapa., 100 ng/ml), or dexamethasone (Dex., 1 μg/ml) for 3 h. Total RNA was extracted, and 10 μg of each sample was subjected to Northern blot analysis probed for PDE4B. The membrane was stripped and reprobed for β-actin as an internal control. Experimental details are described in *Materials and Methods*.

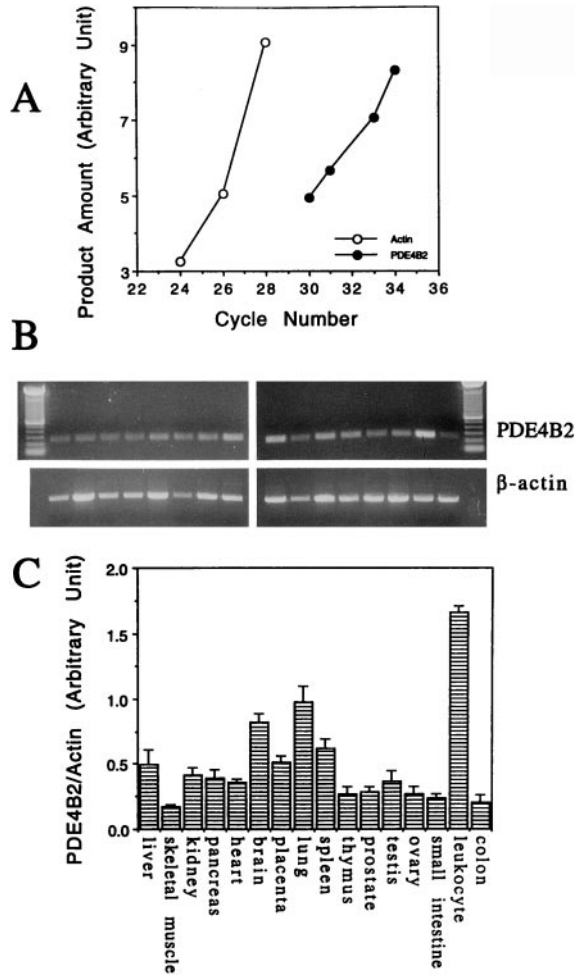

Fig. 4. Tissue distribution of PDE4B2 in humans. A, the raw data on quantified PDE4B2 and β-actin levels in leukocyte cDNA from a typical experiment after various cycles of PCR are presented to show that the tissue distribution study was conducted in linear ranges of PCR product accumulation. B, the raw data on PDE4B2 and β-actin levels in cDNAs of various tissues from a typical experiment after 31 (PDE4B2) or 26 (β-actin) cycles of PCR are shown. C, summarized data on tissue distribution of PDE4B2 are shown. The plotted data are mean ± S.E.M. from four experiments, using the raw data generated after 31 (PDE4B2) or 26 (β-actin) cycles of PCR. Experimental details are described in *Materials and Methods*.

Despite the differential regulation of gene expression, the relative levels of PDE4B2 mRNA are very high in both cells, accounting for as much as 95% to 100% of total cellular PDE4 mRNA in neutrophils and in LPS-stimulated monocytes. To our knowledge, this is the first report showing almost exclusive presence in a particular cell type of a single molecular species of PDE4, despite the fact that there are four different PDE4 subtypes, each composed of multiple variants (Bolger, 1994). Moreover, leukocytes express PDE4B2 at the highest level among all the tissues tested, suggesting that PDE4B2 may be a specific target for anti-inflammation. Almost all known PDE4 inhibitors are active against multiple PDE4 subtypes (Muller et al., 1995; Torphy, 1998). This lack of specificity of the known PDE4 inhibitors for PDE4 subtypes may be a contributing factor to their observed side effects. Apparently in neutrophils and monocytes, PDE4B2-specific compounds should retain the beneficial anti-inflammatory effects while having reduced potential for the emetic side effect associated with almost all known PDE4 inhibitors (Palfreyman and Souness, 1996; Torphy, 1998), because the expression level of PDE4B2 is relatively low in brain.

In summary, PDE4B2 may be an appropriate target for discovering anti-inflammatory drugs for those inflammatory diseases in which monocytes and/or neutrophils play an important pathological role. It is noteworthy that among the various PDE4B variants, B2 is the only "short form" lacking UCR1, and UCR1 has some regulatory functions (Huston et al., 1996; Bolger et al., 1997; Owens et al., 1997; Torphy, 1998). These differences between the "short" and "long" forms might indicate the feasibility of discovering inhibitors specific for PDE4B2 over B1 and B3 variants.

