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Expression and regulation of human and rat phosphodiesterase type IV isogenes

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Abstract

Type IV phosphodiesterases (PDE IV) specifically hydrolyze cAMP and are inhibited by rolipram. RT-PCR was applied to analyze the expression patterns of mRNAs for four cloned human and rat phosphodiesterase type IV isogenes (PDE IV-A, -B, -C and -D). Although these patterns were mostly coincident for the human and rat PDE IV genes, some differences were found between the two species. PDE IV-A expression was detectable in human blood but not in rat blood, suggesting a species-specific difference in the expression of this PDE IV isogene. PDE IV-C was neither detected in human or rat blood nor in different cell populations of the human immune system. It is further demonstrated that the PDE IV isogene expression is differentially regulated by cAMP in different cell types.

Key words: cAMP; RT-PCR; SH-SY5Y cell; U937 cell; Jurkat cell

1. Introduction

Cyclic nucleotide phosphodiesterases (PDEs) are involved in the regulation of cellular processes by modulating intracellular cyclic nucleotide levels. The superfamily of phosphodiesterases can be divided into eight types or subfamilies [1–3]. They are characterized by their substrate affinities and specificities or by particular inhibitors [2,4,5]. The subfamilies are Ca^{2+}/Calmodulin-dependent PDEs (PDE I), cGMP-stimulated PDEs (PDE II), cGMP-inhibited PDEs (PDE III), cAMP-specific PDEs (PDE IV), cGMP-specific PDEs (PDE V), a photoreceptor PDE (PDE VI), a high affinity cAMP-specific PDE (PDE VII), and a PDE with similar K_m values for cAMP and cGMP (PDE VIII). In addition, multiple related genes as well as different mRNA splice forms create various isozymes within these subfamilies [1,6–10].

We have focused on the cAMP-specific phosphodiesterase subfamily (PDE IV). Recently, four rat and four human cDNA isoforms of this subtype were identified [6,11–14]. They are characterized by their selective low-K_m hydrolysis of cAMP and their sensitivity to rolipram and Ro20–1724 [1,4]. As for other PDE-types different splice variants have been described for most of the cloned PDE IV isoforms [6,7,11,13,14].

The existence of a large number of PDE isozymes suggests a specific involvement in multiple mechanisms governing the regulation of cAMP levels. PDE IV enzymes are of particular interest because they have been demonstrated to be involved in the regulation of processes like learning and memory [15,16], cell survival [17], and activation of the immune response [18]. Increased levels of PDE IV were found in monocytes of individuals with atopic dermatitis [19].

PDE IV activity can be regulated by cAMP analogues, agents that increase intracellular cAMP levels or hormones such as follicle-stimulating hormone (FSH) [20–23]. Here, we present the results of an extensive RT-PCR analysis comparing human and rat PDE IV isogene expression in various tissues. The results of the distribution analysis revealed a differential expression pattern of three isogenes and the absence of one isoform in cells of the immune system. Additionally, the influence of cAMP on PDE IV mRNA expression was determined in three different human cell types. PDE IV-A,-B and PDE IV-D, but not PDE IV-C expression was differentially stimulated by cAMP in cells of the immune system. Interestingly, a similar regulation was not apparent in the neuronal cell line analyzed.

2. Material and methods

2.1. Oligonucleotides

PE21: 5′-TCAGAGCTGGCGCTTATGTAC-3′; PE32: 5′-CCGTATGCTTGTCACACAT-3′; hB1: 5′-AGCGGTGGTAGCGGTGACTC-3′; hB2–601: 5′-GCTGCGTGCAGGCTGTTGTG-3′; hD1: 5′-GCAAGATCGAGCACCTAGCA-3′, hD2: 5′-ACCAGACAACTCTGCTATTC-3′; rA1: 5′-GCGGCAAGAGCCTGCGCAAG-3′; rA2: 5′-ATGCCAGAGCTCAAGCTGT-3′; rB1: 5′-TCTCAGAGATGAGCAGATCA-3′; rB2–601: 5′-GCTGCGTGCAGGCTGTTGTG-3′; rC1: 5′-CTGCGGAAGTCTTGCCACAC-3′; rC2: 5′-AGACTCAGTTTCTGCTTGG-3′; rD1: 5′-CTGTCTCTTCGCAGAGGAGG-3′; rD2: 5′-GTGCATGATAACAGTCAGAGGC-3′; β-actin-5′: 5′-ATGGATGATGATATCGCCGCG-3′; β-actin-3′: 5′-GATCATGGCTGGGGTGTTGAA-3′.

