Expression of the transcription factor GATA3 in the postnatal mouse central nervous system

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Abstract

GATA binding protein 3 (GATA3) is an important regulator of central nervous system (CNS) development, but its expression pattern in the postnatal CNS has not been studied. In the present study, we examined the distribution of GATA3 mRNA in the mouse CNS at different postnatal stages by in situ hybridization. During the first 2 weeks of postnatal development, numerous GATA3-expressing cells were found in the intergeniculate leaf, ventral lateral geniculate nucleus, pretectal nucleus, nucleus of the posterior commissure, superior colliculus, inferior colliculus, periaqueductal grey, substantia nigra and raphe nuclei. Few notable changes in the profile of GATA3 expression occurred over this time period. As postnatal development progressed, however, GATA3 expression weakened, and was maintained in only a few regions of the adult CNS. Throughout the brain, we found that GATA3-expressing cells were NeuN-positive, and no colocalization with glial fibrillary acidic protein (GFAP) was observed. In the substantia nigra, GATA3 was exclusively expressed in cells of the reticulate part and some of which were found to be GABAergic. This study presents a comprehensive overview of GATA3 expression in the CNS throughout postnatal life, and the dynamics that we observed provide insights for further investigations of the roles of GATA3 in postnatal development and the maintenance of the mature CNS.

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Keywords: GATA3; In situ hybridization; Brain; Postnatal development; Mouse

Abbreviations: 3V, third ventricle; 4V, fourth ventricle; APT, anterior pretectal nucleus; Aq, aqueduct; Cb, cerebellum; Cx, cerebral cortex; CNS, central nervous system; Co, cochlear nucleus; DT, dorsal tegmental nucleus; DLG, dorsal lateral geniculate nucleus; DrMe, deep mesencephalic reticular formation; DR, dorsal raphe nucleus; GAD67, glutamic acid decarboxylase 67; GATA3, GATA binding protein 3; GFAP, glial fibrillary acidic protein; Hi, hippocampus; Hy, hypothalamus; IC, inferior colliculus; IGL, intergeniculate leaf; DT, dorsal tegmental nucleus; LL, nucleus of the lateral lemniscus; IP, interpeduncular nucleus; MG, medial geniculate nucleus; MnR, median raphe nucleus; Mo5, motor nucleus of the trigeminal nerve; PAG, periaqueductal grey; PC, nucleus of the posterior commissure; PB, parabrachial nucleus; Pn, pontine nucleus; Po, posterior thalamic nucleus; Pp5, principal sensory trigeminal nucleus; py, pyramidal tract; R, red nucleus; RLi, rostral linear raphe nucleus; RMg, raphe magnus nucleus; RPa, raphe pallidus nucleus; RPF, retroparafascicular nucleus; SC, superior colliculus; SN, substantia nigra; SNC, substantia nigra pars compacta; SNR, substantia nigra pars reticulata; SpO, oral subnucleus of the spinal trigeminal nucleus; SPO, superior paraolivary nucleus; TH, tyrosine hydroxylase; VLG, ventral lateral geniculate nucleus; VPM, ventral posteromedial thalamic nucleus; Tph2, tryptophan hydroxylase 2; VTA, ventral tegmental area; ZI, zona incerta.

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1. Introduction

The GATA family transcription factors play critical roles in development, including cell-fate specification, cell proliferation and differentiation in various tissues (Simon, 1995; Patient and McGhee, 2002). The six members are divided into two subfamilies on the basis of sequence homology: GATA1-3 and GATA4-6 (Simon, 1995; Patient and McGhee, 2002). GATA1-3 are primarily expressed in the haematopoietic stem cells where they regulate gene expression in differentiating T-lymphocytes, erythroid cells and megakaryocytes (Pevny et al., 1991; Tsai et al., 1994; Pandolfi et al., 1995; Maeno et al., 1996). GATA4-6 are expressed in various mesoderm- and endoderm-derived tissues, such as heart, liver and gut, and are involved in the differentiation of the heart and viscerae (Laverriere et al., 1994; Kuo et al., 1997). Among the GATA factors, only GATA2 and GATA3 are expressed in the CNS, in partially overlapping patterns, from early embryonic stages (Maeno et al., 1996; Nardelli et al., 1999; Pata et al., 1999; Murphy and Reiner, 2002).

