Identification of novel cyclic nucleotide phosphodiesterase gene cDNAs in the brain of guinea pig

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Abstract: Objective Cyclic nucleotide phosphodiesterase (PDE) is a critical component of the nitric oxide (NO) signaling pathway and plays critical roles in cognition and learning, Parkinson's disease, attention deficit hyperactivity disorder, psychosis and depression. The PDEs in the brain of guinea pig have not yet been reported. The present study aimed to detect the unknown *Pde* cDNAs in the brain of guinea pig. **Methods** Reverse transcription polymerase chain reaction (RT-PCR) and sequence comparison analysis were performed to detect the expression of *Pde* cDNAs and to assess the identity rates of cDNA and amino acid sequences between guinea pig and human or mouse, respectively. The RT-PCR primers were located on the conserved region of human *PDE* and mouse *Pde* cDNAs. **Results** Eleven novel *Pde* cDNAs were detected in the brain of guinea pig (*Cavia porcellus*), including *CpPde1a*, *CpPde1b*, *CpPde2a*, *CpPde4a*, *CpPde4d*, *CpPde5a*, *CpPde6c*, *CpPde7b*, *CpPde8a*, *CpPde9a*, and *CpPde10a*. The identity rates of the *Pde* cDNA sequences between guinea pig and human ranged from 83.8% to 94.3%, and those of the amino acid sequences ranged from 91.9% to 100%. The identity rates of *Pde* cDNA sequences between guinea pig and mouse ranged from 84.6% to 92.1%, and those of amino acid sequences ranged from 91.2% to 99.2%. The average identity rate of the 11 *Pde* cDNA sequences between guinea pig and human was significantly higher ($P < 0.01$) than that between guinea pig and mouse. The putative partial amino acid sequences of guinea pig contained at least one of the conserved domains of human and mouse PDE proteins. **Conclusion** These results indicate that the brain-expressed *Pde* genes are identified in guinea pig, which lays the foundation for further investigating the physiological roles of PDE proteins in the brain.

Keywords: cyclic nucleotide phosphodiesterase; the central nervous system; nitric oxide; guinea pig; cross-species comparison

1 Introduction

Nitric oxide (NO) is an important signaling molecule in the nervous system. NO is characterized as an intercellular

messenger that modulates brain development and synaptic plasticity, and it is also involved in neurological disorders^[1]. NO may exert its effects on the functions of various proteins through increasing the levels of second messengers including adenosine 3' 5'-cyclic monophosphate (cAMP) and guanosine 3' 5'-cyclic monophosphate (cGMP).

Cyclic nucleotide phosphodiesterase (PDE) is a critical component of the NO signaling pathway. It regulates the

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cellular levels of cAMP and cGMP by controlling their degradation rates^[2]. A total of 22 PDE isoforms have been identified in mammalian tissues^[3]. These isoforms differ in three-dimensional structure, kinetic property, inhibitor sensitivity, tissue distribution and cellular localization, and they modulate the NO signaling pathway in the cell^[4]. Almost all the known PDE isoforms are expressed in mammalian brain and are believed to play critical roles in cognition and learning, Parkinson's disease, attention deficit hyperactivity disorder, psychosis and depression^[5]. Therefore, the PDEs have become a target for the development of specific therapeutic agents against neurological disorders.

The guinea pig (*Cavia porcellus*) is a species of rodent that serves as an important laboratory animal for studying the disease mechanisms and the potential therapeutic agents^[6,7]. Therefore, it is very important to identify PDE expression in various tissues of guinea pig. The expression of *Pde* cDNAs in guinea pig cochlea has been identified through conserved homologous sequences^[8], but the expression of PDE isoforms in the brain of guinea pig has not yet been reported. The aim of this study was to identify the unknown *Pde* cDNAs in the brain of guinea pig by reverse transcription polymerase chain reaction (RT-PCR), and to characterize the cDNAs and the putative amino acid sequences by bioinformatics analysis.

2 Materials and methods

2.1 RT-PCR primers The conserved homologous exons of guinea pig *Pde* genes were obtained by comparing homologous sequences between human and mouse, and the primer pairs used to detect unknown *Pde* cDNAs of guinea pig were synthesized as previously reported^[8].

