Expression of MARCKS mRNA in lateral geniculate nucleus and visual cortex of normal and monocularly deprived macaque monkeys

NORIYUKI HIGO, TAKAO OISHI, AKIKO YAMASHITA, KEIJI MATSUDA, and MOTOHARU HAYASHI

1Neuroscience Research Institute, National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan
2Department of Anatomy, Nihon University School of Medicine, Tokyo, Japan
3Department of Cellular and Molecular Biology, Primate Research Institute, Kyoto University, Inuyama, Japan

(Received June 18, 2002; Accepted August 13, 2002)

Abstract

We performed a nonradioactive in situ hybridization histochemistry (ISH) study of the lateral geniculate nucleus (LGN) and the primary visual area (area 17) of the macaque monkey to investigate mRNA expression of the myristoylated alanine-rich C-kinase substrate (MARCKS), a major protein kinase C (PKC) substrate. In the LGN, intense hybridization signals were observed in both magnocellular neurons (layers 1 and 2) and parvocellular neurons (layers 3 to 6). Double labeling using ISH and immunofluorescence revealed that MARCKS mRNA was coexpressed with the α-subunit of type II calcium/calmodulin-dependent protein kinase, indicating that MARCKS mRNA is also expressed in koniocellular neurons in the LGN. GABA-immunoreactive neurons in the LGN did not contain MARCKS mRNA, indicating that MARCKS mRNA is not expressed in inhibitory interneurons. The signals were generally weak in area 17, and intense signals were restricted to large neurons in layers IVB, V, and VI. GABA-immunoreactive neurons in layers II–VI of area 17 did not contain MARCKS mRNA. Double-label ISH revealed that MARCKS mRNA was coexpressed with mRNA of GAP-43, another PKC substrate, in neurons of both the LGN and area 17. To determine whether the expression of MARCKS mRNA is regulated by retinal activity, we performed ISH in the LGN and area 17 of monkeys deprived of monocular visual input by tetrodotoxin. After monocular deprivation for 5 to 30 days, MARCKS mRNA was down-regulated in the LGN, but not in area 17. These results suggest that MARCKS mediates the activity-dependent changes in the excitatory relay neurons in the LGN.

Keywords: Growth-associated protein, Interneurons, Relay neurons, Monocular deprivation, Activity-dependent regulation

Introduction

Protein kinase C (PKC)-associated signal transduction is thought to be activated during activity-dependent synaptic plasticity (for review, see Nishizuka, 1986, 1995; Kaczmarek, 1987; Tanaka & Nishizuka, 1994). We previously reported that GAP-43, a major neuron-specific PKC substrate, is expressed in the monkey lateral geniculate nucleus (LGN) in an activity-dependent manner (Higo et al., 2000). In the primary visual area of the neocortex (area 17 of Brodmann), the expression of GAP-43 mRNA is weaker than that in the LGN, and is not affected by neuronal activity (Higo et al., 1999, 2000b). These results have enhanced our understanding of the molecular basis of activity-dependent plasticity in the monkey geniculocortical system.

In the present study, we focused on gene expression of the myristoylated alanine-rich C-kinase substrate (MARCKS), another major neuron-specific PKC substrate. By regulating interaction between actin filaments and plasma membrane, MARCKS has a role in PKC- and calcium-calmodulin-mediated structural alteration of neurons (for review, see Aderem, 1992; Chakravarthy et al., 1999). MARCKS associates with the membrane of axon terminals and small dendrites (Aderem et al., 1988; Ouimet et al., 1990; Swierczynski & Blackshear, 1995), and crosslinks filamentous actin (Hartwig et al., 1992). When MARCKS is phosphorylated by PKC or bound with calcium-calmodulin, it is pulled off the plasma membrane (Wang et al., 1989; Thelen et al., 1991; Swierczynski & Blackshear, 1995; Arbuzova et al., 1997, 1998), and loses the capacity to crosslink filamentous actin (Hartwig et al., 1992).

Like GAP-43, MARCKS protein and its mRNA are expressed in high levels in developing brain (Patal & Klingman, 1987; McNamara & Lenox, 1998). While the expressions decrease
in most regions of mature brain (Patal & Klingman, 1987; McNamara & Lenox, 1998), high levels of expression remain in specific regions such as the hippocampus, the amygdala, and the olfactory cortex, which may be associated with a high degree of plasticity (Ouimet et al., 1990; McNamara & Lenox, 1997, 1998; Higo et al., 2002). The expression pattern of MARCKS is similar to, but different from that of GAP-43 (McNamara & Lenox, 1997; Higo et al., 1998, 1999, 2002). Moreover, gene expression of MARCKS, but not GAP-43, is increased after chick imprinting (Meberg et al., 1996). These findings indicate that MARCKS and GAP-43 may collaborate in some types of plasticity and not in others.

