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Cloning, genomic organization and functionality of 5-HT$_7$ receptor splice variants from mouse brain

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ABSTRACT

The serotonin (5-HT) 5-HT$_7$ receptors are expressed in both the central nervous system and in peripheral tissues. Receptor distribution studies and pharmacological studies have established that 5-HT$_7$ receptors play an important role in the control of circadian rhythms and thermoregulation. Selective 5-HT$_7$ receptor ligands have potential therapeutic applications for the treatment of pain and migraine, schizophrenia, anxiety, cognitive disturbances and inflammation.

We have cloned two novel C-terminal splice variants of the 5-HT$_7$ receptor from mouse brain. These two new splice variants have almost identical sequences as the rat 5-HT$_{7(b)}$ and 5-HT$_{7(c)}$ splice variants and so were given the same name. Ligand binding assays ([^{3}H]5-CT), membrane localization and functional studies in transiently transfected cells indicated that all three splice variants are well expressed on the membrane and no major differences in their respective pharmacology and their ability to activate adenylyl cyclase were observed. This is in analogy with previous reports comparing either the rat or the human variants.

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1. Introduction

Serotonin (5-HT, 5-hydroxytryptamine) is one of the most common neurotransmitters in the brain. Its biological effects are mediated by at least 14 different serotonin receptors, of which 13 belong to the superfamily of membrane-spanning G-protein-coupled receptors (GPCRs).

5-HT$_7$ receptors are expressed in both the central nervous system and in peripheral tissues. In the central nervous system, they are found in the thalamus, the hypothalamus (including the suprachiasmatic nucleus), the hippocampus (all CA regions), cerebral cortex (both on pyramidal and GABAergic neurons), amygdala and dorsal raphe nucleus (Bonaventure et al., 2004; Thomas and Hagan, 2004). Most 5-HT$_7$ receptors are localized on both the soma and axon terminals of GABAergic neurons. The use of specific agonists and antagonists and a knock-out mouse helped to better define the roles of the 5-HT$_7$ receptors, e.g. the control of circadian rhythms and thermoregulation (Lovenberg et al., 1993; Tsou et al., 1994; Guscott et al., 2003; Hedlund et al., 2003). Involvement of 5-HT$_7$ receptors in learning and memory is suggested by the observed decrease in synaptic plasticity in CA1 and contextual fear conditioning in 5-HT$_7$ receptor −/− mice (Thomas and Hagan, 2004). Importantly, 5-HT$_7$ receptor agonists can be useful in the treatment of dysfunctional memory in age-related decline and Alzheimer's disease (Perez-Garcia and Meneses, 2005). Functional studies have also implicated 5-HT$_7$ receptors in depression and the activation of rapid-eye-movement (REM) sleep via the modulation of suprachiasmatic nucleus neurons (Thomas and Hagan, 2004). Finally, other observations have led to suggestions that selective 5-HT$_7$ receptor ligands may have potential therapeutic applications for treatment of pain, migraine, schizophrenia, anxiety, cognitive disturbances and inflammation (Thomas and Hagan, 2004).

In the periphery, 5-HT$_7$ receptors are found primarily in smooth muscle cells of blood vessels, but also in the gastrointestinal tract, where they have been proposed to be involved in peristalsis, and in rat lumbar dorsal root and sympathetic ganglia. Lower levels have been detected in kidney, liver, pancreas and spleen, based on mRNA expression (Bard et al., 1993). The importance of the 5-HT$_7$ receptor in smooth muscle relaxation might indicate involvement in diseases such as irritable bowel syndrome or angina (Coupar et al., 2007).

