Receptor-mediated stimulation of lipid signalling pathways in CHO cells elicits the rapid transient induction of the PDE1B isoform of Ca^{2+}/calmodulin-stimulated cAMP phosphodiesterase

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Chinese hamster ovary cells (CHO cells) do not exhibit any Ca^{2+}/calmodulin-stimulated cAMP phosphodiesterase (PDE1) activity. Challenge of CHO cells with agonists for endogenous P_2-purinoceptors, lysophosphatidic acid receptors and thrombin receptors caused a similar rapid transient induction of PDE1 activity in each instance. This was also evident on noradrenaline challenge of a cloned CHO cell line transfected so as to overexpress α_{1B}-adrenoceptors. This novel PDE1 activity appeared within about 15 min of exposure to ligands, rose to a maximum value within 30 min to 1 h and then rapidly decreased. In each case, the expression of novel PDE1 activity was blocked by the transcriptional inhibitor actinomycin D. Challenge with insulin of either native CHO cells or a CHO cell line transfected so as to overexpress the human insulin receptor failed to induce PDE1 activity. Reverse transcriptase-PCR analyses, using degenerate primers able to detect the PDE1C isoform, did not amplify any fragment from RNA preparations of CHO cells expressing PDE1 activity, although they did so from the human thyroid carcinoma FTC133 cell line. Reverse transcriptase-PCR analyses, using degenerate primers able to detect the PDE1A and PDE1B isoforms, successfully amplified a fragment of the predicted size from RNA preparations of both CHO cells expressing PDE1 activity and human Jurkat T-cells. Sequencing of the PCR products, generated using the PDE1A/B primers, yielded a novel sequence which, by analogy with sequences reported for bovine and murine PDE1B forms, suggests that the PDE1 species induced in CHO cells through protein kinase C activation and that expressed in Jurkat T-cells are PDE1B forms.

INTRODUCTION

Cyclic nucleotide phosphodiesterases (PDEs) are a multienzyme family in which at least 14 distinct genes encode over 20 different isoforms. These isoforms have been grouped into seven distinct classes depending on sequence criteria coupled with the expression of specific biochemical and pharmacological properties. These classes are: PDE1, Ca^{2+}/calmodulin (CaM)-stimulated; PDE2, cGMP-stimulated; PDE3, cGMP-inhibited; PDE4, cAMP-specific and rolipram-inhibited; PDE5, cGMP-specific; PDE6, cGMP-specific photoreceptor enzymes; PDE7, cAMP-specific but insensitive to inhibition by the non-selective PDE inhibitor isobutylmethylxanthine and the PDE4 specific inhibitor rolipram [1–9].

Regulation of PDE activity provides an equally complex and important route for controlling intracellular levels of the second messenger cAMP as does regulation of adenylate cyclase activity [1,4–8,10,11]. Rapid regulatory changes can be elicited by the phosphorylation of PDE3 enzymes [9,11–16] and isoforms of PDE4 enzymes [17,18]. Longer-term changes, through up-regulation, have also been defined as adaptive responses to chronic increases in intracellular cAMP levels [5,7], and this has been defined in particular detail for splice variants arising from the *PDE4D* gene [5,19,20].

We have recently demonstrated [21] that challenge of Chinese hamster ovary (CHO) cells with phorbol 12-myristate 13-acetate (PMA) led to the induction of novel Ca^{2+}/CaM-stimulated PDE1 activity. This occurred rather rapidly, with transcripts and PDE1 activity being evident some 15 min after exposure of cells to PMA. Intriguingly, PDE1 activity was found to be present in CHO cells transfected so as to overexpress either protein kinase C (PKC)-α or PKC-ϵ, but not either PKC-β or PKC-γ, implying that the induction process was mediated through the actions of distinct members of the PKC family. Such a specific induction event implies a precise form of 'cross-talk' occurring between the cAMP- and lipid-signalling pathways in CHO cells.

PDE1 activity has been shown to be encoded by three distinct genes, namely *PDE1A*, *PDE1B* and *PDE1C* [1,22–26]. These yield distinct protein products and, certainly for *PDE1C*, can produce multiple enzyme forms through alternative splicing. Here we show that agonist occupancy of a variety of receptors able to stimulate lipid-signalling pathways in CHO cells caused a remarkably rapid, but transient, induction of PDE1 activity. Reverse transcriptase (RT)-PCR and sequence analysis suggested that it was the PDE1B isoform of Ca^{2+}/CaM-stimulated PDE that was induced in CHO cells.