The aim of the present study was to investigate whether MARCKS as well as GAP-43 is involved in activity-dependent plasticity in the geniculocortical system of the adult macaque monkey. As the first step, we localized the MARCKS mRNA in the LGN and area 17 of the normal adult monkey, using a nonradioactive in situ hybridization histochemistry (ISH). Moreover, we investigated whether MARCKS mRNA was coexpressed with GAP-43 mRNA in a single neuron of these regions. We then performed ISH analysis in both the LGN and area 17 of monkeys deprived of monocular visual input via intraocular injections of tetrodotoxin (TTX) to determine the effect of neuronal activity on the expression of MARCKS mRNA. Preliminary results have been reported elsewhere (Higo et al., 2000a,c).

Methods

Animals and tissue preparation

Brain tissue was obtained from 11 macaque monkeys (six Macaca fuscata and five Macaca mulatta) aged 2 or more years. The animals were purchased from a local provider, or bred in the Primate Research Institute, Kyoto University. To investigate the effect of visual deprivation on the expression of MARCKS mRNA, five monkeys were injected with TTX (Sigma, St. Louis, MO; 15 μg in 10 μl of normal saline) intravitreally into the right eye every fifth day for a total of 5 (n = 2), 10 (n = 2), and 30 (n = 1) days before sacrifice. Injections of TTX were made under anesthesia with ketamine hydrochloride (10 mg/kg, i.m., Ketalar50, Parke-Davis, Somerville, NJ) and pentobarbital sodium (20 mg/kg, i.v., Nembutal, Abbott, North Chicago, IL). The pupillary light reflex of these monocular-deprived monkeys remained suppressed throughout the deprivation period. All animal experiments were approved by the animal care and use committee in the National Institute of Advanced Industrial Science and Technology, and 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.

All animals were pretreated with Ketalar50 (10 mg/kg, i.m.), and deeply anesthetized with Nembutal (35 mg/kg, i.v.). They were then perfused through the ascending aorta with 0.5 l of ice-cold saline containing 2 ml of heparin sodium (1000 units/ml, Novo Heparin, Leo pharmaceutical products, Ballerup, Denmark), followed by 2–5 l of ice-cold fixative: 4% paraformaldehyde (PFA) and 0.1% glutaraldehyde in 0.15 M phosphate buffer (PB; pH 7.4). During perfusion, the heads were chilled with crushed ice. After perfusion, the brains were immediately removed and blocked in the coronal plane (5 mm thick). Then they were immersed in a postfixative solution containing 2% PFA and 5% sucrose in 0.15 M PB for several hours, followed by successive immersions in 10%, 20%, and 30% sucrose in 0.15 M PB. The brain blocks were mounted in O.C.T. compound (Miles Inc., Elkhart, IN) and frozen rapidly in a dry ice/acetone bath, then stored at −80°C until dissection.

In situ hybridization

For the detection of MARCKS mRNA, digoxigenin-labeled RNA probe was used. RNA probe for MARCKS was transcribed from a 484-bp fragment that encompasses 308 bp of the 5’-untranslated region and 176 bp of the protein-encoding region of the human cDNA clone (pBSH80K1.9B; a gift from Dr. D. J. Stumpo, National Institutes of Environmental Health Sciences, Research Triangle park), which we used in our previous study (Higo et al., 2002). An in vitro transcription method was carried out according to the manufacturer’s instructions (DIG-RNA Labeling Kit, Roche Diagnostics, Mannheim, Germany). Before use, the labeled probe was precipitated with ethanol, then washed to remove unincorporated digoxigenin-labeled nucleotides.

The blocks including the LGN or area 17 were sectioned at a 16-μm thickness on a cryostat (CRYOCUT 3000, Leica, Nussloch, Germany). The sections were mounted on slides coated with Vectabond Reagent (Vector Laboratories, Burlingame, CA), dried, and pretreated for ISH by successive incubations in 4% PFA in 0.1 M PB for 15 min at room temperature, 30 μg/ml Proteinase K (Roche Diagnostics; pH 8.0) for 30 min at 37°C, and 4% PFA in 0.1 M PB for 10 min at room temperature. After a wash of 0.1 M PB, the sections were dehydrated through 70%, 80%, 90%, and 100% ethanol series (1 min at each concentration), then dried.