During embryonic development, GATA3 is necessary for the differentiation of caudal serotonergic neurons in the brainstem and for the development of rhombomere 4 (Hikke van Doorninck et al., 1999; Pata et al., 1999). Following these early stages, however, the expression and functions of GATA3 in the brain have yet to be studied. To begin

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Fig. 1. Expression of GATA3 in the pretectal region and midbrain at P7. (A–C) At the level of the posterior commissure (pc), numerous GATA3-expressing cells are present in the intergeniculate leaf (IGL), ventral lateral geniculate nucleus (VLG) and anterior pretectal nucleus (APT), but not in the dorsal lateral geniculate nucleus (DLG) or posterior thalamic nucleus (Po). Arrowhead in (A) points to a cluster of GATA3-expressing cells in the dorsomedial hypothalamus. (B) and (C) show higher magnification of (A). (D–F) At the level of the superior colliculus, many GATA3-expressing cells are observed in the superior colliculus (SC), periaqueductal grey (PAG), red nucleus (R) and reticular part of substantia nigra (SNR), but not the compact part of the substantia nigra (SNC), ventral tegmental area (VTA) or medial geniculate nucleus (MG). (E) and (F) show higher magnification of (D). 3V, third ventricle; Aq, aqueduct; Hi, hippocampus; Hy, hypothalamus; ZI, zona incerta. Scale bars: in D, 500 μm (applies also to A); in F, 200 μm (applies also to B, C, E, F).
investigating the role of GATA3 in the postnatal CNS we have completed a comprehensive time-course analysis of GATA3 mRNA expression in the brain from birth to adulthood. These results suggest that GATA3 may function in the postnatal development of certain types of neurons as well as in the maintenance of their normal functions in adult brain.

2. Materials and methods

2.1. Animals and section preparation

Staged C57B6 mice were anesthetized by placing on ice [postnatal day (P) 0, P0, and P7] or by intraperitoneal injection of sodium pentobarbital [P15, P25 and P60]. Mice were then transcardially perfused with 0.01 M phosphate buffered saline (PBS; pH 7.4) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4). Brains and spinal cords were removed and placed in PBS containing 15% sucrose overnight, 14 μm-thick transverse sections were then cut on a cryostat and mounted onto polylysine-coated glass slides.

2.2. In situ hybridization

A fragment of GATA3 was amplified by PCR from a mouse brain cDNA library with the primers: 5’ primer: 5'-CTCATCTTCAGCCCAACA-3’; 3’ primer: 5’-TTCCTTA TCTCCATTATCCC-3’. The fragment (approximately 1.1 kb) was then subcloned into pGEM-T Vector (Promega, USA). Digoxigenin-labeled antisense and sense RNA riboprobes for GATA3 were generated and in situ hybridization was performed as described previously (Guo et al., 2007). The anatomical nomenclature of Paxinos and Franklin (Paxinos and Franklin, 2001) was adopted in the description of GATA3 expression in the mouse CNS.

2.3. In situ hybridization and immunohistochemistry double labeling

In situ hybridization for detection of GATA3 mRNA was performed to completion first, followed by immunohistochemical labeling. Primary antibodies used were: rabbit anti-NeuN antibody (1:500; Chemicon, USA), rabbit anti-GFAP antibody (1:1000; GeneTex, USA), rabbit anti-tyrosine hydroxylase (TH; 1:2000; Sigma, USA), rabbit anti-tryptophan hydroxylase 2 (Tph2; 1:2000; gift from Dr. Klaus-Peter Lesch) and rabbit anti-GAD67 (1:1000; Chemicon). All signals, except GAD67, were developed by sequen-

