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Postnatal changes in 5HT and NK1 receptors in rat trigeminal motor nucleus and surroundings

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Abstract

Our previous study showed that developmental changes to serotonin and substance P coexist in the trigeminal motor nucleus (Vmo), dorsolateral subnucleus (Vmo.dl), ventromedial subnucleus (Vmo.vm) and the area within 300 μm surrounding Vmo (SVmo). This occurred in rats from embryonic day (E)19 to postnatal day (P)70, with density of these terminals peaking at P7 in these three areas. The present study examined postnatal development of serotonergic 1A receptor ($5HT_{1A}$ receptor) and substance P receptor (NK1 receptor) expression in Vmo and SVmo in rats from E19 to P70. No significant changes in percentages of $5HT_{1A}$ and NK1 receptor-expressing cells were seen between E19 and P70 by immunohistochemical study or *in situ* hybridization. In a real-time PCR study, quantities of $5HT_{1A}$ and NK1 receptor expression peaked at P7, indicating that expression of these receptors was maximized in each neuronal cell body in the Vmo and SVmo at P7. This result corresponds with postnatal changes in serotonin/substance P-coexisting terminals found in our previous study. Furthermore, $5HT_{1A}$ and NK1 receptors displayed very similar patterns of expression, which may support the hypothesis that potentiation of serotonin and substance P are involved in excitability regulating trigeminal motor functions, including mastication and breathing.

Keywords: Mastication; Respiration; Aging; Jaw muscles; Receptor

1. Introduction

The trigeminal motor nucleus (Vmo) contains somatic motoneurons innervating the jaw muscles, and can be divided cytoarchitectonically into the ventromedial subnucleus (Vmo.vm) and dorsolateral subnucleus (Vmo.dl), with the Vmo.dl containing jaw-closing motoneurons and the Vmo.vm containing jaw-opening motoneurons (Jacquin et al., 1983; Mizuno et al., 1975). Trigeminal motoneurons are related to masticational jaw movements that drive trigeminal rhythmic activity. Generation of this activity is induced by input from premotoneurons located in the area within 300 μm surrounding the Vmo (SVmo) (Enomoto et al., 2002). Serotonin has been shown to excite trigeminal motoneurons (Katakura and Chandler, 1990; Riberio-do-Valle et al., 1991) and excitation of the serotonergic 1A receptor ($5HT_{1A}$ receptor) is facilitated by the generation of trigeminal rhythmic activity (Mori et al., 2002). Previous investigations have reported morphological evidence that $5HT_{1A}$ receptors are expressed in trigeminal motoneurons (Manaker and Zucchi, 1998; Tally and Bayliss, 2000). Furthermore, Manaker and Zucchi (1998) reported that substance P receptor (NK1 receptor) is expressed in adult rat trigeminal motoneurons. In our previous study, about 89% of serotonergic terminals in the Vmo (including Vmo.vm and Vmo.dl) and SVmo also contained substance P. The density of serotonin- and substance P-immunoreactive terminals peaked at P7 in rats from embryonic day (E)19 to postnatal day (P)70 in these three areas, and very similar densities of these terminals were seen in the Vmo.vm and Vmo.dl (Nakamura et al., 2006). However, postnatal development of $5HT_{1A}$ and NK1 receptor expressions has not been clarified. The present study therefore examined embryonic and postnatal expression of 5HT and NK1 receptors in the Vmo and SVmo.

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2. Materials and methods

All procedures were performed in accordance with the guidelines of the Animal Ethics Committee at Matsumoto Dental University.

2.1. Expression of immunoreactive 5HT and NK1 receptors

Healthy male and female Wistar rats at E19 and P0, 4, 7, 14, 21, 28 and 70 ($n = 8$ at each age) were anesthetized with ether or ketamine and transcardially perfused with heparinized physiological saline followed by 0.1 M phosphate buffer (pH 7.4) containing 4% paraformaldehyde and 0.5% glutaraldehyde. The brainstem was removed as one piece, immersed in 10% sucrose in the same fixative for 12 h, and then stored for 24–48 h at 4 °C in a 0.1 M phosphate buffer containing 30% sucrose. Two sets of alternate serial transverse sections of brainstem were cut at 30-μm intervals using a cryostat. Endogenous peroxidase activity in sections was quenched by incubation in 0.3% H_2O_2 in 0.1 M phosphate-buffered saline containing 0.2% Triton X-100 (PBS-Triton) for 1 h. To block nonspecific binding sites, sections were pre-incubated with 3% sheep serum (Sigma, USA) in PBS-Triton for 1 h at room temperature. We used two primary antibodies, comprising rabbit polyclonal antisera to $5HT_{1A}$ receptors (ZYMED, USA) and to NK1 receptors (Sigma). At each age of each primary antibody, 4 rats were used for experiment. One set of sections was incubated with each primary antibody. One more set of sections was mounted on glass slides without immunoreactive procedure, and stained with 1% neutral red. All antisera were diluted 1:2000 in PBS-Triton containing 3% sheep serum. Incubations were maintained for 36 h at 4 °C. After incubation, sections were washed in PBS-Triton and treated with biotinylated sheep anti-rabbit immunoglobulin (1:400; Chemicon, USA) for 3 h, followed by more washes and exposure to ExtrAvidin peroxidase conjugate (1:1000; Sigma) for 3 h. After 3 additional washes (30 min each), peroxidase was visualized using 0.5 mg/ml 3,3′-diaminobenzidine tetrahydrochloride and 0.01% hydrogen peroxide in PBS-Triton. Sections were mounted on silanized slides, coverslipped with Permount (Fisher Chemical, USA) and examined under light microscopy. One section, which was dissected at the one-third caudal level of the Vmo, was selected for analysis under a light microscope. Numbers of neuronal cell bodies in the Vmo and SVmo were counted from counterstained sections, and numbers of immunoreactive neuronal cell bodies in each region were counted. The ratio of immunoreactive neuronal cell bodies was calculated from these counts. Neuronal cell bodies were counted from digital micrographs on a computer monitor.