Sections were prehybridized in 50% formamide, 600 mM NaCl, 1x Denhardt’s solution, 0.25% SDS, 10 mM Tris-HCl (pH 7.6), 1 mM EDTA, and 200 μg/ml tRNA for 3 h at 50°C. Following prehybridization, sections were transferred to fresh hybridization buffer containing an additional 10% dextran sulfate and 1 μg/ml digoxigenin-labeled RNA probe. Hybridization was performed for at least 16 h at 50°C. The hybridized sections were rinsed three times in 5x SSC (standard saline citrate: 3 M sodium chloride and 0.3 M sodium citrate) at room temperature and washed three times in preheated 2x SSC, 50% formamide at 48°C, each time for 10 min. The sections were then treated with 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.5 M NaCl containing RNase A (Roche Diagnostics; 30 μg/ml) for 30 min at 37°C, and washed in 2x SSC for 20 min at 50°C and twice in 0.2x SSC at 50°C, each time for 20 min.

The buffer was changed to 0.1 M maleic acid, 0.15 M NaCl, and 0.2% Tween 20 (pH 7.5) for 10 min at room temperature. Sections were then incubated in 2% blocking reagent (DIG Nucleic Acid Detection Kit, Roche Diagnostics), 0.1 M maleic acid, and 0.15 M NaCl (pH 7.5) for 1 h at room temperature, then incubated in diluted (1:500) anti-digoxigenin Fab-fragments conjugated with alkaline phosphatase (DIG Nucleic Acid Detection Kit, Roche Diagnostics), 2% blocking reagent, 0.1 M maleic acid, and 0.15 M NaCl (pH 7.5) for 3 h at room temperature. Unbound antibody conjugate was removed by washing three times for 10 min each with 0.1 maleic acid, 0.15 M NaCl, and 0.2% Tween 20 (pH 7.5). Finally, the sections were preincubated in 0.1 M Tris-HCl buffer (pH 9.5) containing 0.1 M NaCl and 0.05 M MgCl₂ for 5 min, then incubated in the same buffer containing the substrates nitroblue tetrazolium (NBT, 340 μg/ml; Roche Diagnostics) and 5-bromo-4-chloro-3 indolyl phosphate (BCIP, 170 μg/ml; Roche Diagnostics) for 20 h in the dark. Color development was stopped by incubation in 10 mM Tris-HCl buffer (pH 7.5).
containing 1 mM EDTA for 10 min. The sections were incubated in 4% PFA in 0.1 M PB for 10 min at room temperature to prevent fading. Following this, sections were dehydrated through 70%, 80%, 90%, and 100% ethanol series (1 min at each concentration), transferred to xylene for three washes of 5 min each, then covered with coverslips and Permount histological mounting medium (Fisher Scientific, Fair Lawn, NJ).

The specificity of the probes was confirmed by Northern blot analysis, in which a specific band for MARCKS mRNA was observed, as in the previous study (Higo et al., 2002). In addition, control sections were hybridized using the same method described above, with the sense probes for MARCK mRNA. These control sections showed no specific signals (Fig. 1C).

**Double labeling using ISH and immunofluorescence**

To investigate whether MARCKS mRNA is coexpressed with the α-subunit of type II calcium/calcmodulin-dependent protein kinase (CAMKII-α) or GABA, we conducted double-labeling experiments using nonradioactive ISH and immunofluorescence. We first performed immunofluorescence, captured the image of immunofluorescence, then performed nonradioactive ISH. The sections were rinsed three times in 0.1 M phosphate-buffered saline (PBS; pH 7.4), and preincubated in 1% bovine serum albumin (BSA), 0.02% sodium azide, and 0.6% Triton X-100 in PBS for 4 h at room temperature. Sections were then incubated in the primary antibodies, mouse monoclonal antibody for CAMKII-α (6G9; BIOMOL Research Laboratories, Plymouth Meeting, PA) diluted 1:100 in 0.1 M PBS containing 1% BSA, 0.02% sodium azide, and 0.3% Triton X-100, or in mouse monoclonal antibody for gamma-aminobutyric acid (GABA) (GB69; Sigma) diluted 1:800 in the same buffer for 60 h at 4°C. After washing three times for 15 min each with 0.1 M PBS, the sections were incubated in the secondary antibody, Alexa 488 goat anti-mouse IgG (H+L) conjugate (Molecular Probes, Eugene, OR), diluted 1:400 in 0.1 M PBS containing 1% BSA, 0.02% sodium azide, and 0.3% Triton X-100 for 12 h at room temperature. After washing three times for 15 min each with 0.1 M PBS, the sections were covered with coverslips and 0.1 M PBS/glycerol solution. Images were captured with a fluorescence microscope (BX60; Olympus, Tokyo, Japan) equipped with an illuminator (BX-FLA; Olympus), using a 3CCD color video camera (DXC-950; Sony, Tokyo, Japan). The sections were incubated in 0.1 M PBS to remove the coverslips, and hybridized with digoxigenin-labeled probes of MARCKS, as described above.