2.2. RNA Isolation and RT-PCR

mRNA from different rat tissues was isolated using the guanidinium/acid phenol method [24] with 1 ml homogenization buffer per 100 mg of tissue. Human mRNA was obtained from Clontech. All human tumor cell lines used for RT-PCR analysis are available from the American Type Culture Collection (Rockville, MD). To reduce contamina-

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tion by genomic DNA, 20 μg RNA were treated for 15 min at 37°C with 10 units DNase I (RNase free, Boehringer Mannheim), followed by phenol/chloroform extraction and ethanol precipitation. Reverse transcription was performed with M-MLV reverse transcriptase (BRL) in PCR buffer. PCR reactions were performed with *Taq* DNA polymerase under the conditions recommended by the manufacturer (Boehringer Mannheim). For all four rat and human PDE IV isogenes, specific primers were designed and used under similar cycle conditions with 0.2 μCi [^{32}P]dCTP added to each reaction (34 cycles: 30 s at 95°C, 30 s at 55°C, 60 s at 72°C; rat PCR primers: rA1/rA2 for rPDE IV-A, rB1/rB2 for rPDE IV-B, rC1/rC2 for rPDE IV-C, and rD1/rD2 for rPDE IV-D; human PCR primers: PE21/PE32 for hPDE IV-A and -C, hB1/hB2 for hPDE IV-B, and hD1/hD2 for hPDE IV-D). Restriction of the PE21/PE32-derived PCR fragments either with *Bst*XI (hPDE IV-A specific) or *Eco*RV (hPDE IV-C specific) allowed the two different human isogenes to be distinguished. After electrophoretic separation of PCR products on 4% agarose gels (NuSieve Agarose, FMC), the gels were dried and exposed to X-ray film (Amersham) for 1 h. The expected sizes of the PCR fragments corresponding to the different rat isoforms were: PDEIV-A = 654 bp, rPDEIV-B = 470 bp, rPDEIV-C = 584 bp and rPDEIV-D = 492 bp. For the human isoforms hPDEIV-A and -C a 440 bp fragment was predicted which could then be digested either with *Bst*XI (hPDEIV-A 330 bp + 110 bp fragments) or with *Eco*RV (hPDEIV-C 345 bp + 95 bp fragments) as mentioned above. PCR with hPDEIV-B specific primers resulted in a 680 bp band and with hPDEIV-D specific primers in a 495 bp fragment.

Semi-quantitative PCR was performed under the same conditions. β-actin specific primers were used as internal standard. To avoid errors, 10 μl aliquots were taken after 25, 28, 31 and 34 amplification cycles. Radioactivity incorporated into the PCR-products was quantified by phosphorimaging (Molecular Dynamics) after electrophoretic separation. Because of the sensitivity of competitive RT-PCR experiments to amplification efficiency, we did not calculate absolute values of transcriptional induction.

2.3. Tissue culture

Human neuroblastoma SH-SY5Y cells were cultivated in DMEM with 10% FCS, the human acute leukemia T-cell line (Jurkat JKE6–1) in RPME-1640, 10% FCS and 0.1 mM β-mercaptoethanol. The same medium without β-mercaptoethanol was used for human histiocytic lymphoma cells (U937).

For PDE IV induction studies, 0.5×10^{-3} M dibutyryl-cAMP (dbcAMP; Sigma) was added to the medium 18 h before harvesting. $2–3 \times 10^6$ cells were used for each experiment. Prior to RNA purification the cells were washed with 1 × PBS.

