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Ontogenetic Distribution of 5-HT_{2C}, 5-HT_{5A}, and 5-HT_7 Receptors in the Rat Hippocampus

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It is known that serotonin exerts its different nociceptive and motor functions by interacting with distinct receptors subtypes, which could be either G-protein coupled or ionotropic. Previous reports demonstrated the early activation of serotonin receptor transcripts during rat development, suggesting a potential role of the serotoninergic system during ontogeny. In this study we have compared the cellular distribution of three serotonin receptor subtypes: 5-HT_{2C}, 5-HT_{5A}, and 5-HT_7. Immunocytochemical methods were used in slices of rat hippocampus obtained during the postnatal development. 5-HT_{2C} immunoreactivity was strong at all developmental stages in the CA1 region, whereas differences were observed between P0 and P5 in the CA3 region. The 5-HT_{5A} receptor immunosignal in CA1 and CA3 was strong at P0, decreased at P11, and then increased in the adult. The immunoreactivity to 5-HT_7 receptors was high in all regions at P0 and then decreased progressively during postnatal development; the signal was stronger for 5-HT_{2C} than for 5-HT_{5A} and 5-HT_7 receptors. Changes in the expression level of each receptor may result in differences in functional and pharmacological properties of the cells expressing them as well as in the hippocampal neuronal network. The distribution of the three serotonin receptor subtypes studied varied during the ontogeny, which supports their potential role during development and will help to understand their mechanisms.

Key words: Serotonin receptors; 5-HT_{5A} receptors; 5-HT_7 receptors; Hippocampus development

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter that has been implicated in many diverse functions, such as control of locomotion, pain perception, sexual activity, and vascular contraction in the central nervous system, and dysfunction of the serotonergic system has been associated with the pathogenesis of emotional disorders (18,19,28). To date, at least 15 different 5-HT receptor subtypes have been identified and classified into seven families (5-$HT_{1–7}$) according to their DNA sequence, affinity for serotonin, and mechanisms of action (1,6). Six of the serotonin receptor families are seven transmembrane domain receptors that couple to G proteins, whereas the 5-HT_3 receptor is a ligand-gated ion channel structurally related to the ionotropic nicotinic ACh and $GABA_A$ receptors. The metabotropic 5-HT_{2C}, 5-HT_{5A}, and 5-HT_7 receptor genes are differentially transcribed over the adult rodent brain (8,10,13).

The early appearance of 5-HT receptor mRNA (3) and of monoaminergic neurons during the ontogenetic development of the brain is related to the periods of neurogenesis and synapse formation. This suggests that monoamines and their receptors play a role in controlling the development of their target areas (7). Furthermore, serotonergic activity is necessary for the generation of new neurons in the adult hippocampus (2,12,23). The distribution of different serotonin receptors in the rat hippocampus has been previously examined using several methods such as immunohistochemistry for 5-$HT_{2,3,5,7}$ receptors (5,11, 15–17,19), in situ hybridization for 5-$HT_{1,3–7}$ (10,22, 24,25), and isotopic labeling for 5-$HT_{1,2,4,7}$ (14). Nev-

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ertheless, studies on the expression of the different types of serotonin receptors during development are still incomplete. Here we describe the distribution of three types of serotonin receptors (5-HT_{2C}, 5-HT_{5A}, and 5-HT_7) during the postnatal development of the rat hippocampus.

MATERIALS AND METHODS

Sprague-Dawley rats of postnatal ages P0, P5, P11, and adult were anesthetized with sodium pentobarbital (40 mg/kg, IP) and then decapitated. The brains were rapidly removed and fixed for 2 h in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7) and then cryoprotected overnight in 30% sucrose in 0.1 M phosphate buffer. Specimens were then embedded in tissue tek and 12-μm sections were obtained over super frost slides using a cryostat (Leica CM 1850). The hippocampi were examined according to the Paxinos atlas (21). For immunostaining, the following polyclonal antisera were used: anti-5-HT_{5A} (17-34, Sigma), anti-5-HT_7 (8-23, Sigma), and anti-5-HT_{2C} (C-TERM H85, Santa Cruz Technologies). Endogenous peroxidase was blocked by 1-h pretreatment with 1% H_2O_2 followed by 1-h 5% incubation nonfat milk to block unspecific sites. Tissue sections were incubated overnight with anti-5-HT_{5A}, anti-5-HT_7, or anti-5-HT_{2C} serum (1:100) and then with biotin-conjugated goat anti-rabbit antibody (1:100) for 2 h. Complex for enzymatic staining was obtained with ABC-horseradish peroxidase conjugates and color development by reaction with diaminobenzidine and H_2O_2. The sections were examined with an Axiostar Zeiss microscope and scanned through an MRC Axiocam for digitization. Quantification of the intensity values was computed on digitized images of 497×407 pixels on gray scale with the KS300 software (Carl Zeiss). Controls without the primary antibodies were used to normalize density values at each age. The ANOVA test and Tukey posttest were used to determine statistical differences in optical densities.

