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Research Report

Similar in vitro pharmacology of human cannabinoid CB_1 receptor variants expressed in CHO cells

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ABSTRACT

Through alternative splicing, the human cannabinoid CB_1 receptor gene encodes three variants of protein products (hCB_1, hCB_{1a}, and hCB_{1b}) that differ in amino acid sequence at the N terminus of the receptors. By semi-quantitative PCR from human adult and fetal brain mRNA, we demonstrated that the transcript encoding hCB_1 is the major transcript, and estimated that those of hCB_{1a} and hCB_{1b} represent fewer than 5% of the total human cannabinoid CB_1 receptor transcripts. We characterized the three variants stably expressed in CHO cells. In the contrary to the study by Ryberg et al. (FEBS Lett 579[1], 259-64), we did not find substantial difference among the three variants according to the binding affinity, functional potency, and efficacy of meth-anandamide, 2-arachidonoyl glycerol, virodhamine, Noladin ether, docosatetraenylethanolamide, CP55940, AM251, and compound 35e (an acyclic class human CB_1 receptor inverse agonist similar to MK-0364). The functional significance of different human cannabinoid CB_1 receptor variants remains to be clarified.

1. Introduction

The cannabinoid CB_1 receptor gene is widely and highly expressed in the central nervous system (Matsuda et al., 1990; Herkenham et al., 1990; Tsou et al., 1998). Low level expression of the receptor has also been identified in a variety of peripheral organs (Pertwee, 2001; Croci et al., 1998; Di Marzo et al., 2001, Szabo et al., 2001; Wagner et al., 2001; Wang et al., 2004). Based on the results from studies of the cannabinoid CB_1 receptor gene knock-out mice, CB_1 receptor plays a role in regulating body temperature, nociceptive sensation, and feeding behavior (Zimmer et al., 1999; Ledent et al., 1999; Di Marzo et al., 2001).

The cannabinoid CB_1 receptor is a member of the seven-membrane-spanning receptor family and interacts with the heterotrimeric G proteins to regulate ion channel activities, cAMP production, and to activate the mitogen-activated protein kinase cascade (Matsuda et al., 1990; Gerard et al., 1991; Mackie et al., 1995; Bouaboula et al., 1995). Multiple endocannabinoid and small molecule ligands can interact with cannabinoid CB_1 receptor as agonist or inverse agonist. Anandamide and 2-arachidonoyl glycerol are endogenous cannabinoid ligands, which are fatty acid derivatives (Devane et al., 1992; Mechoulam et al., 1995). Noladin ether, virodhamine, and docosatetraenylethanolamide, which are also fatty acid derivatives, have been reported as ligands for cannabinoid CB_1 receptor (Hanus et al., 1993, 2001; Porter et al., 2002. In additional to the classical cannabinoid (delta-9-THC and HU-210), the non-classical cannabinoid (CP55940) and the

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aminoalkylindole (WIN55212-2) also are cannabinoid CB_1 receptor agonists (Huffman et al., 1996; Melvin et al., 1995; D'Ambra et al., 1992; Luk et al., 2004). SR141716A, and the closely related analog AM251, are cannabinoid CB_1 receptor inverse agonists (Rinaldi-Carmona et al., 1994; Bouaboula et al., 1997). MK-0364 and compound 35e were newly identified cannabinoid CB_1 receptor inverse agonists, which belong to the acyclic class of inverse agonist (Lin et al., 2006).

Through alternative splicing the human cannabinoid CB_1 receptor gene encodes three variants of protein products (hCB_1, hCB_{1a}, and hCB_{1b}), differing in amino acid sequence at the N terminus of the receptors (Shire et al., 1995; Rinaldi-Carmona et al., 1996; Ryberg et al., 2005). It has been reported that Noladin ether and anandamide exhibited little affinity or potency for hCB_{1a} and hCB_{1b} variants and that 2-arachidonoyl glycerol functioned as an inverse agonist at hCB_{1a} and hCB_{1b} variants, when the variants were transiently