In 1993, the 5-HT$_7$ receptor gene was discovered independently in several different laboratories and has to date been cloned from mouse

Abbreviations: 5-HT, 5-hydroxytryptamine; 5-CT, 5-carboxamidotryptamine; ANOVA, analysis of variance; BSA, bovine serum albumin; cDNA, DNA complementary to RNA; CHO, Chinese hamster ovary; DTT, dithiotreitol; GPCR, G-protein-coupled receptor; HEK293, human embryonic kidney; HRP, horse radish peroxidase; PBS, phosphate buffered saline; PCR, polymerase chain reaction; PEI, polyethyleneimine; RT-PCR, reverse transcription PCR; SDS, sodium dodecyl sulphate; SDS-PAGE, SDS polyacrylamide gel electrophoresis; TBS, Tris-buffered saline; TBS-T, TBS Tween-20.

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(Plassat et al., 1993), rat (Lovenberg et al., 1993; Meyerhof et al., 1993; Ruat et al., 1993; Shen et al., 1993), man (Bard et al., 1993), guinea pig (Tsou et al., 1994), *Xenopus laevis* (Nelson et al., 1995), pig (Bhalla et al., 2002), *Caenorhabditis elegans* (Hobson et al., 2003) and honeybee (Schlenstedt et al., 2006). The human 5-HT_7 receptor was initially reported to be a protein of 445 amino acids, with 57% homology with the *Drosophila melanogaster* 5-HT_{dro1} receptor in the transmembrane regions, but only 39–53% homology with cloned human 5-HT_1, 5-HT_2, 5-HT_5, and 5-HT_6 receptors. The human 5-HT_7 receptor gene is located on chromosome 10 (q21–q24) (Gelernter et al., 1995) and contains several introns in the coding region (Ruat et al., 1993; Erdmann et al., 1996; Heidmann et al., 1997). The presence of introns in the 5-HT_7 receptor gene is significant in that a number of functional splice variants of this receptor have been identified (Fig. 1). No alternative splicing has been reported for the first intron, which is in the putative IC2 loop of the receptor. Alternative splicing at the second intron, located in the carboxyl terminus of the 5-HT_7 receptor, gives rise to a number of splice variants, namely 5-$HT_{7(a),\ (b),\ (c),\ (e)}$ in rat and 5-$HT_{7(a),\ (b),\ (d)}$ in man (Heidmann et al., 1998; Liu et al., 2001). The 5-$HT_{7(a)}$ and 5-$HT_{7(b)}$ splice variants are homologous in rat and man and are caused by alternative usage of two splice donor sites arranged in-tandem at the end of exon II. The use of the second splice donor site in splice variant 5-$HT_{7(b)}$ results in a 5-bp insert (GTAAG) within the coding sequence, which introduces a premature termination of the open reading frame. This shortens the C-tail of the 5-$HT_{7(b)}$ splice variants by 13 amino acids. The third splice variant in rat, 5-$HT_{7(c)}$, results from the retention of an additional 97-bp cassette, named exon C, inserted between the apparent splice junction of exons II and III. The third human splice variant, 5-$HT_{7(d)}$, also contains an extra 98-bp exon cassette at the exon II–exon III boundary. This human exon cassette, referred to as exon D, shows no sequence homology to rat exon C, despite their similar size. Although a human exon-C-like sequence ψC was found, that shows strong similarity to rat exon C, no 5-$HT_{7(c)}$ mRNA has been detected so far in man. By contrast, sequence alignment did not provide any indication that an exon D homologue or part of that sequence exists in rat. The fourth rat splice variant,

