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PDE7A1 hydrolyzes cCMP

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ABSTRACT

The degradation and biological role of the cyclic pyrimidine nucleotide cCMP is largely elusive. We investigated nucleoside 3′,5′-cyclic monophosphate (cNMP) specificity of six different recombinant phosphodiesterases (PDEs) by using a highly-sensitive HPLC–MS/MS detection method. PDE7A1 was the only enzyme that hydrolyzed significant amounts of cCMP. Enzyme kinetic studies using purified GST-tagged truncated PDE7A1 revealed a cCMP K_M value of 135 ± 19 μM. The V_{max} for cCMP hydrolysis reached 745 ± 27 nmol/(min mg), which is about 6-fold higher than the corresponding velocity for adenosine 3′,5′-cyclic monophosphate (cAMP) degradation. In summary, PDE7A is a high-speed and low-affinity PDE for cCMP.

1. Introduction

Physiological role and metabolism of the pyrimidine nucleotide 3′,5′-cCMP are still largely elusive. The existence of a cCMP-forming cytidylyl cyclase was proposed [1], but technical problems resulted in a false-positive detection of cCMP [2]. The lack of sensitive detection methods hampered research activity in this field for many years. With the advent of highly sensitive and selective mass spectrometry-based detection systems [3], it became possible to detect even very low concentrations of cyclic pyrimidine nucleotides in cells, tissues and enzymatically digested samples [4,5]. cCMP is synthesized by soluble adenylyl cyclase [4], by soluble guanylyl cyclase [6] and by the *Pseudomonas aeruginosa* exotoxin ExoY [7]. It occurs in numerous cell types like human embryonic kidney cell line (HEK-293) cells and rat B103 neuroblastoma cells and primary cells from the neuronal, mesenchymal or epithelial lineage [8]. The activity of hyperpolarization-activated cyclic nucleotide-gated cation channel (HCN) ion channels is modulated by cCMP [9]. Furthermore, cCMP-agarose binds protein kinase A (PKA) [10] and cCMP regulates both PKA and protein kinase G (PKG) in vitro [11]. cCMP causes guanosine 3′,5′-cyclic monophosphate (cGMP) kinase I activation and subsequent relaxation of murine aorta smooth muscle cells [12]. If cCMP is a second messenger like the purine nucleotides adenosine 3′,5′-cyclic monophosphate (cAMP) and cCMP, it needs inactivation mechanisms to switch off the intracellular signal. A so-called "cCMP-specific" phosphodiesterase (PDE) was claimed with K_M values in the millimolar range [13–16]. Moreover, a "multifunctional cCMP-PDE" was proposed with K_M values <200 μM and activity for both 3′,5′- and 2′3′-cNMPs [16–21]. However, the identity of these proteins is still elusive.

We studied nucleoside 3′,5′-cyclic monophosphate (cNMP) specificity of eight recombinant PDEs (PDE1B, 2A, 3A, 3B, 4B, 5A, 8A and 9A), but, despite their broad substrate specificity, none of these enzymes accepted cCMP as a substrate [5]. In this publication we identified PDE7A as a cCMP-degrading PDE.

Abbreviations: A549, human lung carcinoma cell line; AMP, adenosine 5′-monophosphate; cAMP, adenosine 3′,5′-cyclic monophosphate; B103, rat neuroblastoma cells; CMP, cytidine 5′-monophosphate; cCMP, cytidine 3′,5′-cyclic monophosphate; CyaA, *Bordetella pertussis* adenylyl cyclase toxin; ExoY, *P. aeruginosa* exotoxin Y; GMP, guanosine 5′-monophosphate; cGMP, guanosine 3′,5′-cyclic monophosphate; GST, glutathione-S-transferase protein tag; HCN, hyperpolarization-activated cyclic nucleotide-gated cation channel; HEK-293, human embryonic kidney cell line; HIS, hexahistidine tag; HPLC–MS/MS, high performance liquid chromatography tandem mass spectrometry; HuT 78, human lymphoblast cell line; IMP, inosine 5′-monophosphate; cIMP, inosine 3′,5′-cyclic monophosphate; MRP5, multidrug resistance-associated protein 5; cNMP, nucleoside 3′,5′-cyclic monophosphate; PDE, phosphodiesterase; PKA, protein kinase A; PKG, protein kinase G; Sf9, *Spodoptera frugiperda* insect cell line; TMP, thymidine 5′-monophosphate; cTMP, thymidine 3′,5′-cyclic monophosphate; UMP, uridine 5′-monophosphate; cUMP, uridine 3′,5′-cyclic monophosphate; U-937, human promonocytic cell line; XMP, xanthosine 5′-monophosphate; cXMP, xanthosine 3′,5′-cyclic monophosphate