2.2 Total RNA isolation and RT-PCR Total RNA was isolated from the whole brain of guinea pig by using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA elution was quantified by OD_{260/280} readings, and the quality was assessed on 1.0% agarose gel. RT-PCR was conducted using the TaKaRa PrimeScript™ RT Reagent Kit (code No. DRR037S). To get the first strand cDNA, reverse transcription was performed with 2 µg total RNA as the template, using oligo18T primer and random hexamer primer. PCR was conducted at 94

°C for 4 min, followed by 20 cycles (30 cycles for the second PCR) at 94 °C for 30 s, *n* °C (according the most suitable annealing temperature for each gene^[8]) for 30 s, and 72 °C for 3 min, with a final extension step at 72 °C for 10 min. PCR products were visualized by ethidium bromide staining, purified using the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA), and sequenced by ABI3730.

2.3 Nucleotide sequence and amino acid sequence comparison To obtain the partial putative amino acid sequences, the partial cDNA sequences were predicted with a DNA-PROTEIN translation tool on the ExPASy website (<http://www.expasy.ch/tools/dna.html>). The cDNA sequences and putative protein sequences were aligned with the Clustal-X program of vector NTI (Informax; <http://www.informax.com>). The default alignment parameters (gap opening penalty: 15; gap extension penalty: 6.66; gap separation penalty range: 8; score matrix: swgapdnamt) were used for alignment^[9]. The identity rates of cDNA and amino acid sequences between guinea pig and human or mouse were calculated.

2.4 Conserved domain prediction To characterize the unknown guinea pig *Pde* genes, putative conserved domains in the partial amino acid sequences were searched on the NCBI website (<http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>) with search against database “CDD—37303 PSSMs” and an expect value threshold at 0.01^[10].

2.5 Statistical analysis Data of the identity rates were analyzed by the Student's *t* test. *P* < 0.05 was indicated as significantly different.

3 Results

RT-PCR experiments detected 11 *Pde* cDNAs in the brain of guinea pig, homologous to those of human and mouse including *PDE1A*, *PDE1B*, *PDE2A*, *PDE4A*, *PDE4D*, *PDE5A*, *PDE6C*, *PDE7B*, *PDE8A*, *PDE9A*, and *PDE10A* (Fig. 1). The nucleotide sequences and the putative amino acid sequences of these brain-expressed genes were submitted to the NCBI database (NCBI accession numbers: GQ227351, GQ227352, GQ227354, HM452149-HM452156) and named as *CpPde1a*, *CpPde1b*, *CpPde2a*, *CpPde4a*, *CpPde4d*, *CpPde5a*, *CpPde6c*, *CpPde7b*, *CpPde8a*, *CpPde9a*, and *CpPde10a*, respectively. The cDNA sequence and amino acid sequence

Fig. 1 RT-PCR identification of *Pde* (1-11) cDNAs in the brain of guinea pig. The 11 discovered genes were named as *CpPde1a*, *CpPde1b*, *CpPde2a*, *CpPde4a*, *CpPde4d*, *CpPde5a*, *CpPde6c*, *CpPde7b*, *CpPde8a*, *CpPde9a* and *CpPde10a*. M, DNA molecular ladder; +, β -actin.

Table 1. PDE cDNA and putative amino acid sequence identity rates between guinea pig and human or between guinea pig and mouse

PDE gene	cDNA sequence identity rate (%)		Protein sequence identity rate (%)	
	Guinea pig vs Human	Guinea pig vs Mouse	Guinea pig vs Human	Guinea pig vs Mouse
<i>PDE1A</i>	92.8	88.0	99.4	96.6
<i>PDE1B</i>	93.0	90.6	97.2	98.6
<i>PDE2A</i>	94.3	92.1	98.5	97.7
<i>PDE4A</i>	90.6	89.7	95.1	97.1
<i>PDE4D</i>	93.8	89.2	100.0	99.2
<i>PDE5A</i>	91.6	89.4	98.6	95.2
<i>PDE6C</i>	90.5	85.7	92.3	91.2
<i>PDE7B</i>	92.6	89.6	97.4	97.4
<i>PDE8A</i>	90.4	86.5	94.0	86.6
<i>PDE9A</i>	83.8	87.8	91.9	95.5
<i>PDE10A</i>	91.4	84.6	98.4	97.8
Average rate (mean $\pm$ SD)	91.35 $\pm$ 2.83	88.47 $\pm$ 2.22	96.62 $\pm$ 2.84	95.72 $\pm$ 3.72