MATERIALS AND METHODS

CHO cells and those overexpressing human insulin receptors and various protein kinases [27] were a gift from Dr. Richard Roth, UCSF, San Francisco, CA, U.S.A. A cloned CHO cell line that had been transfected so as to overexpress α_{1B}-adrenoceptors was a gift from Dr. K. Horie, National Children's Medical Research Centre, Tokyo, Japan [28,29]. [^{3}H]cAMP was from Amersham

Abbreviations used: CHO cells, Chinese hamster ovary cells; PDE, cAMP phosphodiesterase; CaM, calmodulin; PDE1, Ca^{2+}/CaM-stimulated PDE activity which also known as type-I PDE activity; PKC, protein kinase C; RT-PCR, reverse transcriptase-PCR; PMA, phorbol 12-myristate 13-acetate; FCS, fetal calf serum.

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The sequences reported here have been deposited in GenBank (accession numbers U40585 and U40584).

International (Amersham, Bucks., U.K.). Leupeptin was from Peptide Research Foundation (distributed by Scientific Marketing Associates, London, U.K.). Tris, benzamidine hydrochloride, PMSF, aprotonin, pepstatin A, antipain, EDTA, cAMP, cGMP, Dowex 1 (X8-400; 200–400 mesh; Cl^- form), 3-isobutyl-1-methylxanthine, snake venom (*Ophiophagus hannah*), bovine brain CaM, NaF and BSA were from Sigma Chemical Co. (Poole, Dorset, U.K.). $CaCl_2$ was from BDH (Glasgow, Scotland, U.K.) and all other biochemicals were from Fisons (Loughborough, Leics., U.K.). All cell culture reagents were from Gibco–BRL (Paisley, Scotland, U.K.) except hygromycin B which was from Boehringer (U.K.) Ltd. (Lewes, Sussex, U.K.). Okadaic acid was from Moana Bioproducts (Honolulu, HI, U.S.A.).

Culture of CHO cells

CHO cells were grown as detailed previously [21] in Hams F12 medium supplemented with 10 % (v/v) fetal calf serum (FCS). Confluent cells were harvested in a 10 mM Tris/HCl buffer, pH 7.5, containing 0.1 mM EDTA, 10 mM NaF, 30 mM sodium pyrophosphate and a protease inhibitor cocktail of 0.1 mM PMSF, 2 mM each aprotinin, antipain, pepstatin A, leupeptin and benzamidine. Okadaic acid (100 nM) was also present. Cells were partially lysed by freeze–thawing and subsequently homogenized in this buffer by 25–30 up-and-down strokes in a Dounce homogenizer. The final protein concentration employed in PDE assays was 2.5 mg/ml.

The human follicular thyroid carcinoma cell line FTC133 [30–34] was obtained from Dr. D. Wynford-Thomas, Cancer Research Campaign, University of Wales College of Medicine, Cardiff, U.K. Cells were cultured at 37 °C in an atmosphere of 5 % CO_2/95 % air in a complete growth medium containing 1 % (v/v) RPMI, 10^4 units/ml penicillin, 10^4 μg/ml streptomycin and 1 % (v/v) 200 mM glutamine and supplemented with 10 % (v/v) FCS.

Culture of Jurkat cells

The human T-cell leukaemia line Jurkat (J6) was cultured in RPMI 1640 medium supplemented with 5 % heat-inactivated fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin and 2 mM L-glutamine at 37 °C and 5 % CO_2 in air. Twice weekly, the cells were split and diluted into fresh prewarmed medium in 75 ml tissue culture flasks (1:5 dilution), under sterile conditions.

Protein determination

Homogenate protein content was determined [35] employing BSA as standard.

PDE assay

PDE activity was determined by a modification of the two-step procedure of Thompson and Appleman [36] as detailed previously [37]. This employed 1 μM cAMP as substrate. All assays were conducted at 30 °C and a freshly prepared slurry of Dowex/water/ethanol (1:1:1, by vol.) was used. As before [21], to obviate the possibility that Ca^{2+} arising from cell homogenates might elicit the full activation of any PDE1 activity without added exogenous Ca^{2+}, we also performed assays in the presence of EGTA (2 mM) to determine whether the subsequent addition of Ca^{2+} then caused stimulation. Thus assays for PDE1 activity were performed in the presence of 2 mM EGTA with the stimulatory effect caused by the addition of 5 mM Ca^{2+} and 10 units of CaM to assays taken as an index of PDE1 activity. However, in no instance did we observe any difference between analyses carried out in this fashion and those where Ca^{2+} was added without EGTA. In all instances, activation was dependent on the presence of both Ca^{2+} and CaM. PDE initial rates were determined from linear time courses of activity.