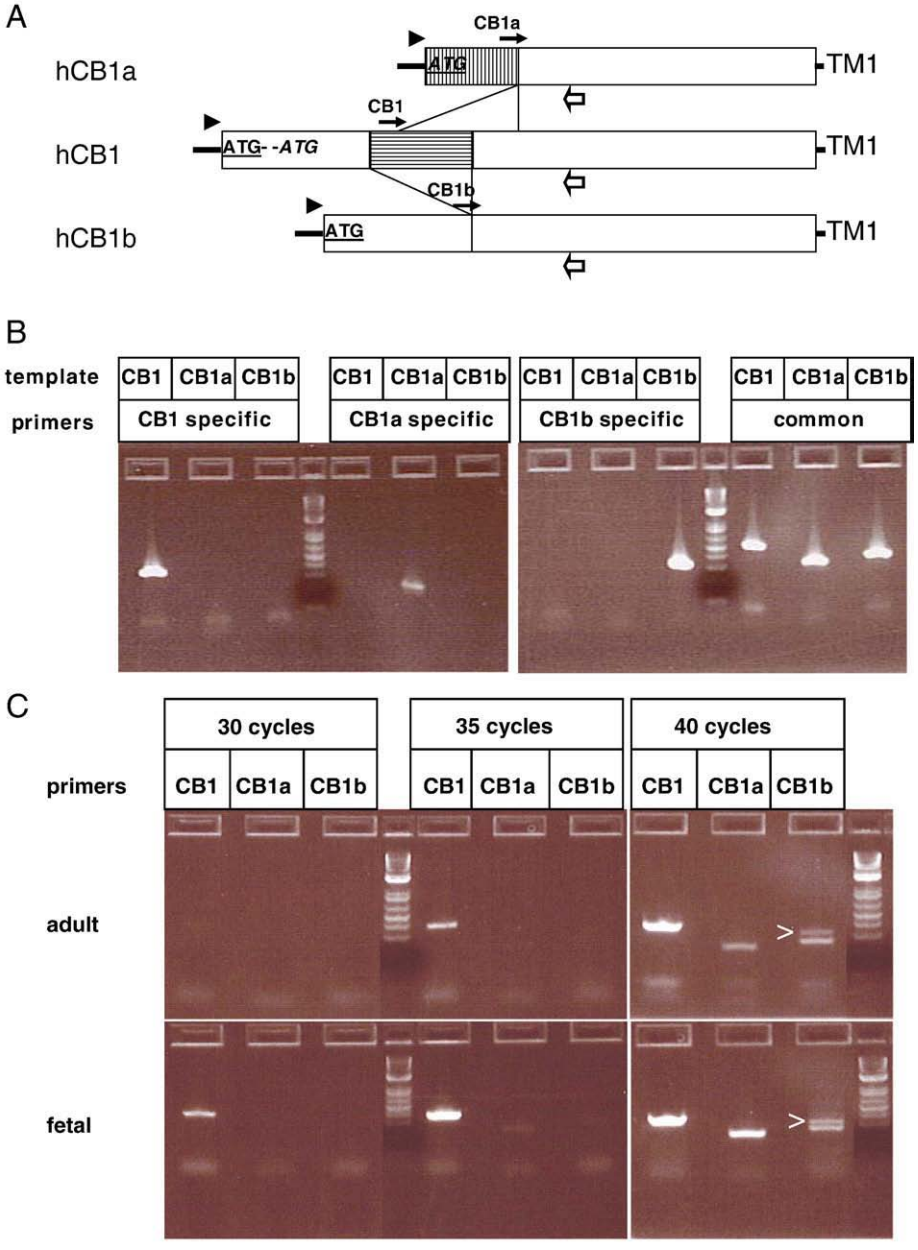

Fig. 1 – Detecting the human cannabinoid CB_1 receptor variants, CB_1, CB_{1a} and CB_{1b}, with variant-specific primer pairs by PCR from adult and fetal human brain cDNA libraries. (A) Schematic representation of the primers used to generate CB_1, CB_{1a} and CB_{1b}, related PCR products. "▶": common forward primer; "⇦": common reverse primer; "⟶": variant-specific forward primers, which cross the RNA splicing site. "ATG": translation initiation for CB_1, and CB_{1b}, "ATG": translation initiation for CB_{1a}; (B) Variant-specific primer pairs only generating PCR products from the specific variant cDNA template (C) Relative abundance of hCB_{1a}, hCB_{1b} and hCB_1 variant transcripts in human adult or fetal brain cDNA libraries. ">": marked the slower migrating band to be CB1b product, confirmed by cloning and sequencing.