available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Postnatal development of GABAergic axon terminals in the rat nucleus of tractus solitarius

Masayuki Yoshioka[a,b], *Yasura Tashiro*[a], *Kiyoharu Inoue*[b], *Yoshinori Kawai*[a,*]

[a]*Department of Anatomy I, Jikei University School of Medicine, 3-25-8 Nishi-Shimbashi Minato-ku, Tokyo 105-8461, Japan*
[b]*Department of Neurology, Jikei University School of Medicine, 3-25-8 Nishi-Shimbashi Minato-ku, Tokyo 105-8461, Japan*

ARTICLE INFO

Article history:
Accepted 1 June 2006
Available online 7 July 2006

Keywords:
GABA
Axon terminal
Synaptic structure
Network formation
Medulla oblongata
Reorganization

Abbreviations:
GABA, γ-aminobutyric acid
GAD, glutamic acid decarboxylase
SYN, synaptophysin
cNTS, caudal nucleus of tractus solitarius
ND, numerical density
BSA, bovine serum albumin
P, postnatal day
PBS, phosphate-buffered saline
PFA, paraformaldehyde

ABSTRACT

The proper function of the brain depends on a precise arrangement of excitatory and inhibitory synapses. Although the caudal nucleus of tractus solitarius (cNTS) plays a pivotal role in cardiorespiratory reflexes, we know little about the formation of the local neural network in the cNTS. In the present study, we have focused on GABAergic axon terminals and investigated postnatal changes in GABAergic synaptic organizations in the rat cNTS immunocytochemically at both light and electron microscopic levels. Counting synaptic and non-synaptic GABAergic axon terminals revealed that GABAergic axon terminal number in the cNTS seemed constant until the second postnatal week and that GABAergic axon terminals were reorganized around postnatal day 10 (P10). Electron microscopic observation revealed that more than 20% GABAergic axon terminals formed axosomatic synapses at P2 to P4, but the number of GABAergic axosomatic synapse on neurons with smaller soma (smaller neurons) decreased considerably after P8. Orphan GABAergic boutons were present around somata of smaller neurons at P10, and axodendritic synapse number on thicker dendrites decreased gradually during postnatal development. These results show that GABAergic axon terminals detach from somata of smaller neurons at the second postnatal week. Such morphologic changes in axon terminals could cause changes in electrophysiological activity and might contribute to reorganization of the local network within the cNTS from neonatal to adult type. These postnatal changes in the cNTS local network might be prerequisite for the cardiorespiratory reflexes of the adult type.

1. Introduction

The autonomic nervous system, which is responsible for homeostasis, contains several nuclei in the brain stem. The caudal nucleus tractus solitarius (cNTS) in the dorsal medulla integrates specifically respiratory, cardiovascular, and gastrointestinal afferents (Dampney, 1994; Spyer, 1994; Chan and Sawchenko, 1998). Efferents from the NTS are regulated by γ-aminobutyric acid (GABA) synapses within the cNTS (Kawai and Senba, 1996). Aberrations of the local neural network within the NTS seem to cause some aspects of sudden infant death syndrome (Schweitzer et al., 1992; Biondo et al., 2004).

* *Corresponding author*. Fax: +81 3 3435 1922.
E-mail address: ykawai@jikei.ac.jp (Y. Kawai).

0006-8993/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2006.06.004

Therefore, the local neural network in the NTS requires precise arrangement of excitatory and inhibitory synapses during development for proper function.

The rat cNTS is a good model to study postnatal development of the local neural network. It has been reported that the morphology of axon terminals (Miller et al., 1983; Rao et al., 1999), dendritic spines and filopodia (Vincent and Tell, 1999), and synaptic density (Lachamp et al., 2002) change during the second postnatal week. Furthermore, morphologic differentiation of the NTS neurons could modify their electrophysiologic properties (Kalia et al., 1993). However, these studies have been done without distinguishing between excitatory and inhibitory synapses.

We focused on GABAergic synapses because electrophysiological study has suggested that developmental changes in GABAergic synaptic activities occur during the second postnatal week (Kawai and Senba, 2000b). The cNTS neurons have been divided into small and large neuron groups according to the somal size (Kawai and Senba, 1996, 1999, 2000a; Okada et al., 2006; Yoshioka et al., 2006). Whereas both glutamatergic and GABAergic postsynaptic currents have been observed in single neonatal small neurons, the activity of spontaneous GABAergic postsynaptic currents decreased remarkably in adult small neurons.