Isolation of RNA from cells

Total RNA was isolated from cultured cells by the Tri-Reagent method. All equipment was sterilized before use so as to be RNase-free and all centrifugation procedures were performed at 4 °C. Cells were grown to 90 % confluency on 10-cm Petri dishes and the culture medium was removed. Each plate was then flooded with 1 ml of Tri-Reagent™ (Sigma), the cell suspension was scraped into an Eppendorf tube and homogenized by ten passages through a Gilson pipette tip. The homogenate was stored at room temperature for 5 min, centrifuged at 12000 ***g*** for 10 min and the supernatant transferred to a new Eppendorf tube. DNA and protein were removed from the preparation by chloroform extraction. To each tube, 0.2 ml of RNase-free chloroform/ml of Tri-Reagent originally used was added. The chloroform and aqueous phases were briefly vortex mixed and stored at room temperature for 3 min. The phases were then separated by centrifugation at 12000 ***g*** for 15 min and most of the upper aqueous phase was transferred to a clean Eppendorf tube. The RNA was precipitated by the addition of 0.5 ml of propan-2-ol/ml of Tri-Reagent originally used at room temperature for 10 min and then pelleted by centrifugation at 12000 ***g*** for 10 min. The RNA was washed using 1 ml of 75 % ethanol and the pellet was dried under vacuum for 15 min. The RNA was resolubilized in 1 ml of diethyl pyrocarbonate-treated water and the A_{260}/A_{280} ratio was determined and was routinely above 1.7. The RNA preparation was stored at −80 °C under 75 % ethanol until use.

RT-PCR and sequence analysis

Preparation of first-strand cDNA was performed using the 'First strand cDNA synthesis kit' (Pharmacia) with a total reaction volume of 33 μl. Briefly, 3 μg of RNA, as determined by its A_{260}, was denatured at 65 °C for 10 min and then incubated with murine reverse transcriptase, 6 mM dithiothreitol, dNTPs and 0.2 μg of the supplied poly(dT)primer [*Not*I–d$(T)_{18}$] for 60 min at 37 °C.

A set of primers (GR18 sense 5′-RYCTYATCARCCGYTT-YAAGATTCC-3′ and GR19 antisense 5′-RAAYTCYTCCAT-KAGGGGCCWTGG) was used as described previously [21] to detect a 601 bp fragment found in all *PDE1A* and *PDE1B* transcripts, including the recently published human *PDE1A* form (U40370) [22] (In these primers, R = A/G, Y = C/T, K = G/T and W = A/T.) We also designed primers specific for phylogenetically stable regions of *PDE1C* on the basis of human and rat sequences deposited in GenBank (human *PDE1C1* (U40371), *PDE1C3* (U40372) [22] and rat *PDE1C2* (L41045) [38]). We used approx. 520 nucleotides (about 1550 to 2067 in human *PDE1C1*) of conserved (> 80 % identity human/rat) unique *PDE1C* sequence found between residues that encode the putative catalytic region and those at the first of the 3′ splice junctions [22,23]. Partially degenerate primers (GR45 sense 5′-CMAAGCGATCAGGTGTCAAG-3′ and GR46 antisense 5′-GTTRGAGTGATCCTTCTTGTCTG-3′) were designed to the ends of this sequence in order to detect an approx. 430 bp fragment common to all the *PDE1C* transcripts currently deposited in GenBank (where M = C/A and GR45 starts at 1609 and GR46 ends at 2039 in *PDE1C1*). These sequences are also found in the mouse *PDE1C* forms recently deposited in GenBank

