Expression of super cervical ganglion-10 (SCG-10) mRNA in the monkey cerebral cortex during postnatal development

Takao Oishia,*, Noriyuki Higoa, Keiji Matsudaa, Motoharu Hayashib

aSystems Neuroscience Group, Neuroscience Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Umezono, Tsukuba, Ibaraki 305-8568, Japan
bDepartment of Cellular and Molecular Biology, Primate Research Institute, Kyoto University, Kanrin, Inuyama, Aichi 484-8506, Japan

Received 9 December 2001; received in revised form 24 January 2002; accepted 1 February 2002

Abstract

We used Northern blot analysis to measure SCG10 mRNA expression in various areas of the monkey cerebral cortex during postnatal development. In all areas, SCG10 mRNA expression was highest immediately after birth, decreased sharply until postnatal day 70 (P70) and then decreased slowly until the adult stage. Expression levels were similar in all areas at all time points during development, including the adult stage. These findings confirm that axonal growth in the monkey cerebral cortex is most vigorous before or during the perinatal period, progresses similarly in all areas until P70, and continues at lower levels during late development.

Keywords: SCG10; Growth-associated protein-43 (GAP-43); Association area; Sensory area; Macaque monkey; Northern blot analysis

We previously studied the developmental time course of growth-associated protein-43 (GAP-43) mRNA in the monkey cortex [12]. GAP-43 mRNA expression is highest in the intermediate fetal period and decreases to the adult level by postnatal day 70 (P70). Expression is higher in the association areas than in the primary sensory areas from the intermediate fetal period to the adult stage.

SCG10 is also a representative membrane-bound neuronal growth-associated protein that is enriched in neuronal growth cones [1,18]. It belongs to the stathmin family [13,14], and promotes neurite outgrowth by inducing microtubule disassembly [16].

Our previous in situ hybridization study revealed the distribution of cells expressing SCG10 mRNA in the infant and adult cerebral cortex of the macaque monkey [5]. In the infant cortex, SCG10 mRNA expression was prominent in layers II–VI. In contrast, adult monkeys had prominent expression of SCG10 mRNA only in layers IV–VI. No obvious difference in the laminar expression pattern of SCG10 mRNA between areas was observed, either in infants or adults. This result is in sharp contrast to the laminar expression pattern of GAP-43 mRNA in the adult cortex. Prominent signals for GAP-43 mRNA were found in layers II–VI of the association areas, and in layers IV–VI of the primary and secondary sensory areas of the adult cortex [5].

The aim of the present study was to determine the developmental time course of SCG10 mRNA in each area of the macaque cerebral cortex, and to determine whether the expression pattern was the same in the association areas and the primary and secondary sensory areas. Preliminary results have appeared in abstract form [4].

Macaques at ages ranging from postnatal day 1 (within 24 h of birth) to adult were used. All monkeys, except those adults purchased from a local provider, were bred in the Primate Research Institute, Kyoto University. Due to limited availability, we used two species of macaque (Macaca mulatta and Macaca fuscata), and the number of animals in each stage was two (Table 1). There were no obvious differences in SCG10 mRNA expression between individuals of the two species at the same age. These monkeys were the same individuals as used in our previous study [12]. All monkeys, except adults, were deeply anesthetized with an overdose of sodium pentobarbital (35 mg/kg, i.p., Nembutal; Abbot, North Chicago, IL) and sacrificed by exsanguination from the carotid artery. Adult monkeys were given an overdose injection of pentobarbital and transcardially perfused with ice-cold saline. All procedures were carried out in accordance with The Guide for the Care and Use of Laboratory Animals established by the Institute of Laboratory Animal Resources and The Guide
for the Care and Use of Laboratory Primates established by the Primate Research Institute, Kyoto University.

The cortical areas of the brains were dissected on crushed ice as quickly as possible using the von Bonin and Bailey classification [20] determined from sulcal patterns as described previously [12]. Three association areas, the prefrontal area (FD), the temporal association area (TE), and the parietal association area (PG), and three sensory areas, the primary somatosensory area (PB), the secondary visual area (OB), and the primary visual area (OC) were sampled. Dissected tissues were frozen immediately with dry ice and stored at $-80^\circ$C until use.