Fig. 2. Expression of GATA3 in the midbrain at P7. (A, B) In the ventral midbrain at the level of the superior colliculus, many GATA3-expressing cells are found in the deep mesencephalic reticular formation (DpMe), red nucleus (R) and substantia nigra (SN). A few labeled cells are also present in the interpeduncular nucleus (IP), but no GATA3 expression is observed in the rostral linear raphe nucleus (RLi). (B) Shows higher magnification of (A). (C–E) In the midbrain at the level of the interior colliculus, many strongly labeled cells are distributed in the inferior colliculus (IC), dorsal raphe nucleus (DR), median raphe nucleus (MnR) and superior paraolivary nucleus (SPO). No GATA3 expression is observed in the cerebral cortex (Cx), cerebellum (Cb), principal trigeminal sensory nucleus (Pr5) or motor nucleus of trigeminal nerve (Mo5). (D) and (E) show higher magnification of (C). Aq, aqueduct. Scale bars: 500 μm in C (applies also to A); 200 μm in E (applies also to B, D).
tial incubation with biotinylated donkey anti-rabbit IgG (1:200; Jackson, USA) for 2 h and Cy2-labeled Avidin D for 1 h (1:1000; Jackson). For GAD67, the Elite ABC kit (1:200 for 1 h; Vector Laboratories, USA) and a diaminobenzidine tetrahydrochloride (0.02% in PBS containing 0.003% hydrogen peroxide) reaction were used in place of Cy2-labeled Avidin D. In situ hybridization signals were imaged in the bright field and immunofluorescence was imaged in the green channel. For overlays, grayscale in situ hybridization images were pseudo-colored cyan and immunofluorescence was shown in red. All images were imported into Adobe Photoshop 7.0 (San Jose, CA, USA) and not manipulated other than slight modifications of the contrast and brightness settings.

3. Results

3.1. Expression of GATA3 in early postnatal CNS

Very few differences in the pattern or intensity of GATA3 expression were seen between P0, P7 and P15 and so, unless otherwise mentioned, the pattern we describe in this section applies to all 3 time-points. During this period, GATA3 expression was most pronounced in the pretectum and midbrain.

![Images of brain regions showing GATA3 expression](imageurl)

Fig. 3. Expression of GATA3 in the pretectal region and midbrain at P15. In the rostral midbrain (A–C), numerous GATA3-expressing cells are located in the ventral lateral geniculate nucleus (VLG), anterior pretectal nucleus (APT), retroparafascicular nucleus (RPF), nucleus of the posterior commissure (PC) and substantia nigra (SN). (B) and (C) show higher magnifications of (A). (D–F) In the midbrain at the caudal level of the superior colliculus, many GATA3-expressing cells are present in the superior colliculus (SC), deep mesencephalic reticular formation (DpMe), peri-aqueductal grey (PAG), median raphe nucleus (MnR) and nucleus of the lateral lemniscus (LL). (E) and (F) show higher magnifications of (D). (G) No staining is observed, including the dorsal raphe nucleus (DG) and raphe magnus nucleus (RMg), when sections are stained with sense RNA in situ probe. Aq, aqueduct; DLG, dorsal lateral geniculate nucleus; pc, posterior commissure; Pr5, principal sensory trigeminal nucleus. Scale bars: 500 μm in G (applies also to A, D); 200 μm in F (applies also to B, C, E).
as well as most raphe nuclei. In the pretectum, expression was restricted to the anterior pretectal nucleus (Fig. 1A, C), retroparafascicular nucleus and the nucleus of the posterior commissure (Fig. 3A, B). In the thalamus and hypothalamus, GATA3 transcripts were observed in the intergeniculate leaf and ventral lateral geniculate nuclei but not in the dorsal lateral geniculate nucleus (Figs. 1A, B, 3A). A cluster of labeled cells was observed in the mediodorsal part of the caudal hypothalamus (arrow in Fig. 1A) but GATA3 was otherwise absent from the hypothalamus and the forebrain, including the olfactory bulb, cerebral cortex, hippocampus and striatum. Sense RNA probe revealed no in situ signal (Fig. 3G).

Expression in the midbrain was widespread. In the dorsal midbrain, GATA3 was expressed by a large number of cells in both the superior (Figs. 1D, F, 2C, 3D, E) and inferior colliculus (Fig. 2C). At P15, however, expression in the inferior colliculus was greatly diminished (Fig. 4A). GATA3 was also observed in both the dorsal and ventrolateral divisions of the pariaqueductal grey (Figs. 1D, F, 2A, C, 3D). In the ventral midbrain, many highly expressing cells were present in the reticulate part of the substantia nigra (Figs. 1D, E, 3A, C), red nucleus, deep mesencephalic reticular formation and interpeduncular nucleus (Figs. 1D, 2A, B). More sparse labeling was observed in superior paraolivary nucleus (Fig. 2C, E) and nucleus of the lateral lemniscus (Fig. 3D, F). The ventral tegmental area did not show GATA3 expression at any time point (Fig. 1D, E).