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2. Materials and methods

2.1. Enzymes and reagents

Recombinant truncated PDE7A1/2 (shared sequence of PDE7A1 and PDE7A2, starting at amino acid 121 of Gene bank #NM_002603) was obtained from *BPS Bioscience* (San Diego, CA, USA). The enzyme was N-terminally GST-tagged, had a purity of >44% and an activity of 221 pmol/min/μg. Full length N-terminally HIS-tagged PDE1A3, 6AB, 7A1, 10A1 and 11A1 (lysates from baculovirus-infected *Spodoptera frugiperda* insect cell line (Sf9) cells, 0.5 pmol/min/μl) and a control lysate from uninfected Sf9 cells were purchased from *sb drug discovery* (Glasgow, UK). Detailed information on the purity and quality of the different PDE preparations is provided in the Supplementary Methods (1.3). Calmodulin was obtained from Calbiochem (Merck, Darmstadt, Germany). The internal standard tenofovir was obtained from the NIH AIDS Research and Reference Reagent Program (Germantown, MD, USA). The selective and competitive PDE7A inhibitor BRL-50481 (5-Nitro-2,N,N-trimethylbenzenesulfonamide) was obtained from *TOCRIS Bioscience* (Bristol, UK) and dissolved in DMSO to yield a 5 mM stock solution. Adenosine 5′-monophosphate (AMP), cytidine 5′-monophosphate (CMP), guanosine 5′-monophosphate (GMP), thymidine 5′-monophosphate (TMP), uridine 5′-monophosphate (UMP), inosine 5′-monophosphate (IMP) and xanthosine 5′-monophosphate (XMP) were from Sigma–Aldrich (Steinheim, Germany). The cyclic 3′,5′-nucleotides cAMP, cCMP, cGMP, inosine 3′,5′-cyclic monophosphate (cIMP), thymidine 3′,5′-cyclic monophosphate (cTMP), uridine 3′,5′-cyclic monophosphate (cUMP) and xanthosine 3′,5′-cyclic monophosphate (cXMP) were purchased from Biolog Life Science Institute (Bremen, Germany). All other reagents were analytical grade and from standard suppliers.

2.2. Screening of enzymes with various cNMPs

Hydrolytic activity of various PDEs was determined in 1x PDE buffer (50 mM Tris–HCl, 1.7 mM EDTA, 8.3 mM $MgCl_2$). For analysis of PDE1A3 activity, 100 μM $CaCl_2$, 100 mM EGTA and 100 nM calmodulin were added. PDE1A3, PDE6AB, PDE7A1 and PDE11A1 were analyzed at a volume activity of 5 pmol/(min ml). PDE10A1 was added at a volume activity of 12.5 pmol/(min ml). GST-tagged and truncated PDE7A was used at a concentration of 0.95 μg/ml, corresponding to 210 pmol/(min ml). Cyclic nucleotides were added to yield a final concentration of 3 μM. Incubation was performed for 1–24 h at 30 °C. Enzyme was inactivated by 15 min incubation at 95 °C. Protein was precipitated by freezing and removed by centrifugation. The supernatant was diluted 1:5 with purified water and then additionally mixed with an equal volume of tenofovir solution (internal standard), yielding a final tenofovir concentration of 50 ng/mL. The samples were analyzed by high performance liquid chromatography tandem mass spectrometry (HPLC–MS/MS) as described in the Supplementary Methods.