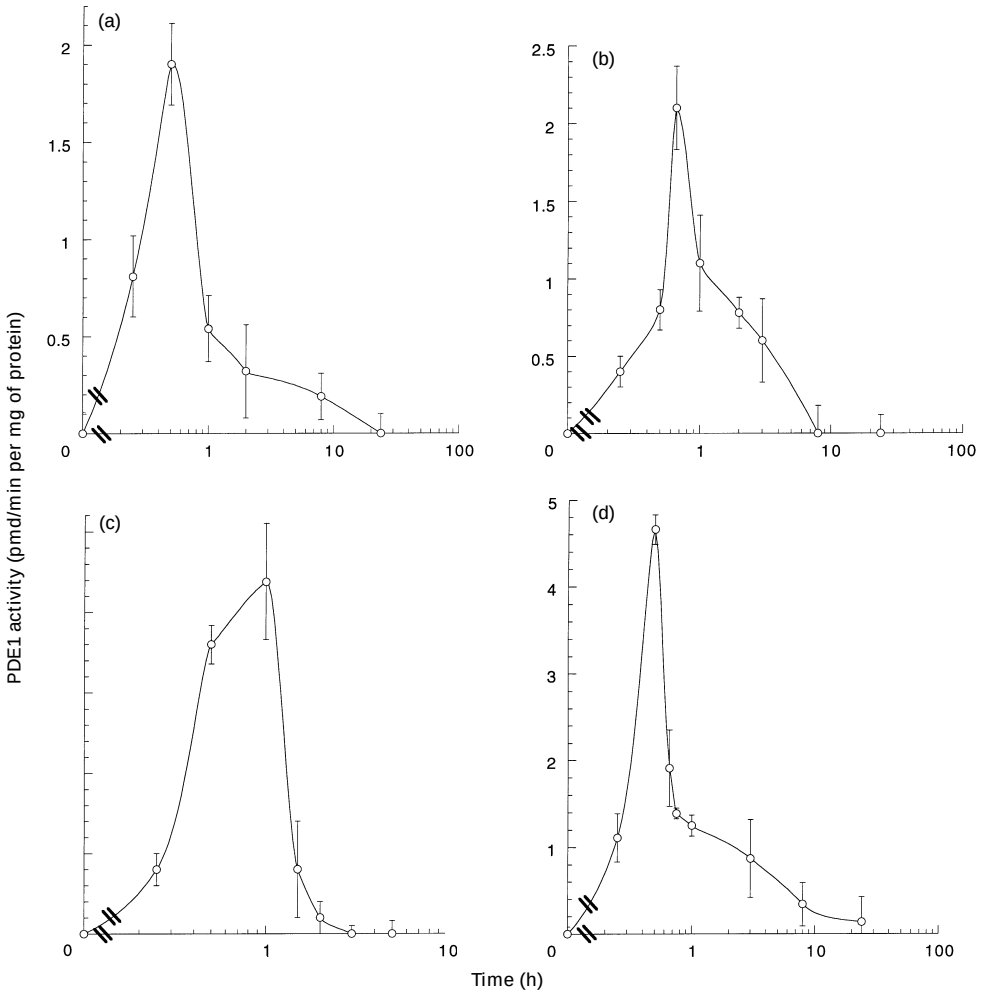

Figure 1 Rapid transient induction of Ca^{2+}/CaM-stimulated PDE1 activity in CHO cells

Cells were treated with ligands for the indicated times before being harvested, washed, homogenized and PDE activity was determined in the presence and absence of added Ca^{2+}/CaM. The increase in PDE activity caused by the addition of Ca^{2+}/CaM is shown here expressed as pmol/min per mg of protein. Data are the average of three separate experiments (mean ± S.D.). All experiments show data using native CHO cells except for experiments using noradrenaline where cells transfected so as to overexpress the α_{1B}-adrenoceptors were employed. Cells were challenged with (**a**) ATP (100 μM), (**b**) lysophosphatidic acid (10 μM), (**c**) thrombin (1 unit/ml) and (**d**) noradrenaline (10 μM).

[one mismatch with each of mouse *PDE1C1* (L76944), mouse *PDE1C4* (L76947) and mouse *PDE1C5* (L76946) forms]. However, they are not found in any deposited *PDE1A* or *PDE1B* sequences [bovine *PDE1A* (M90358), human *PDE1A* (U40370), bovine *PDE1B* (M94867) and mouse *PDE1B* (M94538)]. Amplification was performed in 1 × PCR buffer (50 mM KCl, 20 mM Tris/HCl) containing 200 μM each dNTP, 0.5 μM each primer (except for 1.5 μM GR18 and GR19), 1.5 mM $MgCl_2$, 5 μl of first-strand cDNA mixture and 5 units of *Taq* DNA polymerase that had first been mixed with 'Taq Start' antibody (Clontech Laboratories, Palo Alto, CA, U.S.A.) for 'Hot-Start' PCR according to the manufacturer's instructions. Then 0.5 unit of the PCR additive 'Perfect Match' (Stratagene, La Jolla, CA, U.S.A.) was added to a total volume of 50 μl. The amplification protocol consisted of 40 cycles of denaturation for 60 s at 94 °C, annealing for 70 s at 51 °C (except for PCR with GR18 and GR19 where the annealing segment was 80 s at 50 °C) and extension for 70 s at 72 °C. A 10 μl aliquot from each reaction mixture was resolved by electrophoresis on a 2 % agarose gel and visualized with ethidium bromide under UV light.

RT-PCR products were separated by electrophoresis through low-melting agarose gels and isolated as described previously [39]. Purified DNA fragments were blunt-ended by incubating with Pfu polymerase (Stratagene) in the presence of all four dNTPs (Pharmacia). Blunt-ended fragments were subsequently cloned into pCRscript using the pCRscript Amp SK cloning kit (Stratagene). *Eschericia coli* strain NM522 was used for routine plasmid transformation and propagation [40]. Plasmid DNA was purified using the Wizard™ Miniprep kit (Promega) and inserts sequenced using the PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing kit (Perkin–Elmer). Sequence analysis was performed on a model 373 automated sequencer (Applied Biosystems).