comparisons showed that the identity rates of *Pde* cDNA sequences between guinea pig and human ranged from 83.8% to 94.3% and those of the amino acid sequences ranged from 91.9% to 100% (Table 1). The identity rates of *Pde* cDNA sequences between guinea pig and mouse ranged from 84.6% to 92.1% and those of amino acid sequences ranged from 91.2% to 99.2%. The average identity rate of the 11 *Pde* cDNA sequences between guinea pig and human was significantly higher ($P < 0.01$) than that between guinea pig and mouse, suggesting that guinea pig has a closer genetic relationship with human than with mouse. However, the average identity rate of amino acid sequences between guinea pig and human was not significantly different from that between guinea pig and mouse.

Conserved domain prediction showed that all the 11 PDE isoforms from human and mouse had an HDc superfamily domain, which is a metal-dependent phosphohydrolase with a conserved 'HD' motif. Besides, other domains were also found among these isoforms (Table 2). The 11 putative amino acid sequences identified from the brain of guinea pig all contained at least one of the conserved domains of human and mouse PDE proteins, suggesting that all of them were PDE isoforms.

4 Discussion

The NO signaling pathway has been well characterized in the nervous system of guinea pig^[11-13]. NO mediates various physiological processes in the brain. However, PDE, an

Table 2. Comparison of conserved domains of the partial PDE amino acid sequences of guinea pig with the full-length PDE amino acid sequences of human and mouse

Name	Guinea pig	Human	Mouse
PDE1A	HDc superfamily	HDc superfamily PDEase_I_N superfamily	HDc superfamily PDEase_I_N superfamily
PDE1B	HDc superfamily	HDc superfamily PDEase_I_N superfamily	HDc superfamily PDEase_I_N superfamily
PDE2A	HDc superfamily	HDc superfamily GAF superfamily	HDc superfamily GAF superfamily
PDE4A	HDc superfamily	HDc superfamily	HDc superfamily
PDE4D	HDc superfamily	HDc superfamily	HDc superfamily
PDE5A	GAF superfamily	HDc superfamily GAF superfamily	HDc superfamily GAF superfamily
PDE6C	GAF superfamily	HDc superfamily GAF superfamily	HDc superfamily GAF superfamily
PDE7B	HDc superfamily	HDc superfamily	HDc superfamily
PDE8A	REC superfamily	HDc superfamily REC superfamily PAS superfamily	HDc superfamily REC superfamily PAS superfamily
PDE9A	HDc superfamily	HDc superfamily	HDc superfamily
PDE10A	GAF superfamily	HDc superfamily GAF superfamily	HDc superfamily GAF superfamily

HDc superfamily: metal-dependent phosphohydrolases with conserved 'HD' motif. GAF superfamily: a domain present in phytochromes and cGMP-specific phosphodiesterases. REC superfamily: a signal receiver domain, originally thought to be unique to bacteria (CheY, OmpR, NtrC). PDEase_I_N superfamily: this domain is found in the N-terminus of the calcium/calmodulin-dependent 3'-5'-cyclic nucleotide PDE domain. PAS superfamily: PAS motifs appear in archaea, eubacteria and eukarya. Probably the most surprising identification of a PAS domain is in EAG-like K⁺ channels. PAS domains have been found to bind ligands and act as sensors for light and oxygen in signal transduction.

important component of the NO signaling pathway, is poorly known in the brain of guinea pig. In the present study, we identified 11 novel *Pde* partial cDNAs from the brain of guinea pig. Their putative amino acid sequences share at least one conserved domain with human and mouse PDEs, and the identity rate of amino acid sequences between guinea pig PDEs and human or mouse PDEs is high, showing that they are PDE isoforms. Among these brain-expressed PDEs, PDE1, PDE2 and PDE10 hydrolyze both cAMP and cGMP, while PDE4, PDE7 and PDE8 hydrolyze only cAMP. Besides, PDE5, PDE6 and PDE9 hydrolyze only cGMP^[3]. Furthermore, these PDEs have their unique inhibitors^[3], which allows using their

inhibitors to investigate their physiological roles in brain, helping develop specific therapeutic agents for the treatment of neurological disorders.