References

Baecker PA, Obernolt R, Bach C, Yee C and Shelton ER (1994) Isolation of a cDNA encoding a human rolipram-sensitive cyclic AMP phosphodiesterase (PDE IVD). *Gene* **138:**253–256.

Beavo JA (1995) Cyclic nucleotide phosphodiesterases: Functional implications of multiple isoforms. *Physiol Rev* **75:**725–748.

Becker-Andre M and Hahlbrock K (1989) Absolute mRNA quantification using the polymerase chain reaction (PCR): A novel approach by a PCR aided transcript titration assay (PATTY). *Nucleic Acids Res* **17:**9437–9446.

Bolger GB (1994) Molecular biology of the cyclic AMP-specific cyclic nucleotide phosphodiesterases: A diverse family of regulatory enzymes. *Cell Signal* **8:**851–859.

Bolger G, Erdogan S, Jones RE, Loughney K, Scotland G, Hoffmann R, Wilkinson I, Farrell C and Houslay MD (1997) Characterization of five different proteins produced by alternatively spliced mRNAs from the human cAMP-specific phosphodiesterase PDE4D gene. *Biochem J* **328:**539–548.

Bolger G, Michaeli T, Martins T, St. John T, Steiner B, Rodgers L, Riggs M, Wichael M and Ferguson K (1993) A family of human phosphodiesterases homologous to the dunce learning and memory gene product of *Drosophila melanogaster* are potential targets for antidepressant drugs. *Mol Cell Biol* **13:**6558–6571.

Conti M, Swinnen JV, Tsikalas KE and Jin SLC (1992) Structure and regulation of the rat high-affinity cyclic AMP phosphodiesterases: A family of closely related enzymes. *Adv Second Messenger Prot Phosphoryl Res* **25:**87–99.

Dent G, Magnussen H and Rabe KF (1994) Cyclic nucleotide phosphodiesterases in the human lung. *Lung* **172:**129–146.

Don RH, Cox PT, Wainwright BJ, Baker K and Mattick JS (1991) 'Touchdown' PCR to circumvent spurious priming during gene amplification. *Nucleic Acids Res* **19:**4008.

Engels P, Fichtel K and Lubbert H (1994) Expression and regulation of human and rat phosphodiesterase type IV isogenes. *FEBS Lett* **350:**291–295.

Engels P, Sullivan M, Muller T and Lubbert H (1995) Molecular cloning and functional expression in yeast of a human cAMP-specific phosphodiesterase subtype (PDE IV-C). *FEBS Lett* **358:**305–310.

Fisher DA, Smith JF, Pillar JS, St. Denis SH and JB Cheng (1998a) Isolation and characterization of PDE8A, a novel human cAMP-specific phosphodiesterase. *Biochem Biophys Res Commun* **246:**570–577.

Fisher DA, Smith JF, Pillar JS, St. Denis SH and JB Cheng (1998b) Isolation and characterization of PDE9A, a novel human cGMP-specific phosphodiesterase. *J Biol Chem* **273:**15559–15564.

Frohman MA, Dush MK and Martin GR (1988) Rapid production of full-length cDNAs from rare transcripts: Amplification using a single gene-specific oligonucleotide primer. *Proc Natl Acad Sci USA* **85:**8998–9002.

Gantner F, Tenor H, Gekeler V, Schudt C, Wendel A and Hatzelmann A (1997) Phosphodiesterase profiles of highly purified human peripheral blood leukocyte populations from normal and atopic individuals: A comparative study. *J Allergy Clin Immunol* **100:**527–535.

Gilliland G, Perrin S, Blanchard K and Bunn F (1990) Analysis of cytokine mRNA and DNA: Detection and quantification by competitive polymerase chain reaction. *Proc Natl Acad Sci USA* **87:**2725–2729.

Huston E, Pooley L, Julien P, Scotland G, McPhee I, Sullivan M, Bolger G and Houslay MD (1996) The human cyclic AMP-specific phosphodiesterase PDE-46 (HSPDE4A4B) expressed in transfected COS7 cells occurs as both particulate and cytosolic species that exhibit distinct kinetics of inhibition by the antidepressant rolipram. *J Biol Chem* **271:**31334–31344.