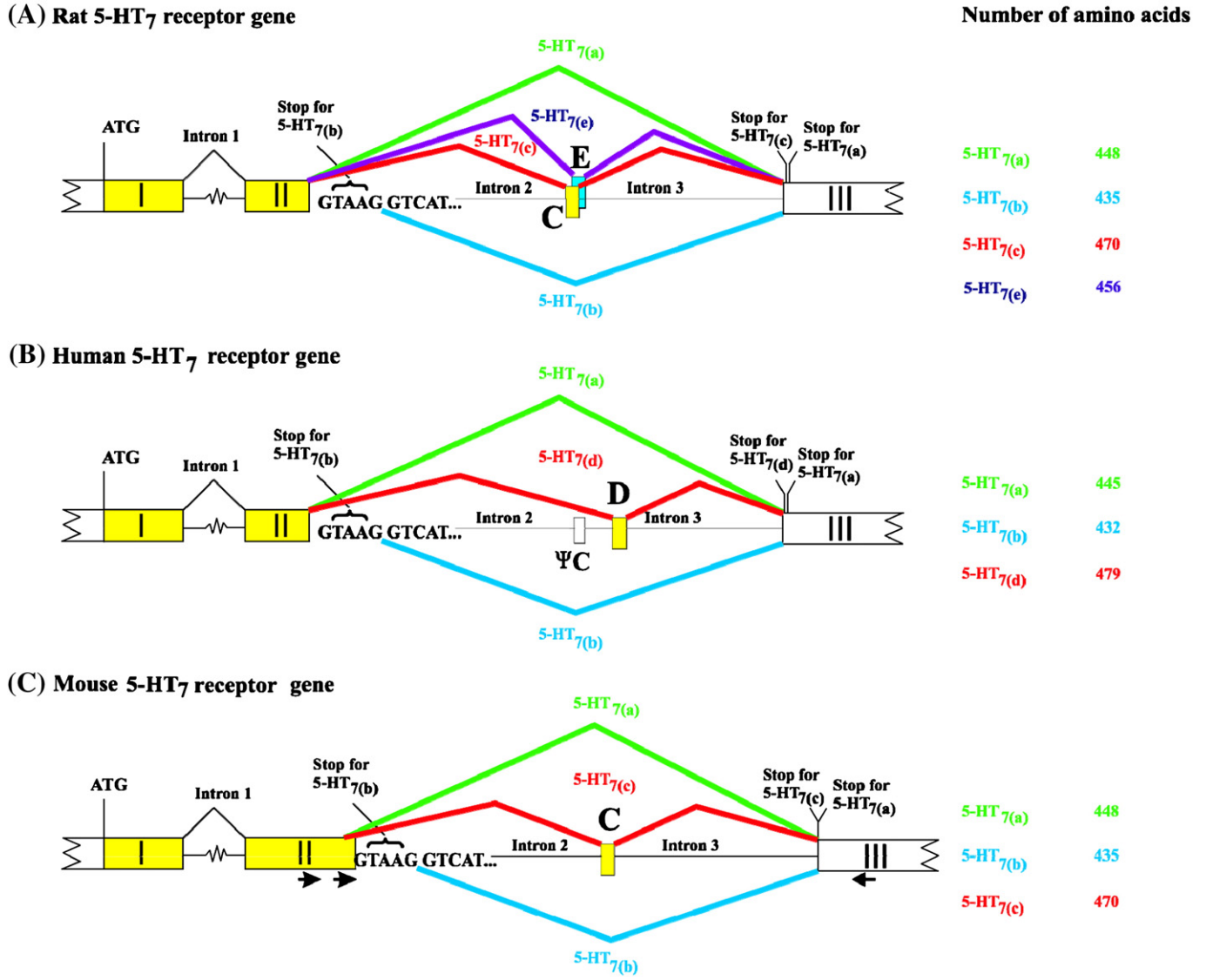

Fig. 1. Schematic overview of the splicing process leading to different rat (A) human (B) and mouse (C) 5-HT_7 receptor mRNA. Exons I, II, III, C, ψC, D and E are indicated by boxes. Those that code for 5-HT_7 splice variants are shown in grey. (C) Primers used to clone the different mouse 5-HT_7 splice variants are indicated by arrows: OFP = outer forward primer, IFP = inner forward primer and RP = reverse primer. Exon I consists of 549 bp, exon II of 755 bp, exon C of 97 bp and exon III of 43 bp. The introns contain 86902 bp (intron 1), 4832 bp (intron 2) and 3907 bp (intron 3). Adapted from Heidmann et al. (1997).

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