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BRAIN RESEARCH

Research Report

Expression of 5-HT$_7$ receptor mRNA in the hamster brain: Effect of aging and association with calbindin-D28K expression

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ARTICLE INFO

Article history:
Accepted 17 January 2007
Available online 20 January 2007

Keywords:
Serotonin receptor
Suprachiasmatic nucleus
Hippocampus
Raphe nuclei
Dorsal raphe
Cingulate cortex

ABSTRACT

Aging affects several processes modulated by the 5-HT$_7$ receptor subtype, including circadian rhythms, learning and memory, and depression. Previously, we showed that aging induces a decrease in the hamster dorsal raphe (DRN) in both 5-HT$_7$ receptor binding and circadian phase resetting responses to 8-OH-DPAT microinjection. To elucidate the mechanisms underlying the aging decrease in 5-HT$_7$ receptors, we investigated aging modulation of 5-HT$_7$ receptor mRNA expression in the DRN, brain regions afferent to the DRN, and brain regions regulating circadian rhythms or memory. In situ hybridization for 5-HT$_7$ receptor mRNA was performed on coronal sections prepared from the brains of young, middle-aged, and old male Syrian hamsters. 5-HT$_7$ receptor mRNA expression was quantified by densitometry of X-ray film autoradiograms. The results showed that aging did not significantly affect 5-HT$_7$ receptor mRNA expression in the DRN or most other brain regions examined, with the exception of the cingulate cortex and paraventricular thalamic nucleus. Within the suprachiasmatic nucleus, the site of the master circadian pacemaker in mammals, 5-HT$_7$ receptor mRNA expression was localized in a discrete subregion resembling the calbindin subnucleus previously described. A second experiment using adjacent tissue sections showed that 5-HT$_7$ receptor mRNA and calbindin mRNAs were concentrated in the same region of the SCN, and as well as in the same region of several other brain structures. The localization of 5-HT$_7$ receptors and calbindin mRNAs within the same regions suggests that the proteins they encode may interact to modulate processes such as circadian timekeeping.

1. Introduction

The 5-HT$_7$ receptor subtype, the most recently cloned of the serotonin receptor subtypes, modulates many physiological and behavioral functions, including circadian rhythms (Ehlen et al., 2001; Duncan and Davis, 2005; Duncan et al., 2004), rapid eye movement (REM) sleep (Hagan et al., 2000; Thomas et al., 2003; Monti and Jantos, 2006), body temperature (Hedlund et al., 2003), learning and memory (Roberts et al., 2004), and behavior (Guscott et al., 2005; Hedlund et al., 2005). For

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Abbreviations: Cg, cingulate cortex; LS/LSV, lateral septum (ventral); TS, triangular septal nucleus; BNST, bed nucleus of the stria terminalis; CPu, caudate putamen; SCN, suprachiasmatic nucleus; AH, anterior hypothalamus; Ce, central amygdaloid nucleus; MHb, median habenula; PVA, anterior paraventricular thalamic nucleus; ZI, zona incerta; PMV, ventral premammillary nucleus; IGL, intergeniculate leaflet; DG, dentate gyrus; CA1, CA1 region of the hippocampus; CA2, CA2 region of the hippocampus; CA3, CA3 region of the hippocampus; MRN, median raphe nucleus; DRN, dorsal raphe nucleus; LPAG, lateral periaqueductal gray

0006-8993/$ – see front matter
doi:10.1016/j.brainres.2007.01.044

example, administration of the 5-HT$_7$ receptor-selective antagonists, SB-269970-A or DR-4004, to hamsters blocks phase advances of circadian locomotor activity rhythms induced by the serotonergic agonists, 8-OH-DPAT or 5-carboxamidotryptamine, demonstrating that pharmacological activation of 5-HT$_7$ receptors stimulates circadian phase resetting (Ehlen et al., 2001; Duncan et al., 2004). Furthermore, administration of 5-HT$_7$ receptor-selective antagonists to guinea pigs or rats selectively decreases REM sleep, suggesting that endogenous serotonin acting at 5-HT$_7$ receptors tonically potentiates REM sleep (Hagan et al., 2000; Thomas et al., 2003; Monti and Jantos, 2006). Furthermore, studies in mice have shown that deletion of the 5-HT$_7$ receptor gene impairs memory and learning (Roberts et al., 2004), reduces immobility in the Porsolt swim test, similar to antidepressants (Guscott et al., 2005; Hedlund et al., 2005), and inhibits hypothermia induced by 8-OH-DPAT (Hedlund et al., 2003).

Aging deleteriously affects some of the functions modulated by 5-HT$_7$ receptors, most notably circadian rhythms and memory. Furthermore, some age-related changes in these processes have been associated with decreases in 5-HT$_7$ receptor expression. For example, significant attenuation of serotonergic induction of circadian phase shifts was observed in hamsters by 17–19 months of age (Penev et al., 1995; Duncan et al., 2004), the same age at which a significant reduction of specific 5-HT$_7$ receptor binding was exhibited in the dorsal raphe nucleus (DRN) (Duncan et al., 1999). Furthermore, age-related memory deficits are exhibited by rodents and humans, and decreased expression of 5-HT$_7$ receptor mRNA in the ventral CA3 of the hippocampus has been observed in old rats (Kohen et al., 2000) [but see also (Yau et al., 1999)].