Total RNA was prepared by the method of Chomczynski and Sacchi [2]. The quantity and purity of RNA in the samples was determined by spectroscopic measurement at 260, 280, and 230 nm. Extracted RNA was divided into 1.25–2.5 µg samples and denatured by incubating at 60 °C for 15 min with 50% formamide, 2.2 M formaldehyde, and 0.5 M 3-Morpholinopropanesulfonic acid buffer (pH 7.0), and then stored at $-30^\circ$C until use. The samples were electrophoresed on a 0.9% agarose gel containing 2.2 M formaldehyde, and blotted onto a nylon membrane (Hybond-N, Amersham Pharmacia Biotech, Piscataway, NJ) by capillary blotting with 20× standard saline citrate (SSC: 3 M sodium chloride and 0.3 M sodium citrate). Membranes were ultraviolet-irradiated and prehybridized overnight at 42 °C in 250 µg/ml sheared salmon sperm DNA, 50% formamide, 5× SSC, 50 mM phosphate buffer (pH 6.5), and 1× Denhardt’s solution containing 0.02% Ficoll (Type 400; Amersham Pharmacia Biotech), 0.02% polyvinylpyrrolidone, and 0.02% bovine serum albumin.

Human SCG10 cDNA (pSG601, 550 bp; a gift from Dr. N. Mori of the National Institute for Longevity Sciences, Japan), and human glyceraldehyde-3'-phosphate dehydrogenase (G3PDH) cDNA (1.1 kb; Clontech, Palo Alto, CA) were labeled with [32P]dCTP using the random primer method (Roche Diagnostics, Mannheim, Germany).

Hybridization was performed overnight in 2.5 ng (105 cpm)/ml of radiolabeled probe, 250 µg/ml sheared salmon sperm DNA, 50% formamide, 5× SSC, 50 mM phosphate buffer (pH 6.5), and 1× Denhardt’s solution at 42 °C. Then, membranes were washed several times at room temperature with 2× SSC and 0.2% sodium dodecyl sulfate (SDS), rinsed twice at 55 °C with 0.1× SSC and 0.2% SDS, sealed in a plastic bag, and the radioactivity was measured with an image analyzing system (BAS 1500; Fuji Film, Tokyo, Japan).

In all regions of the cortex, the probe hybridized to two specific RNA transcripts of 2 and 1 kb. As the signal was more intense for the 2-kb transcript, and a 2-kb transcript is specifically associated with neurite outgrowth [3], we measured the amount of 2-kb band (Fig. 1A). Although there are numbers of stathmin family molecules that have high homology with SCG10, we concluded from its length that the 2-kb band corresponded to SCG10 mRNA [1,3,6,14,18].

To calibrate the total RNA applied, we employed a two-step standardization method as described in our previous study [12]. First, to compensate for differences in signal intensity caused by different specific activities in different experiments, we electrophoresed serially diluted standard total RNA extracted from large volumes of cerebral cortex and experimental samples on the same plate. The amount of SCG10 mRNA in each sample was represented as a multiple of the standard total RNA. Second, to compensate for the error in the amount of total RNA applied, we used G3PDH mRNA (Fig. 1A) as an internal control. G3PDH is a housekeeping gene, and is frequently used as an internal control of

<table>
<thead>
<tr>
<th>M. fuscata</th>
<th>P1</th>
<th>P8</th>
<th>P30</th>
<th>P70</th>
<th>P183</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. mulatta</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 1
Number of monkeys used at each age

Fig. 1. (A) Autoradiograms of SCG10 mRNA and G3PDH mRNA in area FD and area OC. (B) Developmental time course of the amount of SCG10 mRNA in six cortical areas. A closed circle and short horizontal bars indicate the mean value and standard deviation of the mRNA amount of subjects at each time point. Days after birth are shown on the horizontal axis.
growth-associated mRNA measurement [10,19]. G3PDH mRNA expression did not show obvious changes with age, as in our previous study [12]. In this study, we divided the value of SCG10 mRNA by the value of G3PDH mRNA. Thus, the normalized values of SCG10 mRNA are indicated as a multiple of the ratio of SCG10 mRNA to G3PDH mRNA in the standard total RNA from brain homogenate. Measurements were performed at least twice for each RNA sample.