2.3. Time course of PDE7A activity

Time course experiments were performed in PDE buffer + 0.05% (m/v) BSA at a cAMP- or cCMP-concentration of 3 μM. The purified GST-tagged and truncated PDE7A1/2 was added at a concentration of 0.95 μg/ml, corresponding to 210 pmol/(min ml). Crude PDE7A1-containing Sf9 cell lysate was added to yield a final activity of 5 U/ml. The samples were incubated at 30 °C under constant shaking. Aliquots were drawn at appropriate times, processed as described for the enzyme screening experiments (Section 2.2) and analyzed by HPLC–MS/MS as described in the Supplementary Methods. In all experiments, the samples were repeatedly subjected to short centrifugations (at least every 30 min) to avoid concentration changes by evaporation- and condensation processes.

2.4. Determination of cCMP K_M and V_{max} for the GST-tagged purified PDE7A1/2

Michaelis Menten kinetics was studied in PDE buffer + 0.05% (m/v) BSA at an enzyme concentration of 0.95 μg/ml, corresponding to 210 pmol/(min ml) in the presence of increasing concentrations of cyclic nucleotide. Enzyme-free control samples were run in parallel for each individual cyclic nucleotide concentration. After an incubation of 1 h at 30 °C, the samples were stopped, processed and analyzed by HPLC–MS/MS as described in Section 2.2 and in the Supplementary Methods. Only samples with <40% substrate hydrolysis were included into data analysis.

A rough estimation of the kinetics of PDE7A1/2-mediated cAMP hydrolysis was performed by incubating 50 nM, 75 nM, 100 nM and 250 nM of cAMP for 6 min at 30 °C in the presence of 0.06–0.24 μg/ml of enzyme. Only samples with <60% substrate degradation in at least one of the duplicates were included into analysis.

2.5. Determination of the effect of the competitive inhibitor BRL-50481 on PDE7A activity

The effect of the competitive inhibitor BRL-50481 on PDE7A1/2 activity was studied at concentrations between 10 nM and 100 μM in PDE buffer + 0.05% (m/v) BSA. Incubations were performed for 15 min (cAMP) or 4 h (cCMP) at 30 °C in the presence of 500 nM (cAMP) or 10 μM (cCMP) of cyclic nucleotide. The GST-tagged PDE7A1/2 was used at 0.48 μg/ml, corresponding to 105 pmol/(min ml) for cAMP hydrolysis. For cCMP experiments 0.95 μg/ml enzyme were used, corresponding to 210 pmol/(min ml). DMSO content of all samples was adjusted to 2%.

2.6. Quantitation of cyclic nucleotides (cNMPs) and nucleotide monophosphates (NMPs) by HPLC–MS/MS and data analysis

The concentrations of the cNMPs and NMPs were determined by HPLC–MS/MS, consisting of an UFLC HPLC system (Shimadzu, Kyoto, Japan) and the QTRAP5500™ triple quadrupole mass spectrometer (ABSciex, Framingham, MA, USA). A detailed description of HPLC configuration, eluent composition, *m/z* values, retention times and data analysis is provided in the Supplementary Methods (1.1 and 1.2).

2.7. Preparation of whole cell homogenates and Western blots

The preparation of whole cell homogenates and Western blotting for Suppl. Fig. 1 are described in detail in the Supplementary information (Suppl. Methods 1.4 and 1.5).

3. Results

3.1. Analysis of substrate specificity and identification of PDE7A1 as cCMP-hydrolyzing PDE

The substrate specificity of PDE1A3, 6AB, 7A1, 10A1 and 11A1 (HIS-tagged full-length enzymes) was characterized in crude Sf9 cell lysates. The PDEs were incubated in the presence of 3′,5′-cAMP, -cGMP, -cCMP, -cUMP, -cXMP, -cIMP and -cTMP (3 μM each) for 24 h. In case of <100 % hydrolysis, the velocity of the enzymatic reaction was calculated. When 100 % of the specific cNMP was hydrolyzed within 24 h, incubation was shortened until hydrolysis was <100%. Since the enzymes were not purified, a lysate from uninfected Sf9 cells was used as a negative control. The PDE-overexpressing Sf9

cell lysates were diluted several-fold by the manufacturer to yield the specified activity. Thus, the control lysate should show the maximum possible background activity, and we only interpreted PDE activities higher than the corresponding activity of the control sample.

