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cAMP, cGMP, cCMP and cUMP concentrations across the tree of life: High cCMP and cUMP levels in astrocytes

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HIGHLIGHTS

- cCMP and cUMP occur in numerous mammalian cell types.
- Astrocytes possess high cCMP and cUMP levels.
- The concentrations of cUMP and cCMP vary in different cells.
- Prokaryotes, fungi, amoeba and invertebrates lack cCMP and cUMP.

GRAPHICAL ABSTRACT

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ABSTRACT

Adenosine 3′,5′-cyclic monophosphate (cAMP) and guanosine 3′,5′-cyclic monophosphate (cGMP) are well-established second messengers, whereas the physiological role of the cyclic pyrimidine nucleotides cytidine 3′,5′-cyclic monophosphate (cCMP) and uridine 3′,5′-cyclic monophosphate (cUMP) is poorly understood. Certain mammalian nucleotidyl cyclases (NCs) and bacterial NC toxins can generate cCMP and cUMP. Human HEK293 cells and rat B103 neuroblastoma cells are of neuronal origin and possess high basal concentrations of cCMP and cUMP that can be attributed to soluble adenylyl cyclase activity. These data prompted us to conduct a systematic analysis of basal nucleoside 3′,5′-cyclic monophosphate (cNMP) concentrations across the tree of life. cCMP and cUMP were identified in many mammalian cell lines and primary cells. cNMP patterns varied broadly among cells, and in several systems, cCMP and cUMP concentrations were quite high. Prokaryotes, fungi, amoeba and invertebrates lacked cCMP and cUMP, whereas cAMP was found across the tree of life. High cCMP and cUMP concentrations were found in astrocytes. The distinct cNMP patterns support specific second messenger roles of cCMP and cUMP, specifically in astrocytes.

Abbreviations: cAMP, adenosine 3′,5′-cyclic monophosphate; cGMP, guanosine 3′,5′-cyclic monophosphate; cCMP, cytidine 3′,5′-cyclic monophosphate; cNMP, nucleoside 3′,5′-cyclic monophosphate; cUMP, uridine 3′,5′-cyclic monophosphate; pGC, particulate guanylyl cyclase; mAC, membranous adenylyl cyclase; AC, adenylyl cyclase; sAC, soluble adenylyl cyclase; GC, guanylyl cyclase; sGC, soluble guanylyl cyclase; NC, nucleotidyl cyclase; HPLC-MS/MS, high performance liquid chromatography tandem mass spectrometry; HPLC–MS/TOF, high performance liquid chromatography quadrupole time of flight mass spectrometry; LLOQ, lower limit of quantitation; NC, nucleotidyl cyclase.

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1. Introduction

Adenosine 3′,5′-cyclic monophosphate (cAMP) and guanosine 3′,5′-cyclic monophosphate (cGMP) constitute well-established second messengers that regulate numerous cell functions across the tree of life [1,2]. cAMP and cGMP are generated by adenylyl cyclases (ACs) and guanylyl cyclases (GCs), respectively [1–7]. In marked contrast to cAMP and cGMP, the roles of the cyclic pyrimidine nucleotides cytidine 3′,5′-cyclic monophosphate (cCMP) and uridine 3′,5′-cyclic monophosphate (cUMP) are only poorly understood [8]. Methodological problems with regard to cCMP and cUMP detection severely hampered development of the field so that little research was performed over the past 30 years [8]. The situation has changed recently with the development of extremely precise high performance liquid chromatography quadrupole time of flight mass spectrometry (HPLC–MS/TOF) methods for unequivocal identification of cCMP and cUMP [9] and sensitive and specific high performance liquid chromatography tandem mass spectrometry (HPLC–MS/MS) methods for quantitation of cCMP and cUMP [10,11]. Taking advantage of these new methods, the bacterial AC toxins edema factor from *Bacillus anthracis* and CyaA from *Bordetella pertussis* were shown to generate cCMP and cUMP, in addition to cAMP [11]. Moreover, the nitric oxide-stimulated soluble guanylyl cyclase (sGC) and the bicarbonate-stimulated soluble adenylyl cyclase (sAC) have been identified as cCMP and cUMP-generating enzymes [9,10,12], whereas particulate guanylyl cyclases (pGCs) and the forskolin-stimulated membranous adenylyl cyclases (mACs) do not generate cCMP and cUMP [10–12]. Human HEK293 cells and rat B103 neuroblastoma cells possess high concentrations of cCMP and cUMP and sAC accounts for these high basal cyclic pyrimidine nucleotide concentrations [12]. Notably, not only B103 cells but also HEK293 cells are of neuronal origin [13]. Lastly, the type III-secretion toxin ExoY from *Pseudomonas aeruginosa* was identified as nucleotidyl cyclase (NC) that generates large quantities of cUMP and cUMP in B103 and HEK293 cells [14].