We examined the postnatal development of GABAergic axon terminals in the cNTS to determine whether morphologic changes in GABAergic synapses underlie decrease of GABAergic postsynaptic activities. Our electron microscopic observation suggested that developmental changes in postsynaptic structures of GABAergic axon terminals occurred and that some GABAergic axon terminals were detached from neurons with smaller soma (smaller neurons). Our findings presumably reflect the postnatal maturation of local networks involving GABAergic synapses that are possibly linked with cardiovascular and respiratory functions.

2. Results

2.1. GABAergic axon terminals were reorganized around P10

The number of GABAergic axon terminals during postnatal development was estimated from immunofluorescence images using an unbiased disector method (West, 1999; Guillery, 2002). The total number of immunopositive dots was obtained by multiplying numerical density (ND) of GABAergic axon terminals by the volume of the cNTS. An immunohistochemical assay for GABAergic axon terminals was performed using anti-glutamic acid decarboxylase 67 (GAD67) primary antibody (Chemicon, Temecula, CA) and a Texas red-labeled second antibody. The numerical densities (NDs) of GAD67-positive dots were 42.6 × 6.1 ± 10^5/mm^3 (mean ± SD) at P2 to P4 and 22.9 ± 2.4 × 10^5/mm^3 at P21. Volumes of neonatal (P2–P4) and adult (P21) cNTS were about 0.19 mm^3 and 0.37 mm^3, respectively. NDs and volumes yield total numbers of 8.09 × 10^5 dots in neonatal and 8.47 × 10^5 dots in adult cNTS (Table 1). Thus, GABAergic axon terminal number in the cNTS seemed constant during postnatal development.

Table 1 – The number of GAD67-positive dots calculated from numerical density (× 10^5/mm^3) and cNTS volume (mm^3) at P2 to P4 and P21

	Numerical density	cNTS volume	Number of GAD67-positive dots
P2–P4	42.6 ± 6.1	0.19	8.09 × 10^5
P21	22.9 ± 2.4	0.37	8.47 × 10^5

Values are means ± SD.

To distinguish synaptic axon terminals from non-synaptic axon terminals, GABAergic axon terminals were co-stained with anti-synaptophysin (SYN) antibody (Chemicon, Temecula, CA) and visualized with FITC. The axon terminals containing SYN were regarded as synaptic axon terminals, and the axon terminals without SYN were regarded as the non-synaptic terminals. SYN-negative GABAergic axon terminals were observed at P10 (Fig. 1A, arrows), but a large number of SYN-positive GABAergic axon terminals were also observed (Fig. 1A, arrowheads). The percentage of GAD67- and SYN-positive dots among all GAD67-positive dots was 97.8 ± 3.0% (mean ± SD) at P2, 98.6 ± 7.8% at P6, 83.0 ± 6.5% at P10, 94.0 ± 4.0% at P14, and 98.4 ± 3.4% at P21 (Fig. 1B). Thus, synaptic GABAergic axon terminal number decreased temporarily at P10. Although GABAergic axon terminal number in the cNTS seemed constant during postnatal development, synaptic GABAergic axon terminal number decreased temporarily around P10.

2.2. GABAergic axosomatic synapse number decreased considerably after P8

We focused on axosomatic synapse of GABAergic axon terminals because they could have stronger effects on excitability of the postsynaptic cell (Erick et al., 2000). Electron microscopic images of axon terminals stained with anti-GAD67 or -GABA antibody are presented in Fig. 2. GABAergic axon terminals are distinguished by an immunopositive presynaptic structure containing synaptic vesicles (Fig. 2B, arrowheads) and a flat contact zone (Figs. 2B, D, and E, arrows) between pre- and post-synaptic membranes. A large number of GABAergic axosomatic synapses were observed on somata of smaller neurons, which had thin perikaryal cytoplasm (the distance between nuclear membrane and cytomembrane was less than 1 μm), at P2 to P4 (Fig. 2D) and P8 (Fig. 2E), whereas very few GABAergic axosomatic synapses were found at P21. GABAergic axosomatic synapses on somata of larger neurons, which had thick perikaryal cytoplasm (more than 2 μm), were rarely found at each developmental stage. The remaining types of synapse were mainly axodendritic synapse (data not shown). The percentage of axosomatic synapses among all GABAergic synapses was 24.8% at P2 to P4 (66/266), 22.6% at P8 (28/124), and 4.6% at P21 (5/109) (Fig. 2F). Thus, GABAergic axosomatic synapse number decreased considerably after P8.

2.3. Orphan GABA-positive boutons were present around somata of smaller neurons at P10

Although GABAergic axosomatic synapse number decreased after P8 (Fig. 2F), GABAergic axon terminal number in the cNTS