We confirmed the broad substrate specificity of PDEs (Suppl. Table 1) [5]. PDE1A3 hydrolyzed cXMP and cIMP with similar activity as cAMP and cGMP. PDE6AB hydrolyzed cIMP with similar efficiency as cAMP and cGMP, and PDE7A1 showed some activity for cTMP. Most interestingly PDE6AB and PDE7A1 exhibited activity for cCMP. Hydrolysis of cCMP was undetectable in the control lysate and can therefore be attributed to the over-expressed PDEs. Neither PDE1A3, 10A1, 11A1, nor the eight PDEs analyzed in our previous report [5] degraded cCMP. Thus, PDE6AB and PDE7A1 represent the first PDEs that hydrolyze cCMP. PDE7A1 hydrolyzed cCMP with considerably higher activity than PDE6AB, comparable to its activity for the "standard" second messengers cAMP and cGMP.

Therefore, we focused on PDE7A1 and characterized its cCMP-hydrolyzing activity. To minimize background PDE activity we used a GST-tagged affinity-purified (>44%) and truncated enzyme, representing the consensus sequence of the splice variants PDE7A1 and PDE7A2 (="PDE7A1/2").

3.2. Time course of PDE7A1/2 activity

The time course of PDE7A1-mediated cCMP hydrolysis was determined in the presence of 3 μM of substrate. PDE7A1 Sf9 cell lysate hydrolyzed cCMP, and the concentration of the product CMP increased linearly for up to 8 h (Fig. 1A). The purified GST-tagged and truncated enzyme, PDE7A1/2, also converted cCMP to CMP (Fig. 1B). However, while it took >4 h to degrade 50% of cCMP (Fig. 1B), the corresponding amount of cAMP was converted to AMP in <30 min (Fig. 1C). A comparison of the linear curve parts (Fig. 1D; first 30 min for cAMP and first 60 min for cCMP) shows that the activity of PDE7A1/2 for cCMP was ~13% of its cAMP activity under these experimental conditions. Since the K_M value of PDE7A for cAMP is 100–200 nM [22], the 3 μM of cAMP in our time course experiments should saturate the enzyme, yielding the V_{max} of cAMP hydrolysis. The very slow cCMP hydrolysis, however, suggests that the corresponding K_M value is much higher than 3 μM.

3.3. Determination of K_M and V_{max} of PDE7A1/2 for cCMP and of V_{max} for cAMP

Michaelis–Menten analysis was performed with cCMP concentrations of up to 2 mM. Fig. 2A shows that detection of CMP accumulation allowed a highly sensitive analysis of enzyme activity, yielding K_M and V_{max} values of 135 ± 19 μM and 745 ± 27 nmol/(min mg), respectively (Fig. 2A, Table 1). Measurement of cCMP degradation up to a maximum substrate concentration of 250 μM yielded comparable results (Fig. 2B, Table 1). For comparison, the V_{max} of PDE7A1/2 for cAMP hydrolysis was calculated from Fig. 1D (saturation of PDE7A1/2 with 3 μM of cAMP), yielding 113.8 ± 8.7 nmol/(min mg). We determined the speed of cAMP degradation in the presence of 50, 75, 100 and 250 nM of substrate (2–3 experiments in duplicates, mean ± SEM, data not shown) and