3. Results

3.1. Distribution of human PDE IV isogenes (hPDE IV-A, -B, -C, and -D)

Total mRNA from various tissues and cell lines was reverse transcribed into cDNA. The expression patterns of the four known human PDE IV isogenes were determined by PCR with isogene-specific primers. In most of the human tissues tested, all four isoforms, PDE IV-A, -B, -C, and -D, were detectable as summarized in Table 1. Examples of the PCR experiments are shown in Fig. 1. No expression of PDE IV-C was found in blood. In addition, we analyzed several defined cell lines (e.g. SK-N-SH, SH-SY5Y neuronal like cells, Jurkat acute T cell leukemia, U937 histiocytic lymphoma promonocyte-like) as well as different purified blood cell populations (cosinophils, neutrophils). PDE IV-A PCR products were derived in high amounts from all cells with the exception of neutrophils. In Jurkat T-cells, PDE IV-A was the only detectable isoform.

PDE IV-B was found in all tissues and most of the cell lines analyzed with the exception of Jurkat cells where no PCR product was found. In contrast, PDE IV-B seems to be the dominant PDE IV isogene in neutrophils. No PDE IV-C mRNA was found in any of the immune system cell types tested. PDE IV-D was also not detectable in Jurkat T-cells or Namalwa B-cells, while only very weak PDE IV-D expression was observed in neutrophils.

3.2. Distribution of rat PDE IV isogenes (rPDE IV-A,-B, -C, and -D)

Different rat tissues were analyzed by RT-PCR with rat PDE IV isogene-specific primers. PCR results are summarized in Table 2 and some examples are shown in

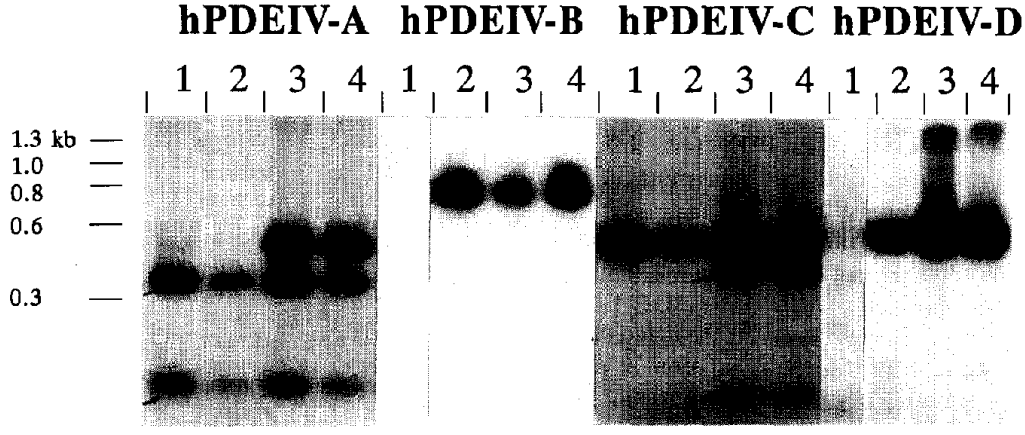

Fig. 1. Distribution of human PDE IV isoforms analyzed by RT-PCR. Human PDE IV isogene specific primers were used for the PCR analysis. hPDE IV-A and -C are recognized by the same PCR primers which give rise a 440 bp product. These two isoforms are then distinguished by digestion with BSTXI (hPDE IV-A) or *Eco*RV (hPDE IV-C) resulting in bands of 330 bp and 110 bp for type A and 345 bp and 95 bp for type C, respectively, as indicated by the arrows. The expected size of PCR fragments for hPDEIV-B was 680 bp and for hPDEIV-D 495 bp. 1, T-cells; 2, blood; 3, brain; 4, kidney.