RESULTS AND DISCUSSION

Induction of PDE 1 activity in CHO cells by endogenous activators of lipid metabolism

As described previously by us [21] in some detail, homogenates of CHO cells contain cAMP-hydrolysing PDE activity where the

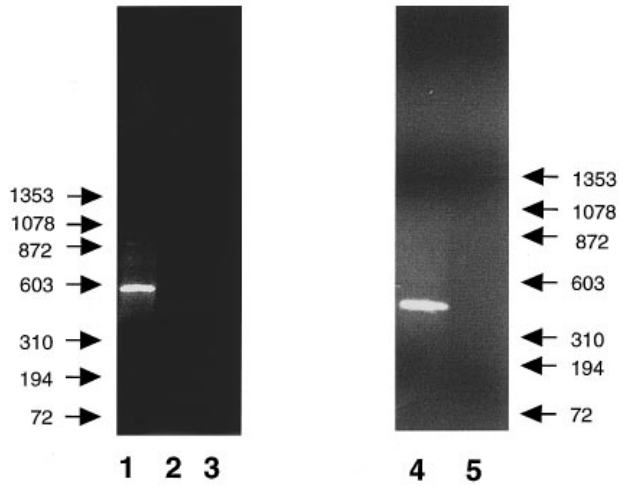

Figure 2 RT-PCR analyses of CHO cells using PDE1-specific primer pairs

Primer pairs designed to detect transcripts for PDE1 isoforms in a manner that was independent of the mammalian source used to prepare RNA (see the Materials and methods section and [21]) were used. Experiments typical of those carried our at least three times are shown. Primer pairs with a predicted preference for either PDE1A/B forms (track 1, 2 and 3) or primer pairs with a predicted preference for the PDE1C form (tracks 4 and 5) were used. Data using RNA from PDE1-induced (100 μM ATP) CHO cells (tracks 1 and 5), FTC133 cells (tracks 2 and 4) and blank (track 3) are shown. Sizes of markers (bp) are shown.

major contribution is provided by PDE4 isoforms and then by PDE3 enzyme activity. Furthermore, we confirm here that, as described previously by us [21], CHO cell homogenate PDE activity was insensitive to activation by Ca^{2+}/CaM (50 μM; 20 ng/ml) ($< 4\%$ change; $n = 9$), indicating the absence of PDE1 activity from these cells. In such experiments, the basal homogenate PDE activity was determined as 5.8 ± 0.6 pmol/min per mg (mean $\pm$ S.D.; $n = 9$). In some instances, Ca^{2+} arising from cell culture media and homogenates can contaminate PDE assays at a level sufficient to activate any PDE1 in full. To obviate this, we performed experiments in the presence of the Ca^{2+} chelator EGTA, such that the addition of exogenous Ca^{2+} would be essential to see PDE1 enzyme activation. In this regard, as we have shown previously [21], when PDE1 activity was induced in CHO cells, this could readily be detected as a Ca^{2+}/CaM-stimulated increase in homogenate PDE activity whether or not assays were carried out in the presence of added EGTA. This signifies the absolute dependence on exogenously added Ca^{2+}/CaM to observe PDE1 activity in homogenates of CHO cells. The lack of PDE1 activity in control CHO cells appears to be due to the absence of enzyme as no transcripts for PDE1 were evident [21].

CHO cells express endogenous P_2-purinoceptors [41] which are able to stimulate inositol phospholipid metabolism and increase intracellular Ca^{2+} [42]. Thus one might expect that the diacylglycerol produced would be capable of activating the endogenous PKC identified [21] in these cells. Employing a concentration of ATP (100 μM), which has been shown to elicit maximal activation of inositol phospholipid metabolism in CHO cells [42], we were able to demonstrate a rapid time-dependent induction of PDE1 activity in CHO cells (Figure 1a). Such an effect was, however, transient in nature with activity rising to a maximum level within an hour and subsequently falling rapidly to extremely low levels within 2 h (Figure 1a). This induction of PDE1 activity by ATP treatment of CHO cells was dramatically attenuated if experiments were performed in the presence of the transcriptional inhibitor actinomycin D: although a challenge of CHO cells with ATP for 30 min led to a Ca^{2+}/CaM-mediated stimulation of homogenate PDE activity of some 2.5 ± 0.6 pmol/min per mg of protein ($n = 6$), this activation was reduced to 0.4 ± 0.1 pmol/min per mg of protein ($n = 3$) when cell incubations were carried out in the presence of actinomycin D (10 μg/ml). This demonstrates that the rapid increase in PDE1 activity elicited by ATP was due to induction of new enzyme, as indicated previously using PMA [21]. However, here we demonstrate that induction of PDE1 activity can be achieved through the stimulation of an endogenous receptor.