To further delineate the localization of GATA3 expression in the substantia nigra, we double labeled P15 and P25 sections for GATA3 and TH. TH-positive dopaminergic neurons were located in the compact part of the substantia nigra, as well as the ventral tegmental area, but not the reticulate part. In contrast, GATA3-expressing cells were exclusively in the reticulate part and no colocalization of GATA3 and TH was detected (Fig. 5A–C). It is known that a large proportion of neurons in the reticulate part of substantia nigra are GABAergic (Windels and Kiyatkin, 2006; Invernizzi et al., 2007). Double labeling sections for GATA3 and GAD67 (the key enzyme for GABA synthesis) revealed that approximately 17.6% of GATA3-expressing cells in the re

Fig. 4. Expression of GATA3 in the caudal midbrain and pons at P15. (A–C) At the level of the inferior colliculus, GATA3-expressing cells are present in the dorsal raphe nucleus (DR), raphe magnus nucleus (RMg), raphe pallidus nucleus (RPa), superior paraolivary nucleus (SPO) and dorsal tegmental nucleus (DT). The cerebellum (Cb), parabrachial nucleus (PB) and oral subnucleus of the spinal trigeminal nucleus (SpO) do not show GATA3 expression. (B) and (C) show higher magnifications of (A). (D, E) At the level of the rostral medulla oblongata, many GATA3-expressing cells are located in the raphe magnus nucleus and raphe pallidus nucleus and a few are seen in the dorsal tegmental nucleus. (E) Shows higher magnification of the raphe nucleus in (A). Aq, aqueduct; Cb, cerebellum; Co, cochlear nucleus. Scale bars: 500 μm in D (applies also to A); 200 μm in E (applies also to B, C).
In the hindbrain (pons and medulla oblongata), only a few regions contained GATA3 mRNA. GATA3 mRNA was strongly expressed by large numbers of cells in all of the raphe nuclei (Figs. 2C and D, 3D, 4) except the linear raphe nucleus in the midbrain, which did not appear to express GATA3 at all (Fig. 2A, B). Double labeling showed that the majority of GATA3-expressing neurons expressed Tph2 (79.1%), the key enzyme for 5-HT synthesis in the brain (Zhang et al., 2004), and vice versa (71.0%). In addition, a small number of GATA3-expressing cells were observed in the medial and superior vestibular nuclei, dorsal tegmental nucleus and surrounding grey matter (Fig. 4A, B, D). No GATA3 expression was found in other regions of the hindbrain, cerebellum (Figs. 2C, 4A, D) or spinal cord (data not shown).

3.2. Expression of GATA3 in adult CNS

Expression of GATA3 throughout the brain at P25 was very similar to that at early stages (P0–P15) but in situ signals had become weak (data not shown). In the adult brain (P60), however, faint GATA3 expression remained only in the pretectal region, superior colliculus, nucleus of lateral lemniscus, pariaqueductal grey, compact part of the substantia nigra and raphe nuclei (Fig. 7), and was absent from all other brain loci. Nowhere in the adult brain did we observe expression of GATA3 where none was found at earlier postnatal stages.

3.3. GATA3 is expressed in neurons, but not glia

To further elucidate the cellular identity of GATA3 expressing cells, we double labeled P15 and P25 brain slices

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Fig. 5. Double labeling for GATA3 and TH in the substantia nigra and for GATA3 and Tph2 in the dorsal raphe nucleus at P15. (A–C) In the substantia nigra, GATA3 mRNA (A) is predominately located in the reticulate part, whereas TH protein (B) is primarily distributed in the compact part. (C) In the overlay of (A) and (B), no colocalization of GATA3 (cyan) and TH (red) is observed. (D–F) In the dorsal raphe nucleus, both GATA3 mRNA (D) and Tph2 protein (E) are highly expressed. (F) Overlay of (D) and (E) shows that the majority of GATA3-expressing neurons (cyan) are Tph2 immunoreactive (red), and vice versa. Double arrows indicate GATA3/Tph2-coexpressing neurons. Arrowheads and arrows point to those expressing only GATA3 or Tph2, respectively. GATA3 expression is revealed by in situ hybridization, and TH and Tph2 are labeled by immunohistochemistry. The scale bar in F represents 200 μm for A–C and 100 μm for D–F.
for GATA3 and the pan-neural marker NeuN or the glial marker GFAP. We found that throughout the CNS almost all GATA3-expressing cells were immunostained with NeuN (Fig. 6B) and, correspondingly, no GATA3-expressing cell was GFAP immunoreactive (Fig. 6C).