Table 1
Summary of the RT-PCR results with different human tissues and defined cell populations

	hPDE-IV-A	hPDE-IV-B	hPDE-IV-C	hPDE-IV-D
Brain	++	++	++	++
Liver	++	++	++	++
Lung	++	++	++	++
Trachea	++	++	++	++
Kidney	++	++	++	++
Placenta	++	++	++	++
Heart	++	++	++	++
Blood	++	++	–	++
Promonocyte like[1]	++	++	–	++
T like cells[2]	++	–	–	–
B like cells[3]	++	++	–	–
Promyelocytic cells[4]	++	++	–	
Neutrophils	±	++	–	±
Eosinophils	++	++	–	++
SH-SY5Y[5]	++	++	++	++
SK-N-SH[5]	++	++	±	++

++, expression; ±, very weak expression; –, no expression; 1, U937 cells; 2, Jurkat cells; 3, Namalwa cells; 4, peripheral blood promyelocytic HL-60 cells; 5, Neuroblastoma cells.

Fig. 2. Most of the tissues also expressed rPDE IV-A. Interestingly, no rPDE IV-A RNA was detected by RT-PCR in blood. Rat PDE IV-B and -D were observed in all analyzed tissues. rPDE IV-C expression was detected in most tissues with the notable exception of blood. Only marginal expression was found in lung and brain (Table 2).

3.3. cAMP dependent stimulation of human PDE IV

We tested the influence of cAMP on the expression levels of different human PDE IV isogenes. Two cell lines derived from the immune system (U937, Jurkat) and one neuronal cell type (SH-SY5Y) were used. The cells were incubated for 18 h with 0.5 mM dbcAMP before RNA was extracted. To detect increased amounts of PDE IV mRNA after dbcAMP-treatment, a semi-quantitative PCR was performed, using β-actin as an internal standard. It has previously been shown that the expression of β-actin is not modulated by cAMP [13]. The results of experiments with untreated and treated cells are summarized in Table 3. PCR experiments performed with U937 cell RNA are shown in Fig. 3 as an example. In U937 promonocytes, which express hPDE IV-A, -B and -D, we observed a more than 2-fold increase in hPDE IV-A and hPDE IV-B expression while no increase was measured for hPDE IV-D (Table 3). Jurkat cells express only hPDE IV-A. These cells showed an approximately 2-fold increase in the PDE IV-A transcript after treatment with cAMP. Interestingly, we found transcripts of hPDE IV-D in stimulated Jurkat T-cells but not in unstimulated cells. In contrast, SH-SY5Y cells express all four isoforms of PDE IV. However, we could not detect a stimulatory effect of cAMP on the transcription of any of these genes.

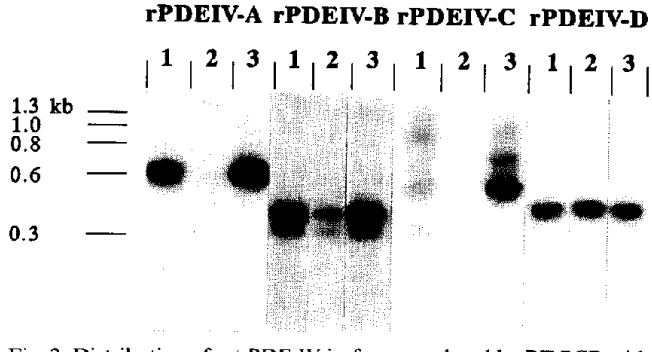

Fig. 2. Distribution of rat PDE IV isoforms analyzed by RT-PCR with rat PDE IV isogene-specific primers. The sizes of the PCR fragments were: rPDEIV-A 654 bp, rPDEIV-B 470 bp, rPDEIV-C 584 bp and rPDEIV-D 492 bp. 1, lung; 2, blood; 3, kidney.