Lysophosphatidic acid, which has also been shown able to stimulate phosphoinositide metabolism in CHO cells [43,44], similarly elicited a rapid highly transient induction of PDE1 activity (Figure 1b). The maximal PDE activity observed caused by stimulation of homogenate PDE activity by Ca^{2+}/CaM, was some 2.2 ± 0.2 pmol/min per mg of protein ($n = 3$). This effect, however, was completely obliterated if cells were incubated in the presence of actinomycin D. In such experiments, after challenge of cells with lysophosphatidic acid together with actinomycin D for 30 min, we singularly failed to observe any stimulatory effect of Ca^{2+}/CaM on homogenate PDE assays ($< 4\%$ change; $n = 3$ separate experiments).

CHO cells also express endogenous thrombin receptors [45]. Using this serine proteinase (1 unit/ml) we were also able to rapidly induce Ca^{2+}/CaM-stimulated PDE1 activity (Figure 1c). After 30 min treatment of cells with thrombin, Ca^{2+}/CaM stimulated homogenate PDE activity by some 3.8 ± 0.6 pmol/min per mg of protein. This action, however, was completely negated when cells were incubated with thrombin together with actinomycin D. In such experiments, Ca^{2+}/CaM addition elicited less than 4% change ($n = 3$ separate experiments) in homogenate PDE activity.

CHO cells do not express endogenous α-adrenergic receptors [28,29]. However, we have also studied a cloned CHO cell line (CHO-α1B cells) which has been transfected so as to overexpress hamster α_{1B}-adrenoceptors [28,29]. Noradrenaline serves as an effective agonist at this receptor and was employed in these studies at a concentration of 10 μM, as this has been shown to be optimal for stimulating lipid signalling in these transfected cells through activation of phospholipase C [28,29]. Noradrenaline does not, however, stimulate either phosphatidylinositol or phosphatidylcholine lipid metabolism in native CHO cells or cells transfected with control vector [28,29]. Consistent with this, when we challenged either native CHO cells or control-vector-transfected cells with noradrenaline (10 μM; 30 min), we failed to observe any stimulatory effect ($< 4\%$ change; $n = 3$) on homogenate PDE activity. In marked contrast with this, challenge of the α_{1B}-adrenoceptor-transfected cells with noradrenaline led to the expression of PDE1 activity in this cell line (Figure 1d). This effect was again remarkably transient, with a stimulatory effect of Ca^{2+}/CaM seen on homogenate PDE activity within 15 min of exposure to noradrenaline but which disappeared some 3 h after exposure ($< 6\%$ change; $n = 3$). Although some 30 min after challenge of CHO-α1B cells with noradrenaline, Ca^{2+}/CaM was able to stimulate homogenate PDE activity by 6.3 ± 0.3 pmol/min per mg, this effect was completely abolished if actinomycin D was added together with noradrenaline ($< 5\%$ change; $n = 3$ separate experiments).

Native CHO cells also express endogenous insulin receptors at levels of about 3000 copies per cell [27]. These have been shown to be biologically functional and can mediate the ability of insulin to stimulate glucose transport and activation of the AP1 transcription factor [27,46]. However, at a concentration of

