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Editing of the serotonin 2C receptor pre-mRNA: Effects of the Morris Water Maze

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Abstract

The pre-mRNA encoding the serotonin 2C receptor, HTR2C (official mouse gene symbol, Htr2c), is subject to adenosine deamination that produces inosine at five sites within the coding region. Combinations of this site-specific A-to-I editing can produce 32 different mRNA sequences encoding 24 different protein isoforms with differing biochemical and pharmacological properties. Studies in humans have reported abnormalities in patterns of HTR2C editing in psychiatric disorders, and studies in rodents show altered patterns of editing in response to drug treatments and stressful situations. To further explore the biological significance of editing of the Htr2c mRNA and its regulation, we have examined patterns of Htr2c editing in C57BL/6J mice after exposure to the hidden platform version of the Morris Water Maze, a test of spatial learning that, in mice, is also associated with stress. In brains of both swimming controls and mice trained to find the platform, subtle time dependent changes in editing patterns are seen as soon as 1 h after a probe trial and typically last less than 24 h. Changes in whole brain with cerebellum removed differ from those seen in isolated hippocampus and cortex. Unexpectedly, in hippocampi from subsets of mice, abnormally low levels of editing were seen that were not correlated with behavior or with editing levels in cortex. These data implicate responses to spatial learning and stress, in addition to stochastic processes, in the generation of subtle changes in editing patterns of Htr2c.

Keywords: 5HT2C receptor; Inosine; Protein isoforms; Primer extension; Spatial learning; Stress

1. Introduction

The serotonin 2C receptor, Htr2c, is unique among the serotonin receptors in that the pre-mRNA is subject to deamination of adenosine to inosine (Burns et al., 1997). This editing occurs at five sites within a 15 nucleotide segment encoding the second intracellular loop (reviewed in Schmauss, 2003; Sanders-Bush et al., 2003). Because inosine is interpreted by the ribosome as guanosine, editing alters individual codons, most often also altering the encoded amino acid. Fig. 1a illustrates the repertoire of codon combinations that can result in a total of 24 different protein sequences. Within the protein sequence, the edited region starts two amino acids downstream from the conserved DRY motif that is believed to be involved in G-protein coupling (Ballesteros et al., 1998; Moro et al., 1993). Both mutation experiments and computational approaches predict that the sequence diversity created by editing will modulate protein function (Ballesteros et al., 1998; Moro et al., 1993; Visiers et al., 2001), as originally suggested by Burns et al. (1997). Indeed, in vitro studies have shown differences among several isoforms in biochemical and pharmacological properties that include affinity

Abbreviations: Htr2c, serotonin receptor 2C; I, inosine; A, adenosine; G, guanosine; MWM, Morris Water Maze; SC, swimming control.