These data prompted us to conduct a systematic analysis of basal nucleoside 3′,5′-cyclic monophosphate (cNMP) concentrations across the tree of life. We studied 17 cell lines from human, two from mouse, and one from rat, hamster, monkey and dog, each (Table 1). We also examined seven primary human cell types, three primary rat cell types and model organisms from invertebrates, amoeba, plants, fungi and prokaryotes (Table 2). Astrocytes were found to exhibit particularly high cCMP and cUMP concentrations.

2. Materials and methods

2.1. Cell culture

Primary rat astrocytes and microglia cells were isolated and cultured as described previously [15]. Cell lines were propagated according to previously published protocols [9,11,12]. Protein concentrations were determined as described using bicinchoninic acid as detection reagent [12].

2.2. Analysis of cNMPs in intact cells

cNMP quantitation in cells and model organisms was performed via HPLC–MS/MS as described using a QTrap5500™ triple quadrupole mass spectrometer (ABSCIEX, Foster City, CA, USA) [9–12]. Ion source settings and collision gas pressure were manually optimized regarding ion source voltage, ion source temperature, nebulizer gas, and curtain gas (ion source voltage of 5500 V, ion source temperature of 400 °C, curtain gas of 30 psi, collisionally activated dissociation gas of 9 psi). Nitrogen was used as collision gas. Chromatographic data were collected and analyzed with Analyst 1.5.1 software (ABSCIEX). The lower limit of quantitation (LLOQ) for cAMP was 0.04 pmol per sample, for cGMP 0.07 pmol per sample, for cCMP 0.07 pmol per sample, and for cUMP 0.4 pmol per sample [16]. In some systems, the identity of cCMP and cUMP was also confirmed by HPLC–MS/TOF [9].

2.3. Statistics

Data are presented as means ± SEM, and are based on 3–6 independent experiments.

3. Results

3.1. Analysis of basal cNMP concentrations in cell culture lines

In all cell culture lines, cAMP was the predominant cNMP (Table 1). However, the absolute cAMP concentrations varied by up to 100-fold among different cell lines. cAMP concentrations were particularly high in HEK293, COS and MDCK cells, and they were particularly low in several leukemia cell lines. cGMP concentrations were always lower than cAMP concentrations, but the ratio varied substantially. For example, in HEK293 cells, cGMP reached about 50% of the levels of cAMP, whereas in HL60 cells, cGMP levels were 30-fold lower than cAMP levels. In 13 out of 23 cell lines, cGMP concentrations were below LLOQ under basal conditions. This was quite surprising given the fact that cGMP is an established second messenger [1,2,6,7] and that the LLOQ for cGMP is low, i.e. cGMP is detected with high sensitivity (see Section 2 and [16]). cCMP was identified in all but two cell lines. In those cell lines that contained both cGMP and cCMP, cGMP concentrations generally surpassed cCMP concentrations. The highest cCMP concentrations were found in HEK293 cells and followed by COS, B103 and MDCK cells. cUMP was identified in 16 out of the 23 cells lines, despite the high LLOQ for this cNMP (see Section 2 and [16]). The highest cUMP concentrations were observed for HEK293 cells, followed by MDCK, COS and B103 cells. In general, cUMP concentrations surpassed cCMP concentrations. Most strikingly, cUMP concentrations surpassed cGMP concentrations in nine systems. In all six mammalian species studied (human, monkey, dog, rat, mouse and hamster), we detected cCMP and cUMP. In HEK293 cells, HeLa cells, B103 cells and MDCK cells we confirmed the presence of cCMP and cUMP by HPLC-MS/TOF (data not shown).

3.2. Analysis of basal cNMP concentrations in primary mammalian cells

Cell lines often possess tumor-like properties and do not reflect the physiological situation. Therefore, we analyzed several primary mammalian cell types from two species (human and rat) (Table 2). In human neutrophils, monocytes and platelets, cAMP was the most abundant cNMP; platelets also exhibited considerable basal concentrations of cGMP. However, neither cell type contained cCMP or cUMP. In contrast, in cultured human Th2 lymphocytes, cCMP and cUMP could be detected. In primary human hepatocytes, pulpa stem cells and endothelial cells, cCMP and cUMP were also found. Most strikingly, rat astrocytes exhibited very high cAMP concentrations, and cUMP surpassed cCMP and cGMP. In marked contrast, in rat microglia cells, only cAMP was detected, and in rat cardiac fibroblasts, low levels of cCMP (but not cUMP) were detected.

3.3. Analysis of basal cNMP concentrations in model organisms

In regenerating tail blastema of *Ambyostoma mexicanum* we detected cAMP and cGMP at low concentrations, but no cCMP or