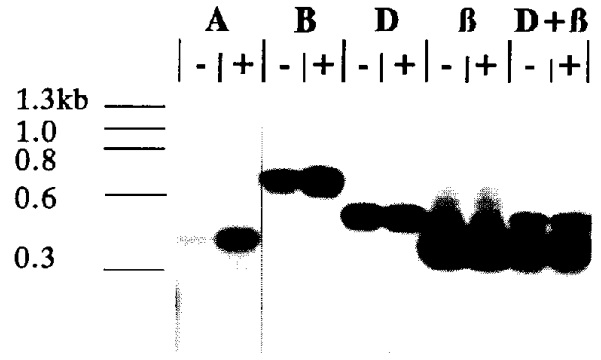

Fig. 3. Semi-quantitative RT-PCR analysis of PDE IV induction by cAMP. RT-PCR was performed with RNAs isolated from U937 cells treated with cAMP and controls. hPDE IV isogenes were recognized by isogene-specific primers and β-actin was used as standard. –, untreated; +, treated; A, hPDE IV-A; B, hPDE IV-B; D, hPDE IV-D; β, β-actin; D+β, mixture of β-actin and hPDE IV-D specific primers for competitive PCR.

4. Discussion

Type IV phosphodiesterases (PDE IV) are involved in the modulation of cellular processes via degradation of the second messenger cAMP. Four different rat and human PDE IV isogenes have been cloned and charac-

Table 2
Summary of the RT-PCR results with different rat tissues

	rPDE IV-A	rPDE IV-B	rPDE IV-C	rPDE IV-D
Brain	++	++	±	++
Liver	++	++	++	++
Lung	++	++	±	++
Kidney	++	++	++	++
Heart	++	++	++	++
Blood	–	++	–	++

++, Expression; ±, very weak expression; –, no expression.

Table 3
Regulation of hPDE IV isogene expression in three different cell lines

	hPDE IV-A	hPDE IV-B	hPDE IV-C	hPDE IV-D
SH-SY5Y			–	–
Jurkat	+	–	–	++
U937	++	++	–	–

Using RT-PCR we analyzed the regulation of PDE IV subtype expression in one neuronal and two immune system derived cell lines after treatment with 0.5×10^{-3} M dbcAMP for 18 h. –, No increase of mRNA; +, ~2-fold increase; ++, >2-fold increase.

terized [6,11–14]. All of these isoforms show a high amino acid homology in the catalytic domain but less at the N- and C-terminal sequences. Comparison of the human and rat amino acid sequences led to the recent analogous nomenclature (human PDE IV-A to -D = rat PDE IV-A to -D) although only rat and human PDE IV-B show a very high homology of 92% over the total sequences [14]. For the other three PDE IV isogenes the homology is less pronounced.

Our extensive distribution analysis showed that the four isogenes are expressed in most of the human and rat tissues tested. This overlapping expression pattern of whole organ mRNA may not reflect the real situation since the isoforms may be differentially expressed in individual cell types [6,10]. Nevertheless, in human and rat blood we found no or only marginal expression of PDE IV-C, suggesting that the isoform plays a minor role in cAMP regulation in blood cells. In contrast to the RNA blotting experiments of McLaughlin et al. [25] we detected transcripts of hPDE IV-B in liver and kidney. These results may be due to the higher sensitivity of RT-PCR versus Northern blotting, although we cannot exclude an interference of mRNA derived from blood cells.

Investigation of defined cell types of the immune system revealed that three of the four known human PDE IV genes display differential expression patterns in various cell types of the immune system, while one isoform (hPDE IV-C) is lacking completely in this system. The distinct isozyme expression in eosinophils, neutrophils and T-cells (all of which are involved in inflammatory reactions) suggests that the isozymes may modulate different pools of intracellular cAMP which are important for the particular responses of these cells. A differential expression of cAMP-PDEs was also described for the rat testis [9,10] where rPDE IV-A and -C are expressed in germ cells whereas rPDE IV-B and -D were found in somatic cells. Furthermore, in rat brain distinct expression patterns of the four isogenes were shown by in situ hybridization with overlapping expression in hippocampus and cerebellum but differences in midbrain and cortex [26]. In human neuronal tissue it was demonstrated that no expression of hPDE IV-C occurred in temporal cortex and glioblastoma cells whereas in SH-N-SK neuroblastoma cells this isoform was detected in RNAse-protection experiments [6]. It seems clear from our data, however, that individual cell types can express several or all PDE IV isoforms. To explain the benefit for the cell of the expression of the multiple isoforms, it needs to be discovered whether functional or regulatory differences between these isoforms exist or whether they are present in different subcellular compartments. In this respect it is interesting to note that rPDE IV-A (RD1) was found as a membrane-bound isoform [27,28] whereas rPDE IV-D was found in soluble fractions of stimulated Sertoli cells by other authors [13,20].