2.2. In situ hybridization

Healthy male and female Wistar rats at E19 and P0, 4, 7, 14, 21, 28 and 70 ($n = 8$ at each age) were used. Rats were killed by decapitation under deep ether

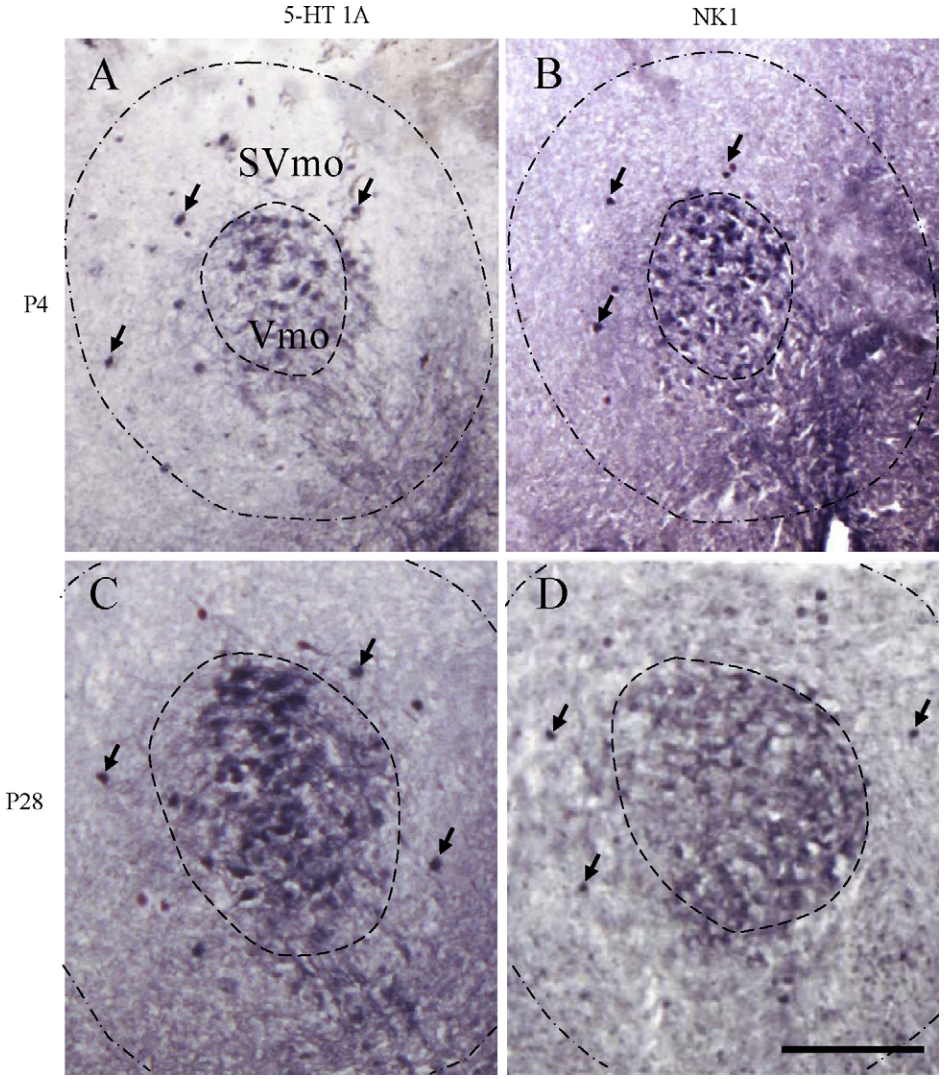

Fig. 1. Immunoreactive photomicrographs of cross-sections through the caudal third of the Vmo in rats at P4 (A, B) and P28 (C, D). Sections in (A) and (C) were incubated with antiserum to $5HT_{1A}$ receptor, while those in (B) and (D) were incubated with antiserum to NK1 receptor. Arrows indicated $5HT_{1A}$- and NK1 positive cells in SVmo. Scale bar, 300 μm.