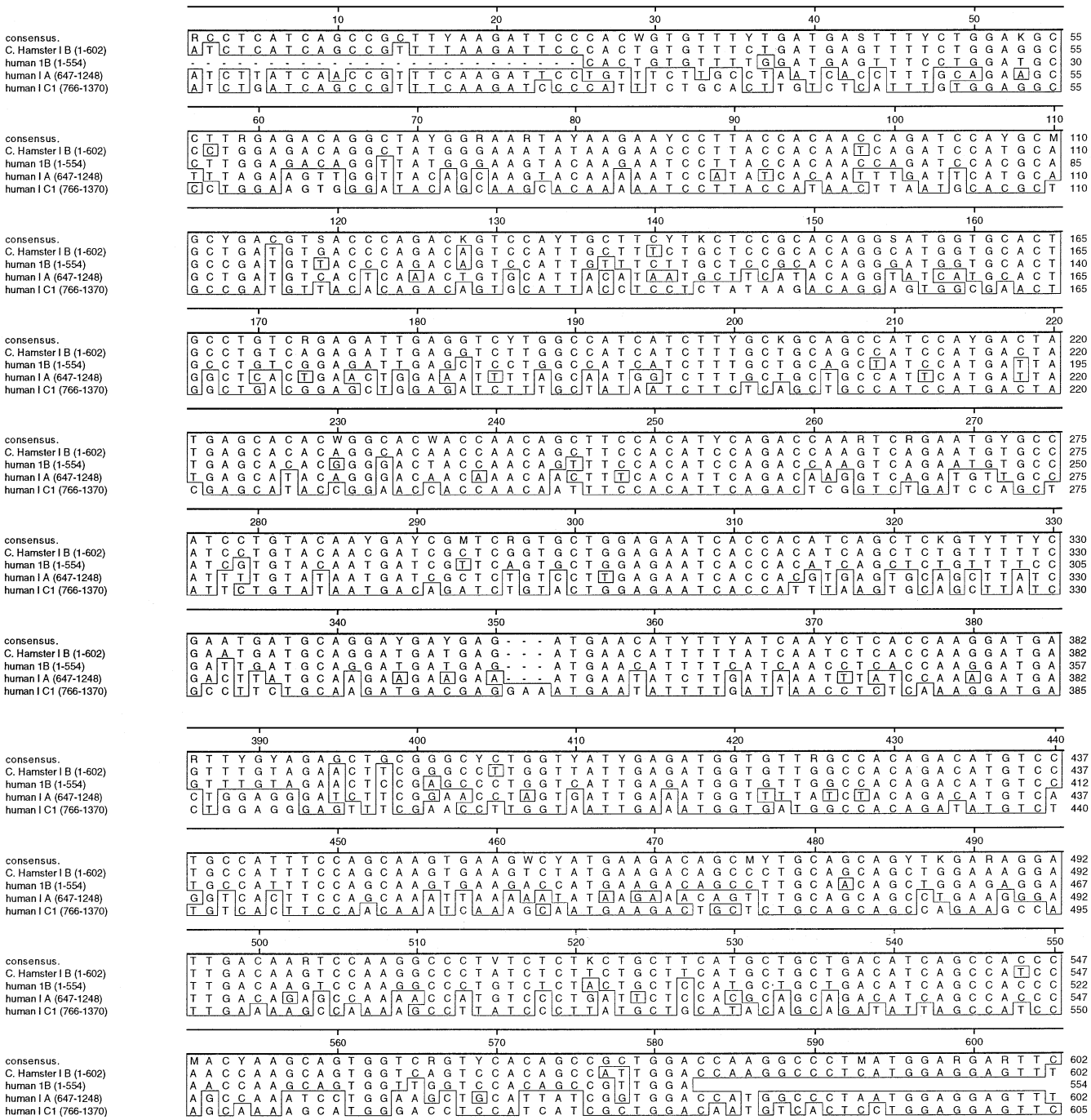

Figure 3 Sequence of PDE1 transcript PCR products from induced CHO cells and from human Jurkat T-cells

Sequences of PCR products amplified using our PDE1A/B-species-independent primers from PDE1-induced CHO cells (GenBank accession number U40585) and from human Jurkat T-cells (GenBank accession number U40584) are shown. Alignments are given for both of these together with sequences for the equivalent region of published data for mouse and bovine *PDE1B* sequences and the *PDE1A* and *PDE1C* sequences. Alignment was carried out with a *PDE1B* consensus sequence [a degenerate consensus of the three full-length *PDE1B* sequences; mouse *PDE1B* form (M94538), rat *PDE1B* form (M94537), bovine *PDE1B* form (M94867), where M = A/C, R = A/G, W = A/T, S = C/G, Y = T/C, K = G/T]. This shows that the novel sequence we present here is highly homologous to these *PDE1B* gene transcripts. Human *PDE1C1* (U40370) and human *PDE1A* (U40371) sequences are shown for comparison. Matches to the consensus are printed within the boxed area.

100 nM, insulin failed ($< 5\%$ change; $n = 5$) to allow Ca^{2+}/CaM to stimulate CHO cell homogenate PDE activity at any time over a period between 15 min and 8 h of incubation of cells with this hormone. In order to see if this was an inappropriate insulin concentration, we studied a range of concentrations of insulin from 1 nM to 1 μM over a period from 15 min to 8 h with similar lack of effect ($< 4\%$ change). We also analysed the CHO-T cell line which was transfected to overexpress the human insulin receptor at levels of approx. 10^6 copies per cell [47]. Similarly, in this cell line, insulin failed to induce Ca^{2+}/CaM-stimulated PDE

activity. Furthermore insulin (10 nM), over a range of time from 30 min to 8 h, did not alter PDE1 activity ($< 5\%$ change; $n = 3$) in CHO cells in which PDE1 had already been induced, i.e. CHO cells transfected to overexpress either PKC-α or PKC-ϵ [21].