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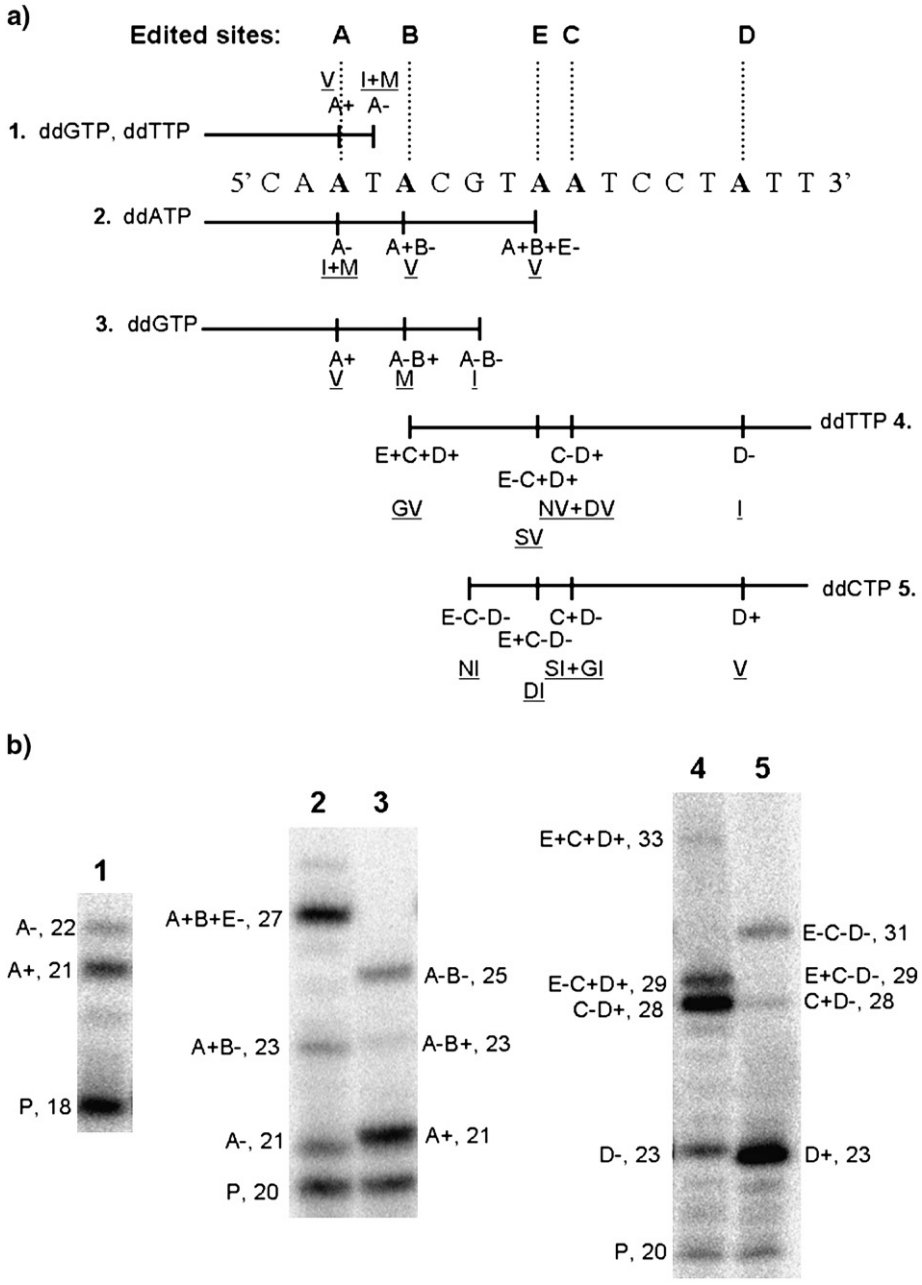

Fig. 1. Primer extension analysis. a) Schematic of primer extension assays. The genomic sequence spanning the edited region is shown in the center, 5′ to 3′. The five A residues that can be edited are named A–E in the order indicated. Primer extension reactions 1–5 are shown with the corresponding dideoxynucleotide chain terminator. Short vertical lines indicate positions at which chain termination may occur. Products obtained in each reaction are named for the edited site combination and the corresponding encoded amino acids (underlined). + indicates the site is edited to inosine; − indicates it remains the genomic adenosine. b) Representative gels for reactions 1–5. Bands are labeled by edited status (site + or − editing) and length in nucleotides. P, primer.

for serotonin, G-protein coupling, and responses to atypical antipsychotics (Niswender et al., 1999, 2001; Herrick-Davis et al., 1999; Fitzgerald et al., 1999; Wang et al., 2000; Price and Sanders-Bush, 2000; Berg et al., 2001; Price et al., 2001; McGrew et al., 2004; Marion et al., 2004).

In vivo studies have shown that most of the 32 possible mRNA variants are produced in human and rodent brain (Burns et al., 1997; Niswender et al., 1999). In a recent work, we have detected 28 of 32 mRNA variants in mouse brain, encoding 20 of 24 possible protein isoforms (Du et al., 2006). However, there are both species-specific and brain region-specific differences in the proportions of the different variants, and only five or six variants typically are found at high frequency, with the remainder together accounting for only a few percent. Abnormalities in patterns of HTR2C editing have been reported in subsets of patients with schizophrenia and depression (Gurevich et al., 2002a; Niswender et al., 2001; Dracheva et al., 2003), and baseline differences have also been reported among different inbred mouse strains (Englander et al., 2005; Hackler et al., 2006; Du et al., 2006). Changes in editing