Currently, at least 20 PDEs have been identified in the mammalian central nervous system^[5], including the 11 PDE homologs identified here in the guinea pig brain. To exclude the possibility that some PDE isoforms could not be detected by RT-PCR, RNA extraction was repeated twice for each tissue and RT-PCR was repeated at least 3 times for each gene until we get the PCR products of all 22 *Pde* genes independently from 10 tissues of guinea pig (brain, heart, kidney, lung, muscle, *etc.*, data not shown) to determine the best

PCR conditions for each gene (such as primers and annealing temperature). PCR products were further confirmed by sequencing. It is possible that the transcripts of other PDE isoforms in guinea pig brain might be too low in level to be detected by RT-PCR, or perhaps some PDE isoforms might not be expressed in brain of guinea pig but in brain of other species.

Based on the cDNA sequence and amino acid sequence comparisons, we propose that guinea pig has a closer relationship with human than with mouse. This result is supported by several studies which show that guinea pig is not a rodent, and the guinea pig and myomorph rodents might diverge before the separation between myomorph rodents and a lineage leading to primates and artiodactyls^[14,15], although guinea pig is traditionally classified as a rodent^[14]. Therefore, we propose that compared to mouse, guinea pig might be a more suitable model for investigating the physiological roles of PDE isoforms in the brain. However, to develop new therapeutic drugs against neurological disorders using guinea pig as a model, further studies are needed, such as cloning the full-length *Pde* cDNAs and characterizing the modulators of these brain-expressed PDEs.

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豚鼠脑中表达的磷酸二酯酶基因 cDNA 序列的鉴定与分析

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摘要: **目的** 环核苷酸磷酸二酯酶(phosphodiesterase, PDE)是一氧化氮(nitric oxide, NO)信号转导途径中的一个关键成员, 在认知与学习、帕金森病、注意缺陷多动障碍、精神病和抑郁症等过程中发挥重要作用。PDE 在豚鼠脑中的表达情况还未见报道。本研究旨在对豚鼠脑中表达的 *Pde* cDNA 序列进行鉴定与分析。**方法** 根据人 *PDE* 和小鼠 *Pde* cDNA 序列的 22 个保守区设计引物, 采用 RT-PCR 法扩增豚鼠脑中表达的未知 *Pde* 基因 cDNA 部分序列, 并采用生物信息学软件分析不同物种间 *Pde* cDNA 序列及氨基酸多肽序列的同源性及其保守功能基序。**结果** 在豚鼠脑中发现了 11 个新的 *Pde* 基因 cDNAs, 包括 *CpPde1a*、*CpPde1b*、*CpPde2a*、*CpPde4a*、*CpPde4d*、*CpPde5a*、*CpPde6c*、*CpPde7b*、*CpPde8a*、*CpPde9a* 和 *CpPde10a*。豚鼠与人之间的 *Pde* 基因 cDNA 序列的同源率在 83.8%-94.3% 之间, 氨基酸序列的同源率在 91.9%-100% 之间。豚鼠与小鼠之间的 *Pde* 基因 cDNA 序列的同源率在 84.6%-92.1% 之间, 氨基酸序列的同源率在 91.2%-99.2% 之间。此外, 这 11 个 *Pde* cDNAs 在豚鼠与人之间的平均同源率显著高于在豚鼠与小鼠之间的同源率($P < 0.01$)。豚鼠的部分氨基酸序列具有至少一个人及小鼠 PDE 蛋白的保守功能区。**结论** 这些发现为进一步研究 PDE 在大脑中的生理功能打下了基础。

关键词: 环核苷酸磷酸二酯酶; 中枢神经系统; 一氧化氮; 豚鼠; 种间比较