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The regional pattern of μ-opioid receptor (MOR1) mRNA in human brain: a real-time PCR assay

J. Becker*, P. Schmidt, M. Fitzenreiter, F. Musshoff, B. Madea

Institute of Forensic Medicine, Friedrich-Wilhelms-University, Bonn, Germany

Abstract

In this study, a reverse transcription/real-time PCR assay was established to semi-quantify the mRNA levels of the human μ-opioid receptor (MOR1) relative to the housekeeping gene β-2-microglobulin in human autopsy brain tissue. Nine selected regions (thalamus, caudate putamen, hypothalamus, ventral tegmentum, hippocampus, amygdala, frontal cortex, nucleus accumbens, putamen) of five human brains were analysed. The observed expression pattern of μ-opioid receptor mRNA correlates with results obtained by in situ hybridisation. The method described here will be useful in further studies on steady-state mRNA levels in the brains of addicts who died due to opiate overdose, in order to elucidate whether chronic opioid abuse is followed by a regulation of μ-opioid receptor expression.

Keywords: mRNA quantification; Real-time PCR; μ-Opioid receptor; Human brain

1. Introduction

The stability of total mRNAs in human post-mortem brain tissue has been shown in several reports [1,2]. Therefore, quantitative RNA-based gene expression can be examined even if samples of varying post-mortem times have to be analysed [3,4].

Here, a reverse transcription/real-time PCR assay was established to semi-quantify the mRNA levels of the human μ-opioid receptor relative to the housekeeping gene β-2-microglobulin. This PCR-driven method allows a quantification of gene transcripts at low levels and in small tissue samples.

* Corresponding author. Tel.: +49-228-738327; fax: +49-228-738339.
E-mail address: jutta.becker@uni-bonn.de (J. Becker).

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2. Material and methods

2.1. RNA preparation/cDNA synthesis

Tissue samples from five human brains (0.5–1 g) were dissected during autopsy, immediately frozen in dry ice and stored at −80 °C. Frozen tissue samples were homogenized in 2 ml Trifast reagent (Peqlab, Erlangen, Germany) and total RNA was extracted following the manufacturer's protocol. DNA was removed by DNAse I treatment (Boehringer, Mannheim, Germany), followed by column-purification with the RNeasy Kit (Qiagen, Hilden, Germany).

200–500 ng total RNA was mixed with 100 pmol random hexamer primers, filled up with DEPC-water to a final volume of 12 μl and incubated for 10 min at 65 °C. After cooling on ice, 8 μl of the Omniscript RT-mastermix (Omniscript RT Kit, Qiagen, Hilden, Germany) was added and cDNA synthesis was performed for 60 min at 42 °C and terminated by incubating for 10 min at 75 °C.

2.2. Quantitative PCR/amplification settings

Real-time PCR was carried out with the LightCycler® Instrument (Roche Molecular Biochemicals, Mannheim, Germany) using the DNA binding dye SYBR Green I for the detection of PCR products. MOR1 and β-2-microglobulin products were generated by separate PCR reactions using the following primers: MOR1: 5′ ccctgtcaaggccttagatt 3′, 5′ tgatgagcactggcataatgaa 3′ (NCBI-Acc. NM_000914, nucl. pos. 731–960); β-2 microglobulin: 5′ tgtctttcagcaaggactgg 3′, 5′ gatgctgcttacatgtctcg 3′ (NCBI-Acc. AF072097, nucl. pos. 1021–1168). For each amplification, 0.5 μl cDNA was used in a 10-μl PCR reaction mix including 4 mM $MgCl_2$, 5 pmol each primer and 1 μl master mix (FastStart DNA Master SYBR Green I, Roche Molecular Biochemicals). The thermal profile of the LightCycler was optimised with an initial denaturation of 10 min at 95 °C (hotstart) and 40 amplification cycles with each 15 s at 95 °C, 5 s at 58 °C and 10 s at 72 °C.

2.3. Data analysis

PCR data was analysed using the LightCycler® Software (Version 3.4). Standard curves were generated by a cDNA template that is used in four different dilutions in each experiment (concentrate, 1:10, 1:100, 1:1000). The relative amounts of MOR1-mRNA were calculated for each sample by expressing the level as a percentage of β-2-microglobulin mRNA levels, which were determined from separate PCR reactions of aliquots of cDNA for different brain regions from each case.

3. Results and discussion

The isolation of intact mRNAs from post-mortem human brain enabled us to evaluate the gene expression in specific areas of the human central nervous system. Discrete nuclei of the human brain were dissected for RNA isolation and were analysed separately. PCR

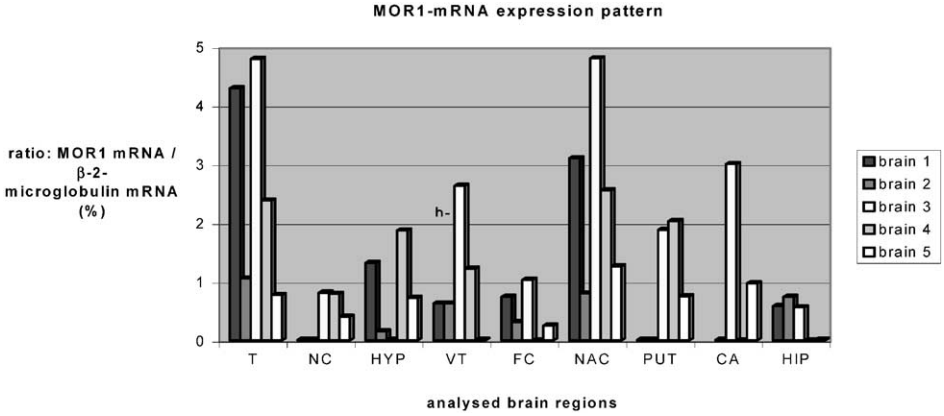

Fig. 1. Results from semi-quantitative analysis of MOR1-mRNA in five human brains. Data are given as ratios of MOR1-mRNA to β-2-microglobulin mRNA (T = thalamus, NC = nucleus caudatus, HYP = hypothalamus, VT = ventral tegmentum, FC = frontal cortex, NAC = nucleus accumbens, PUT = putamen, CA = amygdala, HIP = hippocampus).

products were generated from cDNA synthesized from total RNA with random hexamers as primer instead of oligo-dT, eliminating the requirement for intact [poly A+] tails and allowing the amplification of different genes with the same cDNA. MOR1-mRNA levels obtained by analysis of five different brains are shown in Fig. 1. The observed expression pattern correlates with results obtained by in situ hybridisation study [5]. More brain samples will be needed to fix the distribution of μ-opioid receptors and to find possible alterations caused by chronic opioid abuse.

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