A two-way analysis of variance (ANOVA) was applied to test the differences in mRNA between ages or areas using StatView 4.5 software (Abacus Concepts, Berkeley, CA). The decrease rate in the SCG10 mRNA level between two time points was calculated as the difference in the normalized amounts of SCG10 mRNA divided by the interval. We compared decrease rates successively with the Wilcoxon signed-ranks test (StatView 4.5) to determine when the rate changed.

In all areas examined, SCG10 mRNA expression was highest at the earliest time point (P1 for FDΔ, PB, and OC; P8 for TE, PG, and OB), decreased sharply until P70, and then decreased gradually until the adult stage (Fig. 1B). The overall time course of the developmental change in SCG10 mRNA expression was similar in all areas (P = 0.97, two-way ANOVA). The SCG10 mRNA decrease slowed down significantly at P70 (P < 0.05, Wilcoxon signed-ranks test).

SCG10 induces depolymerization of assembled microtubules, and inhibits polymerization of new microtubules [16]. Moreover, overexpression of SCG10 in PC12 promotes neurite extension [16], and the level of SCG10 mRNA (2-kb) is highly correlated with neurite outgrowth of PC12 in various conditions [3]. These results suggest that SCG10 enhances neurite elongation via dynamic microtubule instability in the growth cones. In case of reactive synaptogenesis, SCG10 mRNA and GAP-43 mRNA are differentially regulated. SCG10 mRNA, but not GAP-43 mRNA, is upregulated when new synapses are formed on dendritic spines of target neurons that are within a few microns of the original degenerating axon after unilateral cortical ablation [11]. In contrast, GAP-43 mRNA, but not SCG10 mRNA, is upregulated when new synaptic contacts are formed at some distance away from the original fiber after combined lesion of the entorhinal cortex and fimbria fornix [17]. Thus, there is a possibility SCG10 and GAP-43 mRNA are related to different aspects of axonal development. The level of SCG10 mRNA expression and the level of GAP-43 mRNA expression may reflect the degree of different kinds of axonal development in each area at each development stage.

In all areas of the macaque cerebral cortex examined, SCG10 mRNA expression was highest immediately after birth, decreased steeply by P70, and then decreased slowly until the adult stage. The pattern was similar to that of GAP-43 mRNA [12]. In various cortical areas of the monkey, the synaptic density reaches a peak at 2–4 months after birth [15]. Therefore, the increase in synaptic density slows down at 2–4 months. The steep decline to the nearly adult level in the expression of both SCG10 mRNA and GAP-43 mRNA until P70 in the cortex suggests that net axon elongation diminishes, and that growth cones are replaced by mature synapses until P70.

The SCG10 mRNA expression level was similar in each area during development, and even after maturation. It suggests that SCG10-related axonal development may occur at a similar rate in each area. This kind of concurrent development among cortical areas in the monkey is also observed with synaptic density [15] and neurotransmitter receptors [9]. As described above, expression of GAP-43 mRNA is similar to, but slightly different from that of SCG10 mRNA. GAP-43 mRNA is slightly more abundant in the association areas than in the primary sensory areas from the late gestation period to adulthood [12]. As GAP-43 is downregulated by myelin-related neurite-growth inhibitors [7], the smaller amount of GAP-43 mRNA in the primary sensory areas than in the association areas during the postnatal period may be related to the precedence of myelination in the primary sensory areas over the association areas, as shown in the callosal axons [8].

Prominent signals for SCG10 mRNA are observed in layers II–VI of all areas at the infant stage (P2–P31) and in layers IV–VI of all areas at the adult stage [5]. A large part of the decrease in SCG10 mRNA expression during development may be attributed to the drastic reduction in SCG10 mRNA expression in layers II and III.

This work was supported by a grant from the National Institute of Advanced Industrial Science and Technology, METI, Japan and the Cooperation Research Program of the Primate Research Institute, Kyoto University. The authors appreciate Dr N. Mori’s generosity in providing the SCG10 cDNA clone. The authors would like to thank Dr K. Kawano and Dr S. Yamane for the valuable discussion they offered during this project, Ms Y. Umino for her participation in the earlier part of this study, and Ms M. Uchiyama, Ms A. Kameyama and Mr T. Takasu for their excellent technical assistance.