4. Discussion

In the present study, we examined the distribution of GATA3-expressing cells in the postnatal mouse CNS. We observed strong expression in neurons, but not glia, of restricted brain regions at early postnatal ages suggesting that GATA3 functions in the postnatal development and maturation of specific types of neurons. Moreover, the present study shows that GATA3 was greatly downregulated throughout the CNS, and maintained expression in only a few specific loci in the adult brain.

GATA3 expression is first detected in rhombomeres 2 and 4 in the mouse CNS at embryonic day (E) 9.0, and its expression is also present in the preptectal region and in a narrow strip of region in the dorsolateral neural tube extending from the midbrain to the spinal cord at E9.5 (Nardelli et al., 1999). Thus, GATA3 expression in the preptectal region was maintained into the postnatal life, and GATA3-expressing neurons in the reticulate part of the substantia nigra and red nucleus are likely derived from GATA3-expressing cells in the ventrolateral neural tube of the midbrain at early embryonic stages (Nardelli et al., 1999).

GATA3 expression is not observed in the tectum at early embryonic stages (E9.0–E11.5), but we found it to be abundantly expressed in the tectum of the early postnatal brain, suggesting that the onset of GATA3 expression in the tectum begins late in embryogenesis. On the other hand, GATA3 is expressed in the olfactory bulb and ventral interneurons of the spinal cord during embryonic development (Nardelli et al., 1999) but was not detected in the corresponding regions of the postnatal brain. In the postnatal CNS, GABA3 expression is restricted to the preptectal region, midbrain and raphe nuclei and is associated with postnatal developmental stages: high expression level in the early postnatal brain but low in matured CNS. These data suggest that GATA3 may play a role in the maturation of neurons in these brain regions.

GATA3 has been shown to be essential for the differentiation of serotonergic neurons in the caudal raphe nuclei (Hikke van Doorninck et al., 1999; Pattyn et al., 2004) and, as we confirmed here, it continues to be expressed in serotonergic neurons during postnatal stages. Since GATA3 knock-out mice die around E11.0 with multiple defects, including brain and spinal cord abnormalities, abnormal liver hematopoiesis and bleeding (Pandolfi et al., 1995; Lim et al., 2000), conditional knock-out strategies are required to study the role of GATA3 in the postnatal brain. For example, mice carrying inducible Cre recombinase transgenes that are specifically expressed in serotonergic neurons of the raphe nuclei have recently been generated (Scott et al., 2005) and it could be used to delete GATA3 gene after crossing with floxed-GATA3 mice (Yamane et al., 2005) to study the effects of eliminating GATA3 from postnatal serotonergic neurons in the raphe nuclei.

The present study also revealed GATA3 expression in neurons of the adult preptectal region and tectum. These regions are important for several brain functions including visual and auditory input-induced motor reflexes and transmission of visual and auditory sensations to higher brain regions. We also showed that GATA3 was expressed in some GABAergic neurons and other subtypes in the reticulate part of the substantia nigra. Although the function of GABA3 in each of these regions is unknown, the GABAergic neurons in the reticulate part of the substantia nigra have been shown to play important roles in the regulating dopaminergic neurons in the compact part (Invernizzi et al., 2007; Tepper and Lee, 2007;
Tsumori et al., 2002; Windels and Kiyatkin, 2006). Thus, determining the role of GATA3 in postnatal life may provide insights into nervous system dysfunctions.

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References


Fig. 7. Expression of GATA3 in adult brain (P60). Sparse expression is seen in the anterior pretectal nucleus (APT), nucleus of the posterior commissure (PC), superior colliculus (SC) and dorsal raphe nucleus (DR). 3V, third ventricle; 4V, fourth ventricle; Aq, aqueduct. Scale bar: 200 μm, applies to all panels.
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