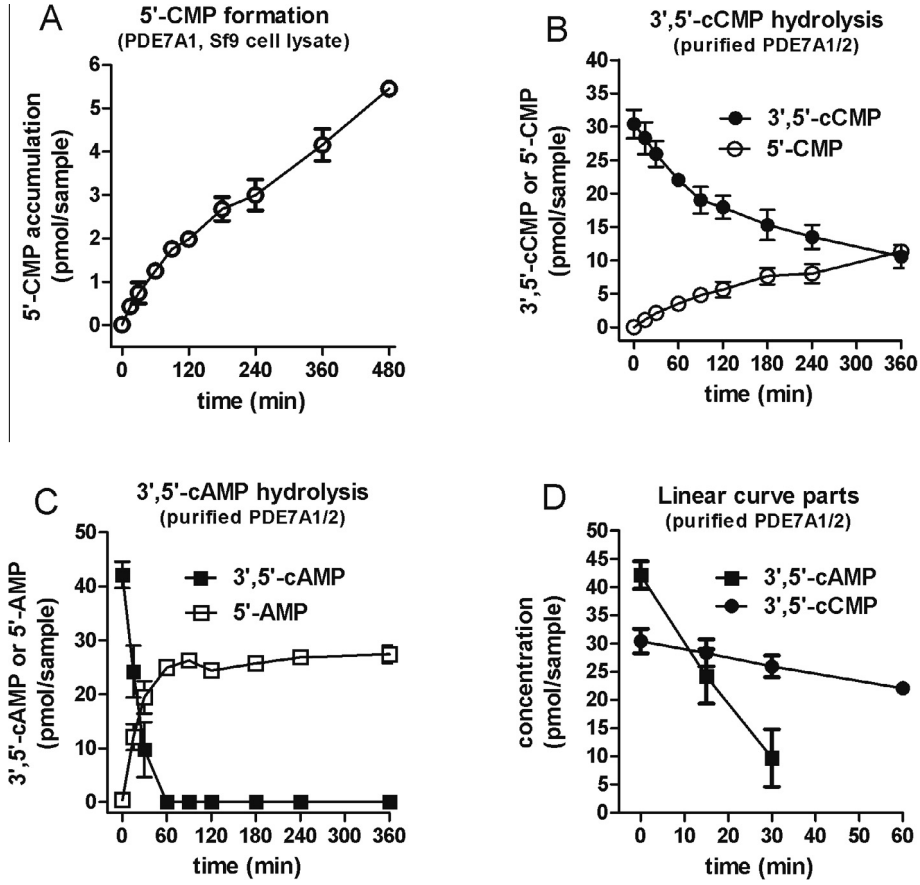

Fig. 1. Time course of PDE7A-mediated hydrolysis of 3′,5′-cAMP and 3′,5′-cCMP. (A) formation of 5′-CMP by a PDE7A1-containing Sf9 cell lysate in the presence of 3 μM of 3′,5′-cCMP; (B) degradation of 3′,5′-cCMP (3 μM) and formation of 5′-CMP by purified GST-tagged PDE7A1/2; (C) degradation of 3′,5′-cAMP (3 μM) and formation of 5′-AMP by GST-tagged purified PDE7A1/2; (D) comparison of the initial linear parts of the curves for 3′,5′-cAMP and 3′,5′-cCMP hydrolysis by purified GST-tagged PDE7A. All experiments were performed as indicated in Section 2. Data are means ± SEM. (A) shows merged data from two independent experiments in duplicates with partially different sampling times; (B–D) are from 2 independent experiments in duplicates with the same sampling.

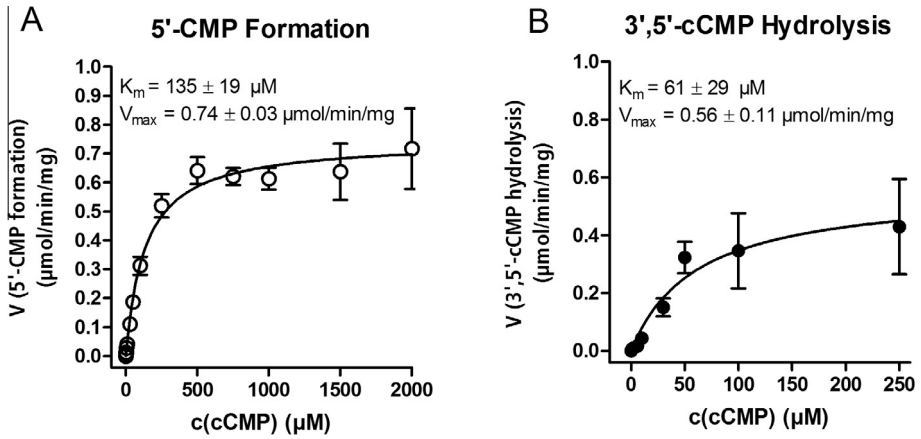

Fig. 2. Michaelis Menten kinetics of PDE7A-mediated hydrolysis of 3′,5′-cCMP. (A) Velocity of 5′-cCMP formation and (B) 3′,5′-cCMP degradation during incubation of GST-tagged PDE7A1/2 with increasing concentrations of 3′,5′-cCMP. All experiments were performed as indicated in Section 2. Data are means ± SEM from 2 to 4 independent experiments in duplicates.