RESULTS AND DISCUSSION

The distribution of serotonin receptors in the postnatally developing rat hippocampus and surrounding areas was examined at days P0, P5, P11, and adult.

The 5-HT_{2C} receptors were widely distributed throughout the rat brain at the four developmental ages examined and at all stages they were more intensely expressed in the hippocampus. The CA1 pyramidal region showed an intense immunoreactivity at P0, which decreased progressively until P11 and then increased slightly to the adult level (Fig. 1). Our observations on the distribution of 5-HT_{2C} receptors in the adult brain confirm and extend those reported previously (14,27).

In the CA2 region the staining was intense in the newborn, became even stronger at P5, and then decreased in the adult. The immunoreactivity in CA3 was intense in the newborn, decreaed substantially at P5, and then declined slightly to the adult level. Interestingly, we observed intense 5-HT_{2C}-immunoreactive cells at P5 in the striatum, as well as in the habenular nucleus at P11 and adult. At P0 we did not find significant differences between the CA1 and CA3 regions whereas at the other ages there were significant differences between the two areas. In the dentate gyrus the strongest signal was found at P0 (Fig. 1).

The 5-HT_{5A} immunoreactivity of both the CA1 and CA3 regions decreased progressively to a minimum at P11 and then increased to the adult level (Fig. 2). In the CA2 and dentate gyrus the immunostaining also decreased from P0 to P11 and then increased in the adult. The cells in the striatum were strongly marked in the newborn while in the habenular nucleus the staining was stronger in the adult. Our observations on the expression of 5-HT_{5A} receptors suggest the same distribution as that found for the human hippocampus (20), although previous Northern blot analyses report contradictory results during rat development (4).

In CA1, CA3, and dentate gyrus, labeling of 5-HT_7 receptors decreased progressively until P11, and then remained at that level (Fig. 3). In CA2 the strongest signal was found in the newborn and then the signal decreased, as for CA1 and CA3 (data not shown). In the present study, 5-HT_7-containing cells were detectable earlier than previously reported (26).

In summary, the expression of the three types of serotonin receptors studied decreased gradually in the hippocampus to a minimum at P11, and while the expression of 5-HT_{2C} and 5-HT_7 receptors remained essentially at that level to the adult, the expression of 5-HT_{5A} receptors was greatly increased from P11 to adult. It is interesting to compare these results with those of Carpenter et al. (3), who found that the genes coding for serotonin receptors in the brain were already transcribed at embryonic day 15 (E15) and the corresponding mRNA, injected into frog oocytes, expressed receptors that activated the phosphoinositide system. The currents generated by oocytes expressing adult brain mRNA elicited serotonin currents that were more than three times greater than those generated by P0 mRNA; those results contrast with our findings, which show a decrease in 5-HT_{2C} receptor immunoreactivity. This suggests the presence of other types of phosphoinositide coupling serotonin receptors.

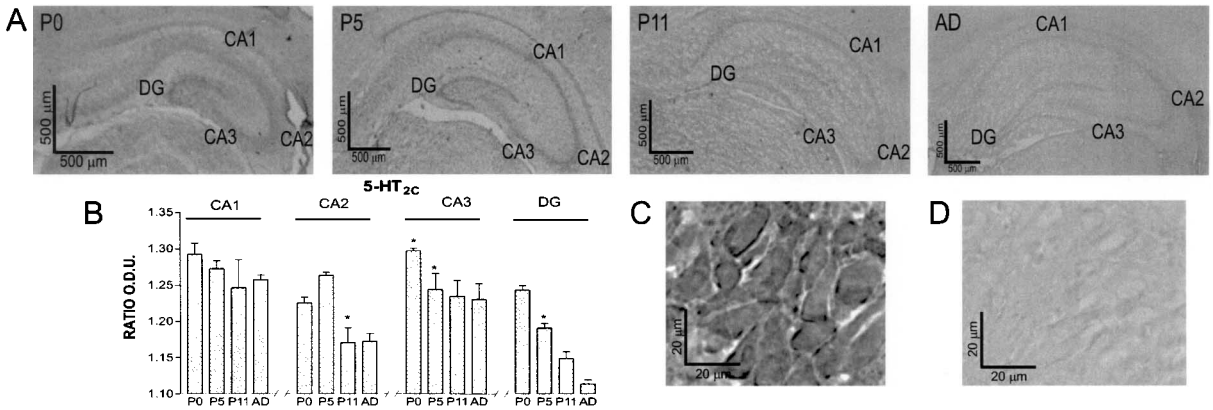

Figure 1. Distribution of 5-HT_{2C} receptors in the rat hippocampus. The ages studied were: newborn (P0), 5 days postnatal (P5), 11 days postnatal (P11), and adult (AD). (A) Immunohistochemistry of coronal sections. (B) Densitometry of immunohistochemical staining in the CA1, CA2, and CA3 regions. Asterisks on the error bars indicate the statistical difference with the previous bar. (C) Coronal section of the CA3 hippocampus at P11. (D) Control section of the CA3 hippocampus at P11 without primary antibody.