The induced Ca^{2+}/CaM-stimulated PDE enzyme in CHO cells is PDE1B

We have previously discussed the design of a set of degenerate PCR primers aimed at detecting transcripts for PDE1 in a species-independent fashion [21]. Employing RT-PCR, these were successful in detecting a species of the predicted size in brain RNA extracts but failed to detect any signal in extracts from CHO cells unless they had either been challenged with PMA or were clones that had been transfected so as to overexpress stably either PKC-α or PKC-ϵ [21]. On this basis we concluded that native CHO cells do no express PDE1 enzyme. Since we designed this set of degenerate primers, a novel sequence for human PDE1A and PDE1C has been reported [22]. From these data it would appear that the set of primers devised in our earlier study [21] would be optimized for the detection of PDE1A and PDE1B but not for PDE1C. In order to address this we have designed a set of degenerate primers which should be capable of detecting PDE1C transcripts. Indeed, using these, we were able to identify a fragment of the predicted size, about 430 bp, using RNA extracts from the human thyroid carcinoma cell line FTC133 but not with RNA from PMA-treated CHO cells (Figure 2), indicating that the Ca^{2+}/CaM-stimulated PDE induced in CHO cells was not the PDE1C isoform. Using our set of original PCR primers, which were optimized for amplification of *PDE1A* and *PDE1B* transcripts, we were unable to amplify any product from FTC133 cells. However, we were successful in amplifying a product of the predicted size from CHO cells that had been challenged with either PMA [21] or receptor ligands able to induce PDE1 activity, such as ATP (Figure 2), or in CHO cells transfected so as to overexpress the α- and ϵ-forms of PKC [21]. It thus seems most probable that the PDE1 species induced in CHO cells is either PDE1A or PDE1B. In the absence of any reported sequence for human PDE1B, it is not possible to design PCR primer pairs that would allow us to discriminate between these two possibilities. However, we have now cloned and sequenced the 601 bp PCR fragment amplified from PDE1-induced CHO cells. From such analyses we obtained an identical sequence (Figure 3; GenBank accession number U40585) for six independent clones using products from both PMA- and ATP-treated cells. In addition, using RNA extracts from Jurkat T-cells we were also able to use this set of primers to amplify a 601 bp fragment whose sequence (Figure 3; GenBank accession number U40584) was distinct from that reported for human PDE1A and PDE1C forms [22] but which bore strong similarity to that obtained using RNA extracts from PMA-treated CHO cells. Pairwise alignments found that the three existing *PDE1B* sequences shared 91–95 % and 90–92 % nucleotide identity with the Chinese hamster sequence and the human Jurkat T-cell sequence respectively, whereas the *PDE1A* and *PDE1C* sequences shown were 68–69 % identical with Chinese hamster and 67–71 % identical with the human sequence. As might be expected, most of the mismatches occur at 'wobble' positions, such that the conservation between the Chinese hamster sequence and the homologous *PDE1B* sequences is even more marked at the amino acid level. We found that the Chinese hamster sequence has 99 %, and the human sequence 96–97 % amino acid identity when compared pairwise with the other *PDE1B* sequences, whereas the Chinese hamster sequence has 65–69 %, and the human sequence 66–70 % amino acid identity when compared with the non-homologous *PDE1A* and *PDE1C* sequences. We are therefore able to assign our sequences as PDE1B forms.

Conclusion

Challenge of CHO cells with a variety of ligands that can serve as agonists for distinct receptors able to stimulate lipid-signalling processes all provoked the extremely rapid and transient induction of Ca^{2+}/CaM-stimulated PDE activity. This novel activity appeared within about 15 min of exposure to ligands, rose to a maximum value within 30 min to 1 h before rapidly decreasing. Certainly the rapidity of PDE1 induction by these various agonists bears the hallmark of an 'immediate early' response such as has been noted consequent on the activation of PKC in a number of cells, leading to the induction of, for example, c-Fos [48]. Responses mediated by Fos and Jun through the AP1 system can show very rapid time courses. In this regard, insulin has been demonstrated to enlist AP1 activation in CHO and CHO-T cells [46]. However, as shown here, insulin singularly failed to induce PDE1 activity in these CHO cell lines and, indeed, induction of PDE1, as noted here, was considerably more rapid than the rate at which AP1 activation can be effected by insulin [46]. Thus regulation of *PDE1B* transcription as a consequence of AP1 action would appear to be unlikely.

The fact that induction of PDE1B can be elicited by the phorbol ester PMA [21] suggests that activation of PKC provides the basis for induction by these various endogenous receptor systems in CHO cells that are all coupled to lipid-signalling processes. That overexpression in CHO cells of either PKC-α or PKC-ϵ, but not various other PKC isoforms, can also induce PDE1 activity and transcript production [21] suggests that the transcriptional regulation of the *PDE1B* gene may show distinct specificity with regards to the action of PKC isoforms. Identification of the molecular basis of this remarkably rapid regulatory transcriptional event may shed light on both the functional roles of PKC isoforms and 'cross-talk' regulation [49] between the lipid- and cyclic-nucleotide-signalling pathways. In this regard, it is certainly most intriguing that agonists for a variety of endogenous lipid-signalling receptors in CHO cells can all elicit the rapid transient induction of a cyclic nucleotide PDE, the activity of which can be stimulated by Ca^{2+}, the intracellular concentration of which is invariably elevated by these agonists.

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