Our comparison of the human and rat tissue distribution revealed significant differences between the two species. It must be assumed that either species-specific differences in expression patterns exist indeed or that the correlation between the cloned human and rat PDE IV isogenes is partly incorrect. The existence of additional, as yet unidentified, PDE IV isogenes would then have to be postulated.

Further experiments were performed to investigate the regulation of the hPDE IV isogenes in different human cell types. It has recently been demonstrated that cAMP phosphodiesterase activity can be increased by cAMP via protein kinase A in myoblasts [29]. PDE IV activity was further found to be elevated in FRTL-5 cells after treatment with thyroid-stimulating hormone (TSH), a process which is mediated by cAMP-dependent phosphorylation [22]. Other authors describe the induction of PDE IV gene expression in Sertoli or C6-glioblastoma cells after treatment of the cells with dbcAMP, forskolin or FSH [20,23,30] where correlation between elevated mRNA levels and protein activity was demonstrated. Therefore, cAMP may mediate PDE activity both via protein modification and induction of transcription.

Our experiments in human cells showed that expression of three of the four human isogenes can be induced in cells derived from the immune system but not in a neuronal cell line. This is particularly important if a long-term treatment of patients with PDE IV inhibitors is envisaged, which has been proposed for various diseases. cAMP-dependent upregulation of PDEs may outbalance the effects of PDE inhibitors in certain tissues. Our data suggest that distinct regulatory mechanisms exist in different cell types. Modulation of cAMP levels may be achieved by differential regulation of expression of PDE IV isozymes. Second messenger-dependent regulation of PDE gene expression has also been found for other PDE subfamilies. For instance, expression of Ca^{2+}/Calmodulin dependent phosphodiesterases is increased by Calmodulin or by cAMP-dependent protein kinase phosphorylation [31]. Other authors described the regulation of nucleotide phosphodiesterases in liver by glucagon and insulin [32].

In conclusion, our results suggest that the PDE IV subtypes fulfil different roles in the regulation of cellular

cAMP-dependent processes. Individual cells may contain one or several PDE IV subtypes which may occur in different intracellular localizations. The specific patterns of PDE IV subtype expression and the differences in their transcriptional regulation may enable the cells to variably regulate cAMP-degradation in different cellular compartments.