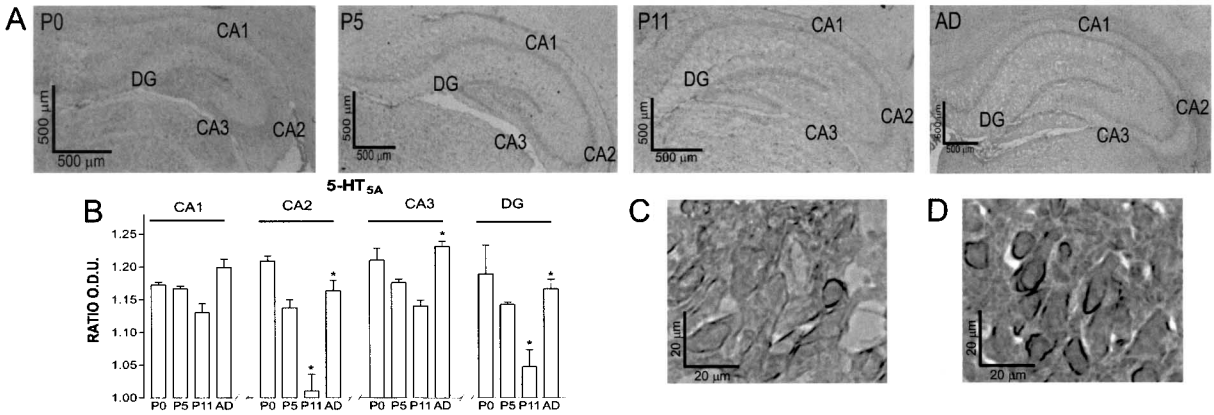

Figure 2. Distribution of 5-HT_{5A} receptors in the rat hippocampus. (A) Immunostaining of coronal sections during development. (B) Semi-quantitation of the 5-HT_{5A} receptor immunosignal in CA1, CA2, CA3, and dentate gyrus during postnatal development. Asterisks on the error bars indicate the statistical difference with the previous bar. (C) Coronal section of the CA3 hippocampus at P11. (D) Coronal section of the adult CA3 region.

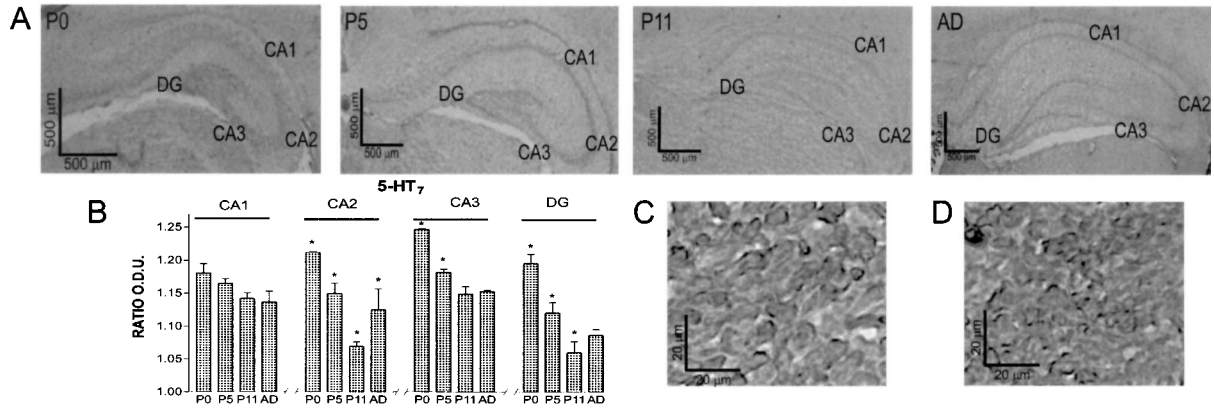

Figure 3. Distribution of 5-HT_7 receptors. (A) Immunohistochemistry of coronal sections during development. (B) Densitometry of CA1, CA2, CA3, and dentate gyrus during development. Asterisks on the error bars indicate the statistical difference with the previous bar. Coronal sections at P0, CA1 (C) and CA3 (D).

It is known that serotonin receptors are important for neurogenesis in the adult dentate gyrus (6,9) and that depletion of serotonin and its receptors delays its progress (2). Therefore, the presence of 5-HT_{2C}, 5-HT_{5A}, and 5-HT_7 receptors in the early hippocampus suggests that they may be involved in its development. However, more work is necessary to determine the timing of gene transcription and the precise location and functionality of the different types of serotonin receptors to assess their role in the development of the brain. The expression of these serotonin receptors in areas where signals for neuronal proliferation are constantly required supports the idea of a possible role in developmental neurogenesis.

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