Molecular cloning and functional expression in yeast of a human cAMP-specific phosphodiesterase subtype (PDE IV-C)

Peter Engels[a], Michael Sullivan[a,**], Thomas Müller[b], Hermann Lübbert[a,*]

[a]*Preclinical Research 386-246, Sandoz Pharma Ltd., 4002 Basel, Switzerland*
[b]*Sandoz Research Institute, Monbijoustr. 115, 3001 Bern, Switzerland*

Received 14 November 1994; revised version received 22 December 1994

Abstract We have recently reported increased survival of dopaminergic substantia nigra neurons by inhibition of phosphodiesterase type IV enzymes. As a first step to unravel the involvement of PDE IV subtypes in this process, we isolated phosphodiesterase type IV cDNAs from human substantia nigra. One isolated partial cDNA clone was most homologous to the partially cloned rat and human PDE IV-C isogene. Distribution analysis revealed that the enzyme is expressed in various tissues but not in cells of the immune system. Isolation of the full-length human PDE IV-C isogene cDNA and expression in a PDE-deficient yeast strain resulted in functional complementation of the yeast heat shock response. Inhibition of the enzymatic activity by rolipram characterized this enzyme as a typical type IV phosphodiesterase.

Key words: Substantia nigra; Rolipram; Yeast complementation; SH-SY5Y cell

1. Introduction

Cyclic nucleotide phosphodiesterases (PDEs) regulate the cellular concentration of cyclic nucleotides by converting cAMP or cGMP to the non-cyclic 5′-NMP [1]. The PDE enzyme family can be divided into 7 subfamilies or classes [2,3], characterized by their substrate affinity and specificity and their sensitivity to specific inhibitors [1,4,5] and activators. The subfamilies are Ca^{2+}/Calmodulin-dependent PDEs (PDE I), cGMP stimulated PDEs (PDE II), cGMP inhibited PDEs (PDE III), cAMP-specific rolipram sensitive PDEs (PDE IV), cGMP specific PDEs (PDE V), photoreceptor PDEs (PDE VI), and high affinity cAMP-specific rolipram-insensitive PDEs (PDE VII). For all the families, multiple related genes as well as different mRNA splice forms create a basis for variable control of cyclic nucleotide concentration in different cells and subcellular compartments [6,7].

One interesting family with respect to therapeutic applications is PDE IV. Inhibitors of this enzyme class may be beneficial in asthma [8] and other inflammatory diseases [9] as well as in diseases of the CNS like depression [10] or multiinfarct dementia [11]. Recently, we demonstrated that cyclic AMP stimulated the dopamine uptake and development of mesencephalic dopaminergic neurons in primary cell cultures [12]. In addition, an elevated intracellular level of cAMP, but not bFGF or IGF-I, prevented the cultured neurons from degeneration and protected them from the dopaminergic neurotoxin MPP^+ [12]. In a previous study with inhibitors for different phosphodiesterase subtypes we found, that PDE type IV specific inhibitors also increased the survival of dopaminergic neurons in primary cell culture and protected mice from the action of the neurotoxin MPTP [13,14].

In order to characterize the PDE IV subtypes involved in that process we isolated PDE IV cDNAs from a human substantia nigra cDNA library. One of the subtypes we found in this tissue had previously only been described as a short partial DNA fragment. We have now cloned and characterized the full-length cDNA. After expression in yeast the recombinant protein displayed cAMP-specific activity and was sensitive to rolipram. The spatial expression of this gene was distinct from other known human PDE IV genes [15,16,17,18].

2. Materials and methods

2.1. Oligonucleotides

PE 1: 5′-GTCCAAACACATGACCCTCCTGGCTGACCTG-3′; *PE 2:* 5′-GCAGGAGGGAGCTGATTGCTGGATGAAG-3′; *PE 3:* 5′-TCAGAGCTGGCGCTTATGTAC-3′; *PE 4:* 5′-CCGTATGCTTGTCACACAT-3′; *PE 5:* 5′-TCAAGCTGCTGCAGCAGAG-3′; *PE 6:* 5′-GAGTCCTTCCTGTACCGCTCA-3′; *PE 7:* 5′-GACTGGAGCCTGCATAATCCG-3′; *PE 8:* 5′-AGGTCAAAGCGCCTGCAGGAGG-3′; *PE 9:* 5′-GCCAGTCTGCGGACCGTT-3′; *PE 10:* 5′-CAGCAATGCCCTAGGAGCAGC-3′.

2.2. Cloning of PDE IV cDNA

To isolate PDE IV isogenes expressed in the substantia nigra, we designed the 30 bp oligonucleotide PE 1 which was complementary to the highly conserved catalytic domain of all published rat and human PDE IV isogenes. With this oligonucleotide probe we screened three million independent clones of a commercial λ-Zap human substantia nigra cDNA library (Stratagene) using standard procedures. DNA sequencing was performed with the T7 sequencing kit (Pharmacia). Using RT-PCR as described below, we found that SH-SY5Y neuroblastoma cells contain mRNA of the particular PDE isogene detected by PE1. To isolate a full-length cDNA clone we extracted mRNA from SH-SY5Y cells. cDNA was prepared from 3 μg poly(A)$^+$ mRNA using the Amersham cDNA kit. The size selected cDNA (> 1.5 kb) was cloned into a modified pBluescript vector as described by Foguet et al. [19]. 25 pools, each containing 4×10^5 independent clones with an average insert size of 2 kb, were obtained after transformation into the *E. coli* strain MC1061 by electroporation (Gene Pulser, Bio-Rad). From each pool, 0.5 μg DNA was used for a PCR analysis which specifically detected the cDNA clone isolated from the sustantia nigra library. Three positive pools were then plated and the clones isolated by standard filter hybridization [20] using oligonucleotide PE 1 as probe.

To extend the 5′ end, double-stranded SH-SY5Y cDNA was synthesized with the primer PE 4. After circularization of the cDNA with T4-DNA ligase, sequential inverse PCRs [21,22] were performed with the nested primer pairs PE 2/PE 3 and PE 2/PE 5. In an additional extension step, inverse PCR was performed using cDNA created with the primer PE 2. Nested primer pairs in this process were PE 6/PE 7, PE 8/PE 9, and PE 8/PE 10. The DNA fragments obtained were cloned

*Corresponding author. Fax: (41) (61) 324-2177.
E-mail: hermann.luebbert@pkfcns.pharma.sandoz.ch

**Present address:* Fisons Pharmaceuticals, Bakewell Road, Loughborough, Leicestershire LE11 ORH, UK.

0014-5793/95/$9.50 © 1995 Federation of European Biochemical Societies. All rights reserved.
SSDI 0014-5793(94)01460-4