Huston E, Lumb S, Russell A, Catterall C, Ross AH, Steele MR, Bolger GB, Perry MJ, Owens RJ and Houslay MD (1997) Molecular cloning and transient expression in COS7 cells of a novel human PDE4B cAMP-specific phosphodiesterase, HSPDE4B3. *Biochem J* **328:**549–558.

Livi GP, Kmetz P, Mchale MM, Cieslinski LB, Sathe GM, Taylor DP, Davis RL, Torphy TJ and Balcarek JM (1990) Cloning and expression of cDNA for a human low-Km, rolipram-sensitive cyclic AMP phosphodiesterase. *Mol Cell Biol* **10:**2678–2686.

Ma D, Wu P, Egan RW, Billah MM and Wang P (1999) Phosphodiesterase 4B gene transcription is activated by lipopolysaccharide and inhibited by interleukin-10 in human monocytes. *Mol Pharmacol* **55:**50–57.

Manganiello VC, Murata T, Taira M, Belfrage P and Degerman E (1995) Diversity in cyclic nucleotide phosphodiesterase isozyme families. *Arch Biochem Biophys* **322:**1–13.

McLaughlin MM, Cieslinski LB, Burman M, Torphy TJ and Livi GP (1993) A low-Km, rolipram-sensitive, cAMP-specific phosphodiesterase from human brain: Cloning and expression of cDNA, biochemical characterization of recombinant protein, and tissue distribution of mRNA. *J Biol Chem* **268:**6470–6476.

Muller T, Engles P and Fozard JR (1995) Subtypes of the type 4 cAMP phosphodiesterases: Structure, regulation and selective inhibition. *Trends Pharmacol Sci* **17:**294–298.

Nicholson CD and Shahid M (1994) Inhibitors of cyclic nucleotide phosphodiesterase isoenzymes: Their potential utility in the therapy of asthma. *Pulm Pharmacol* **7:**1–17.

Obernolte R, Bhakta S, Alvarez R, Bach C, Zuppan P, Mulkins M, Jarnagin K and Shelton ER (1993) Isolation of a cDNA encoding a human rolipram-sensitive cyclic AMP phosphodiesterase (PDE IVD). *Gene* **129:**239–247.

Owens RJ, Catterall C, Batty D, Jappy J, Russell A, Smith B, O'Connell J and Perry MJ (1997) Human phosphodiesterase 4A: Characterization of full-length and truncated enzymes expressed in COS cells. *Biochem J* **326:**53–60.

Palfreyman MN and Souness JE (1996) Phosphodiesterase type IV inhibitors. *Prog Med Chem* **33:**1–52.

Roux KH (1995) Optimization and troubleshooting in PCR. *PCR Methods Appl* **4:**5185–5194.

Soderling SH, Bayuga SJ and Beavo JA (1998a) Cloning and characterization of a cAMP-specific cyclic nucleotide phosphodiesterase. *Proc Natl Acad Sci USA* **95:**8991–8996.

Soderling SH, Bayuga SJ and Beavo JA (1998b) Identification and characterization of a novel family of cyclic nucleotide phosphodiesterases. *J Biol Chem* **273:**15553–15558.

Torphy TJ (1998) Phosphodiesterase isozymes: Molecular targets for novel antiasthma agents. *Am J Respir Crit Care Med* **157:**351–370.

Wahl LM, Katona IM, Wilder RL, Winter CC, Haroui B, Scher I and Wahl SM (1994) Isolation of human mononuclear cell subsets by counterflow centrifugal elutriation (CCE). I. Characterization of B-lymphocyte-, T-lymphocyte-, and monocyte-enriched fractions by flow cytometric analysis. *Cell Immunol* **85:**373–383.

Wang AM, Doyle MV and Mark DF (1989) Quantification of mRNA by the polymerase chain reaction. *Proc Natl Acad Sci USA* **86:**9717–9721.

Wang P, Wu P, Anthes JC, Siegel MI, Egan RW and Billah MM (1994a) Interleukin-10 inhibits interleukin-8 production in human neutrophils. *Blood* **83:**2678–2683.

Wang P, Wu P, Siegel MI, Egan RW and Billah MM (1994b) IL-10 inhibits transcription of cytokine genes in human peripheral blood mononuclear cells. *J Immunol* **153:**811–816.

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