Table 1
Characterization of cCMP hydrolysis by GST-tagged PDE7A1/2.

Parameter	CMP formation	cCMP hydrolysis	AMP formation	cAMP hydrolysis
V_{max}	745 ± 27 nmol/(min mg)	560 ± 110 nmol/(min mg)	n.d.	113.8 ± 8.7 nmol/(min mg)
K_M	135 ± 19 µM	61 ± 29 µM	n.d.	<100 nM (estimation)
pIC_{50} (BRL-50481)	6.98 ± 0.10	7.01 ± 0.14	5.37 ± 0.15	5.31 ± 0.11
K_i (BRL-50481)	98 nM	91 nM	n.d.	n.d.

n.d. = not determined.

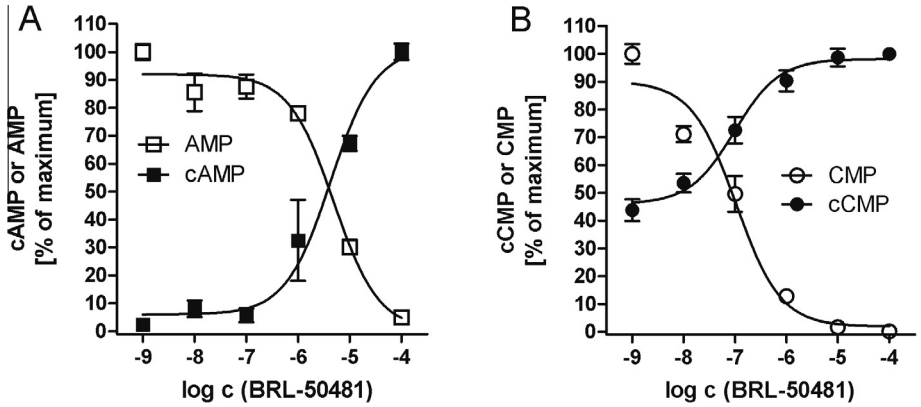

Fig. 3. Inhibition of PDE7A-mediated 3′,5′-cAMP and 3′,5′-cCMP hydrolysis by BRL-50481. (A) 3′,5′-cAMP (500 nM) degradation and 5′-AMP formation in the presence of increasing concentrations of BRL-50481. (B) 3′,5′-cCMP (10 µM) degradation and 5′-CMP formation in the presence of increasing concentrations of BRL-50481. All experiments were performed as indicated in Section 2. Curves were normalized to 100% cyclic nucleotide concentration in the presence of 100 µM of inhibitor (=100% inhibition) and to 100% product formation in the absence of inhibitor (=100% enzyme activity and no inhibition). In both graphs, the data point at log −9 represents the inhibitor-free control. Data are means ± SEM from 3 independent experiments in duplicates.

found velocities of 69 ± 4, 89 ± 2, 117 ± 10 and 132 ± 29 nmol/(min mg), respectively. Thus, under our experimental conditions, the K_M value of PDE7A1/2 for cAMP is probably around 50 nM.

3.4. Inhibition of PDE7A1/2-mediated hydrolysis of cAMP and cCMP by BRL-50481

BRL-50481 is a specific competitive inhibitor of PDE7A-mediated cAMP hydrolysis [23]. PDE7A1/2 activity for cCMP (10 µM) was reduced by 50% in the presence of ~100 nM of BRL-50481 (Fig. 3B, Table 1). By contrast, the enzymatic activity in the presence of 500 nM of cAMP was reduced with an IC_{50} of 4–5 µM for both cAMP hydrolysis and AMP formation (Fig. 3A, Table 1). Despite the long incubation time of 4 h, only 50–60% of the cCMP were hydrolyzed in the absence of inhibitor (Fig. 3B). Under the assumption of steady-state conditions, the K_i value of BRL-50481 (calculated from IC_{50}, cCMP concentration and K_M) was 98 nM and 91 nM for CMP formation and cCMP hydrolysis, respectively (Table 1). Since cAMP hydrolysis was almost 100%, even in the presence of 100 nM of inhibitor, steady-state conditions were not guaranteed any more, and we did not calculate the BRL-50481 K_i value for cAMP hydrolysis.