The specificity of each primary antibody was confirmed in previous studies (Kalyuzhny & Wessendorf, 1998; Tanigawa et al., 1998; Tighilet et al., 1998a,b). Furthermore, control sections were processed using the same method described above, except that the primary antibodies were excluded. These control sections showed no specific signals (data not shown).

**Double-label ISH**

To investigate whether MARCKS mRNA is coexpressed with GAP-43 mRNA in the single neuron, double-label ISH was performed. For this purpose, we synthesized digoxigenin-labeled probe for GAP-43 mRNA and biotin-labeled probe for MARCKS mRNA. To amplify the signals from biotin-labeled probes, we used the technique of tyramide signal amplification (TSA-indirect, NEN Life Science Products, Boston, MA).

Biotin-labeled probe was made with an *in vitro* transcription method according to the manufacturer’s instructions (DIG-RNA Labeling Kit and Biotin RNA Labeling Mix., Roche Diagnostics). Before use, the labeled probes were precipitated with ethanol, then washed to remove unincorporated biotin-labeled nucleotides. The pretreatment, prehybridization, hybridization, and washing of probes were processed in the same manner as described above, except that hybridization was performed in the buffer containing both digoxigenin-labeled probe for GAP-43 mRNA and biotin-labeled probe for MARCKS mRNA. After washing the probes, the buffer was changed to 0.1 M Tris-HCl, 0.15 M NaCl, and 0.05% Tween 20 (pH 7.5) for 15 min at room temperature. Sections were then...
incubated in 0.5% blocking reagent (TSA-indirect, NEN Life Science Products), 0.1 M Tris-HCl, and 0.15 M NaCl (pH 7.5) for 1 h at room temperature. Then, incubated in diluted (1:100) streptavidin-HRP (TSA-indirect, NEN Life Science Products), 0.5% blocking reagent, 0.1 M Tris-HCl, 0.15 M NaCl (pH 7.5) for 1 h at room temperature. Unbound streptavidin-HRP was removed by washing three times for 5 min each with 0.1 M Tris-HCl, 0.15 M NaCl, and 0.05% Tween 20 (pH 7.5). Sections were then incubated in Biotinyl Tyramide Working Solution (TSA-indirect, NEN Life Science Products) for 20 min, then washed three times for 5 min each with 0.1 M Tris-HCl, 0.15 M NaCl, and 0.05% Tween 20 (pH 7.5). The sections were then incubated in diluted (1:100) Streptavidin-Alexa488 (Molecular Probes), 0.5% blocking reagent, 0.1 M Tris-HCl, and 0.15 M NaCl (pH 7.5) for 30 min at 48°C. After washing three times for 10 min each with 0.1 M Tris-HCl, 0.15 M NaCl, and 0.05% Tween 20 (pH 7.5), the sections were covered with coverslips and 0.1 M PBS/glycerol solution. Images were captured with a fluorescence microscope (BX60; Olympus) equipped with an illuminator (BX-FLA; Olympus), using a 3CCD color video camera (DXC-950; Sony). The sections were then incubated in 0.1 M maleic acid, 0.15 M NaCl, and 0.2% Tween 20 (pH 7.5) to remove the coverslips, and digoxigenin-labeled probes for GAP-43 were visualized with NBT and BCIP by the same methods described above.

To evaluate the specificities of biotin-labeled probe for MARCKS mRNA, control sections were hybridized using the same method described above, with the sense probe. These control sections showed no specific signals (data not shown).

**Quantification**

To quantify the expression patterns in the LGN of monocular-deprived monkeys, the optical densities of the in situ hybridized sections or adjacent Nissl- or cytochrome oxidase-stained sections were measured. Images of the sections were captured with the Olympus BX60 microscope using a 3CCD color video camera (DXC-950; Sony), and digitized by an image analysis system.

---

**Fig. 2.** Higher magnification photomicrographs of the normal LGN. Many MARCKS mRNA-positive neurons existed in the magnocellular layers (layers 1 and 2), parvocellular layers (layers 3 to 6), and interlaminar regions. A: Nissl-stained section. B: Localization of MARCKS mRNA. Scale bar = 100 μm.