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References

[1] Beavo, J.A. and Reifsnyder, D.H. (1990) Trends Pharmacol. Sci. 11, 150–155.
[2] Michaeli, T., Bloom, T.J., Martins, T., Loughney, K., Ferguson, K., Riggs, M., Rodgers, L., Beavo, J.A. and Wigler, M. (1993) J. Biol. Chem. 268, 12925–12932.
[3] Mukai, J., Asai, T., Naka, M. and Tanaka, T. (1994) Br. J. Pharmacol. 111, 389–390.
[4] Beavo, J.A. (1988) Adv. Second Messenger Phosphoprotein Res. 22, 1–30.
[5] Nicholson, C.D., Challiss, R.A. and Shadid, M. (1991) Trends Pharmacol. Sci. 12, 19–27.
[6] Bolger, G., Michaeli, T., Martins, T., St. John, T., Steiner, B., Rodgers, L., Riggs, M., Wigler, M. and Ferguson, K. (1993) Mol. Cell. Biol. 13, 6558–6571.
[7] Monaco, L., Vicini, E. and Conti, M. (1994) J. Biol. Chem. 269, 347–357.
[8] Sharma, K.R., Adachi, A.M., Adachi, K. and Wang, J.H. (1984) J. Biol. Chem. 259, 9248–9254.
[9] Sonnenburg, W.K., Seger,D. and Beavo J.A. (1993) J. Biol. Chem. 268, 645–652.
[10] Taira, M., Hockman, S.C., Calvo, J.C., Taira, M., Belfrage, P. and Manganiello, V.C. (1993) J. Biol. Chem. 268. 18573–18579.
[11] Colicelli, J., Birchmeier, C., Michaeli, T., O'Neill, K., Riggs, M. and Wigler, M. (1989) Proc. Natl. Acad. Sci. USA 86, 3599–3603.
[12] Davis, R.L., Takayasu, H., Eberwine, M. and Myres, J. (1989) Proc. Natl. Acad. Sci. USA 86, 3604–3608.
[13] Swinnen, J.V., Joseph, D.R. and Conti, M. (1989) Proc. Natl. Acad. Sci. USA 86, 5325–5329.
[14] Obernolte, R., Bhakta, S., Alvarez, R., Bach, C., Zuppan, P., Milkins, M., Jarnagin, K. and Shelton, E.R. (1993) Gene 129, 239–247.
[15] Chen, C.N., Denome, S. and Davis, R.L. (1986) Proc. Natl. Acad. Sci. USA 86, 3599–3603.
[16] Qiu, Y.H. and Davis, R.L. (1993) J. Mol. Biol. 222. 553565.
[17] Hulley, P., Hartikka, J., Abdel'Al, S., Engels, P., Buerki, H.R., Mueller, T., Goger, C., Lowe, D. and Luebbert, H. (1994) submitted.
[18] Molnar-Kimber, K.L., Yonno, L. and Heaslip, R.J. (1992) Mediators of Inflammation 1, 411–417.
[19] Chan, S.C., Reifsnyder, D., Beavo, J.A. and Hanifin, J.M. (1993) J. Allergy Clin. Immunol. 91, 1179–1188.
[20] Conti, M., Swinnen, J.V., Tsikalas, K.E. and Jin, S.-L.C. (1992) in: Advances in Second Messenger and Phosphoprotein Research, Vol. 25 (Strada, J. and Hidaka Eds.) pp. 165–175, Raven Press, New York.
[21] Conti, M., Toscano, M.V., Petrelli, L., Geremia, R. and Stefanini, M. (1983) Mol. Cell. Endocrinol. 113, 1845–1853.
[22] Sette, C., Iona, S. and Conti, M. (1994) J. Biol. Chem. 269, 9245–9252.
[23] Swinnen, J.V., Joseph, D.R. and Conti, M. (1989) Proc. Natl. Acad. Sci. USA 86, 8197–8201.
[24] Chomczynski, P. and Sacchi, N. (1987) Analyt. Biochem. 162, 156–159.
[25] McLaughlin, M.M., Cieslinski, L.B., Burman, M., Torphy, T.J. and Livi, G.P. (1993) J. Biol. Chem. 268, 6470–6476.
[26] Engels, P., Abdel'Al, S., Hulley, P. and Lübbert, H. (1994) in preparation.
[27] Shakur, Y., Pryde, J.G. and Houslay, M.D. (1993) Biochem. J. 292, 677–686.
[28] Kithas, P.A., Artman, M., Thompson, W.J. and Strada, S.J. (1989) J. Mol. Cell Cardiol. 21. 507–517.
[29] Kovala, T., Lorimer, I.A.J., Brickenden, A.M., Ball, E.H. and Sanwal, B.D. (1994) J. Biol. Chem. 269, 8680–8685.
[30] Swinnen, J.V., Tsikalas, K.E. and Conti, M. (1991) J. Biol. Chem. 266, 18370–18377.
[31] Sharma, R.K. and Wang, J.H. (1985) Proc. Natl. Acad. Sci. USA 82, 2603–2607.
[32] Houslay, M.D. and Kilgour, E. (1990) in: Cyclic Nucleotide Phosphodiesterases: Structure, Regulation and Drug Action (Beavo, J.A. and Houslay, M.D. Eds.) pp. 185–218, John Wiley and Sons, Chichester, New York.