3.5. Validation of integrity and identity of the PDE7A used in the experiments

In order to confirm the presence and integrity of PDE7A1 in the Sf9 cell lysate and of PDE7A1/2 in the recombinant preparation, we performed a Western blot with the enzyme preparations. PDE7A1 was detected in the corresponding Sf9 cell lysate (Suppl. Fig. 1, lane

1), while no band was visible in the equally concentrated PDE7A1-free control lysate (Suppl. Fig. 1, lane 2). PDE7A1 from Sf9 cells migrated as two bands and slightly faster than the expected molecular weight of 57 kDa. This may be caused by a different glycosylation pattern in insect cells. Analysis of the recombinant purified GST-tagged PDE7A1/2 (Suppl. Fig. 1, lane 3) showed the predicted molecular mass of 60–70 kDa.

Next we aimed at identifying a cell line with high PDE7A1 expression, which may be suited for future functional studies. Since high PDE7A1 expression was reported for human lymphoblast cell line (HuT 78) lymphoma cells, in contrast to rather low expression levels in human promonocytic cell line (U-937) cells [23], we chose these two cell lines for Western blotting. In fact, U937 cells (Suppl. Fig. 1, lane 4) exhibited only a very weak signal, while HuT 78 cells (Suppl. Fig. 1, lane 5) showed an intense band at the molecular weight corresponding to PDE7A1.

4. Discussion

Out of 13 enzymes (five enzymes analyzed in this paper and eight in Ref. [5]), we identified PDE7A1 as the first PDE that hydrolyzes cCMP. Except for a very low activity of PDE6AB, the other enzymes described in this paper did not hydrolyze cCMP. However, they partially showed activity for "exotic" cyclic nucleotides like cXMP, cIMP and cTMP.

Only PDE7A1 and – to a lesser extent – PDE6AB hydrolyze cCMP. Due to its low V_{max} for cAMP, it has been suggested that PDE7A may need additional factors to be activated or may simply play a role in regulating "basal" cAMP concentrations [22]. Our findings suggest that PDE7A may be additionally involved in the degradation of intracellular cCMP. Analysis of HEK-293 and B103 cells has demonstrated that cCMP is generated by soluble adenylyl cyclase in mammalian cells, reaching concentrations comparable to the established second messenger cGMP [4]. Since cCMP is biologically active [9,11,12], it is conceivable that its intracellular concentration has to be regulated, e.g. by the activity of PDEs. PDE7A1/2 is a low-affinity PDE for cCMP with a K_M value around 135 μM. Thus, the question arises, whether this activity is relevant under physiological conditions. In fact, cCMP occurs in mammalian cells, and HEK-293 cells contain ~30 pmol of cCMP per 10^6 cells under basal conditions [8]. Since the volume of a HEK-293 cell is ~1000 femtoliters [24], this corresponds to a cCMP concentration around 30 μM. This calculation, however, disregards the fact that in many cell types a large part of the volume is occupied by the nucleus. Thus, if cCMP is confined to the cytoplasm, its actual concentration may be even higher. In summary, it is likely that cCMP concentrations in human cells can reach levels, where PDE7A1/2 activity becomes relevant.