In order to further elucidate the mechanisms responsible for the age-related reduction of 5-HT$_7$ receptor binding in the aging hamster brain, we investigated 5-HT$_7$ receptor mRNA expression in young, middle-aged, and old hamsters. We focused on the DRN, based on our previous identification of age-associated loss of 5-HT$_7$ receptor binding and functional responses in this region (Duncan et al., 1999, 2004) and on brain regions known to send afferent projections to the DRN. We also investigated several other brain regions involved in the regulation of circadian rhythms, memory, or mood. In the course of investigating the expression of 5-HT$_7$ mRNA in the hamster suprachiasmatic nucleus, we observed that its expression was concentrated in a small subregion previously reported to express calbindin mRNA. Therefore, in this project, we also investigated the effect of aging on calbindin mRNA expression in the SCN and other brain regions.

2. Results

2.1. *Experiment 1. The effect of aging on 5-HT$_7$ receptor mRNA expression in the dorsal raphe and other discrete regions of the midbrain and the forebrain*

Expression of 5-HT$_7$ receptor mRNA was observed in many brain regions, similar to previous findings in rats (Neumaier et al., 2001). The DRN exhibited a relatively low level of 5-HT$_7$ receptor mRNA expression that was not significantly affected by aging (Fig. 1 and Table 1). Furthermore, no effect of aging on 5-HT$_7$ receptor mRNA expression was observed in most of the midbrain or forebrain regions examined, with the exception of the cingulate cortex and paraventricular thalamic nucleus (Fig. 1 and Table 1). Distinct but faint labeling was detected in a small circular region of the ventral mid-caudal SCN of young hamsters, but appeared to be absent from this region in most of the middle-aged and old animals.

2.2. *Experiment 2. The effect of aging on 5-HT$_7$ receptor mRNA expression in the calbindin-expressing region of the SCN and on calbindin mRNA expression*

Previous studies have indicated that the ventral mid-caudal region of the hamster SCN is unique in its expression of the calcium-binding protein calbindin, and furthermore, this region is essential for generation of circadian rhythms (LeSauter et al., 2002; Hamada et al., 2003; Kriegsfeld et al., 2004; Antle and Silver, 2005). This experiment investigated if the expression of 5-HT$_7$ receptor mRNA occurs in the same region of the SCN that expresses calbindin mRNA, and if aging affects the expression of 5-HT$_7$ receptor mRNA or calbindin mRNA within this region. The results showed that the ventral mid-caudal region of the hamster SCN expressed calbindin mRNA, as expected. Furthermore, 5-HT$_7$ receptor mRNA was

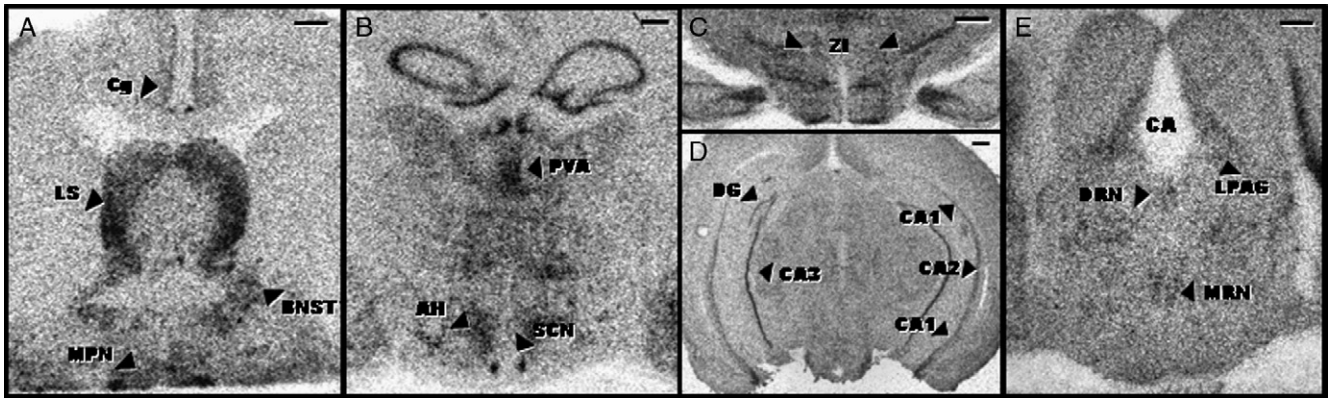

Fig. 1 – Autoradiograms representing expression of 5-HT$_7$ receptor mRNA in the forebrain and midbrain of young adult hamsters. Scale bar depicts 500 μm.