The cCMP degrading activity of PDE7A may even become more important during bacterial infections, since bacterial toxins like edema factor (EF) [25,26] or adenylyl cyclase toxin CyaA [26], both from *Bacillus anthracis*, produce cCMP. In addition, the *P. aeruginosa* type III secretion protein ExoY, which shows highly toxic effects in the rat model [27], produces a significant amount of cCMP in B103 and human lung carcinoma cell line (A549) cells [7]. If a cell is flooded by cCMP during a bacterial infection, detoxifying processes are required. PDE7A1-mediated cCMP hydrolysis may be one of them, in addition to the very recently described export of cCMP by the multi-drug resistance protein MRP5 [28]. Both PDE7A1 and MRP5 represent low-affinity and high-capacity mechanisms for the removal of cCMP, which additionally supports a potential function in the detoxification of exceedingly high cCMP concentrations. cCMP-degrading activity has been described in several types of cells and tissues [13–21] and was attributed to a "cCMP-specific PDE" and a "multifunctional PDE" (Suppl. Table 2). However, the molecular identity of these activities is still elusive. We have identified PDE7A1 as cCMP-hydrolyzing PDE, but the properties of PDE7A1 are quite different from the features reported for "cCMP-specific PDE" and "multifunctional PDE" (Suppl. Table 2). While "cCMP-specific PDE" hydrolyzes only cCMP with a K_M value in the millimolar range [13–15], the PDE7A1/2 in our study shows a >10-fold lower K_M-value and hydrolyzes also 3′,5′-cAMP. The "multifunctional PDE" [17–20] has a K_M value of ~180 μM for 3′,5′-cCMP, which is close to the 135 μM of PDE7A1/2. However, "multifunctional cCMP-PDE" also accepts 2′,3′-cNMPs as substrates [21], but we did not detect such 2′,3′-cNMP-hydrolytic activity for PDE7A1/2 (data not shown). Moreover, PDE7A1 and PDE7A2 have molecular masses of 57 and 50 kDa, respectively [29], while the "multifunctional PDE" was purified as a protein with 33 kDa [21] and the molecular weight of "cCMP-specific PDE" is only 28 kDa. Therefore, we conclude that we have discovered a third type of cCMP-hydrolyzing PDE, which is distinct from the cCMP-hydrolyzing activities reported to date (Suppl. Table 2).

The PDE7-specific competitive inhibitor BRL-50481 [23] inhibited PDE7A1/2-mediated cCMP hdrolysis with a K_i value of 98 nM or 91 nM for CMP formation or cCMP hydrolysis, respectively. This corresponds well to the K_i value of 180 nM that was previously reported for inhibition of cAMP hydrolysis by PDE7A1 expressed in Sf9 cells [23]. This suggests that cCMP may bind to the same binding site of PDE7A1 as cAMP. From a different point of view, cCMP could also be regarded as an inhibitor of PDE7A1-mediated cAMP hydrolysis or vice versa.

PDE7A1/2 has a V_{max} value for cCMP, which is 6–7-fold higher than for cAMP. This may result in some cCMP "specificity" of PDE7A1/2. Similarly, although the "cCMP-specific PDE" has K_M values in the millimolar range that are very similar for both cAMP and cCMP (Suppl. Table 2), its "specificity" for cCMP is caused by the ~200-fold higher V_{max} value for cCMP hydrolysis, as compared to cAMP degradation [16].

It may be argued that the observed cCMP-hydrolyzing activity is not caused by PDE7A1, but due to a contamination. However, the following arguments support the notion that PDE7A1 hydrolyzes cCMP: First, the activity was observed in two PDE7A1-containing preparations that were very different (Sf9 cell lysate and purified protein) and from two independent commercial suppliers. Second, as shown in this paper and in our preceding publication [5], the cCMP-degrading activity was absent in all other enzyme preparations from these suppliers (except for the low activity observed with PDE6AB). Finally, the cCMP-hydrolyzing activity was eliminated by the PDE7A1-selective inhibitor BRL-50481 with a K_i value that corresponds to the literature [23].

Our results also show distinct substrate profiles for the investigated PDEs (Suppl. Table 1), suggesting PDE-specific "cNMP signatures". Besides the unique cCMP-hydrolyzing activity of PDE7A1, we also found that PDE1A3 hydrolyzes cXMP and cIMP much faster than any of the other tested enzymes. This finding confirms our previous results with purified recombinant PDE1B [5], indicating that this substrate profile may be a feature shared by all members of the PDE1 family.

In conclusion, PDEs should be re-visited and studied with the complete spectrum of cNMPs and with substrate concentrations up to the micromolar range. Low-affinity- and high-V_{max}-PDE activity as well as PDE activity for "uncommon" cyclic nucleotide substrates may represent a novel and yet unappreciated component of physiological PDE function.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2014.08.005.

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