**Fig. 3.** Adjacent sections of area 17 of the normal monkey (A, B). In area 17, hybridization signals were weaker than those in the LGN, and the intense signals were restricted to large neurons in layers IVB, V, and VI, which were presumed to be Meynert cells (C, D). The expression patterns were similar among animals and throughout the rostral to caudal levels of the coronal sections. A: Nissl-stained section. B: Localization of MARCKS mRNA. C: The MARCKS mRNA-positive large neuron in layer IVB. D: The MARCKS mRNA-positive large neuron in layer VI. Scale bar = 100 μm for A and B; and = 20 μm for C and D.
Optical density was measured in each layer of a 300-μm-wide column that sampled from layer 1 to layer 6 of the LGN, and optical density of the background staining was measured in the neighboring cell-free regions. OD (%Background) was defined as a percentage of optical density to that of the background staining. The layers of in situ-hybridized sections were identified by adjacent Nissl-stained sections.

In area 17 of monocularly deprived monkeys, we showed the relative optical density in each section in a pseudocolor representation. The resolutions of the digitized images were reduced from $1.03 \times 10^3 \text{mm}^2/4096 \times 4096$ pixels to $2.52 \times 10^3 \text{mm}^2/4096 \times 4096$ pixels, and the optical density was measured in each pixel. Using MATLAB version 5.0.0 (The Math Works Inc., Natick, MA) on a Power Macintosh G3 (Apple Computer, Inc., Cupertino, CA), the optical density in each pixel was plotted in a pseudocolor representation (Figs. 9B, 9D, & 9F).

**Results**

**Expression of MARCKS mRNA in the normal LGN and area 17**

In the normal LGN, intense hybridization signals for MARCKS mRNA were observed in the neurons of all layers (Fig. 1B). Signals were also observed in the adjacent perigeniculate nucleus (Fig. 1B). These results were similar among each animal examined ($n = 6$) and throughout the rostral to caudal levels of the coronal sections. Higher magnification photomicrographs revealed that many MARCKS mRNA-positive neurons with intense hybridization signals existed in magnocellular layers (layers 1 and 2), in parvocellular layers (layers 3 to 6), and in interlaminar regions (Fig. 2B). Many MARCKS mRNA-positive neurons were also observed in area 17, but the hybridization signals were generally weaker than those in the LGN (Fig. 3B). The intense signals were often observed in large neurons in layer IVB (Fig. 3C), presumed to be outer Meynert cells, and in layers V and VI (Fig. 3D), presumed to be inner Meynert cells (Lund, 1973; Chan-Palay et al., 1974; Winfield et al., 1981; Payne & Peters, 1989; Peters & Sethares, 1991).

A double-labeling study in the LGN involving nonradioactive ISH of MARCKS mRNA and immunofluorescence of CAMKII-α, a molecular marker of koniocellular neurons (Hendry & Yoshioka, 1994; Hendry & Calkins, 1998), revealed that nearly all CAMKII-α-positive neurons expressed MARCKS mRNA (Figs. 4A–4C).

**Fig. 4.** A–C: Expression of MARCKS mRNA in CAMKII-α-immunoreactive neurons (koniocellular neurons) in the interlaminar region of the LGN. A: Localization of MARCKS mRNA. B: Localization of CAMKII-α in the same section as A. C: The image of B was superimposed on the image of A. CAMKII-α-immunoreactive neurons (green) expressed MARCKS mRNA (dark blue). D–F: Absence of MARCKS mRNA in GABA-immunoreactive neurons in layers 2 and 3 of the LGN. D: Localization of MARCKS mRNA. E: Localization of GABA in the same section as D. F: The image of E was superimposed on the image of D. GABA-immunoreactive neurons (green) did not express MARCKS mRNA (dark blue). Scale bar = 50 μm.

**Fig. 5.** MARCKS mRNA was coexpressed with mRNA of GAP-43, another major PKC substrate, in neurons of both the LGN (A–C) and area 17 (D–F). All MARCKS mRNA-positive neurons (green) expressed GAP-43 mRNA (dark blue), and vice versa. A: Localization of MARCKS mRNA in layer 3 of the LGN. B: Localization of GAP-43 mRNA in the same section as A. C: The image of A was superimposed on the image of B. D: Localization of MARCKS mRNA in layer V of area 17. E: Localization of GAP-43 mRNA in the same section as D. F: The image of D was superimposed on the image of E. Arrows in D–F indicate large neurons that contained intense hybridization signals only for MARCKS mRNA. Scale bar = 100 μm.
The result indicates that koniocellular neurons, as well as magnocellular and parvocellular neurons, contain MARCKS mRNA.

We also conducted a double-labeling experiment of MARCKS mRNA and GABA. In the LGN, MARCKS mRNA was not expressed in GABA-immunoreactive neurons (Figs. 4D–4F). Previous studies confirmed that GABA is expressed in inhibitory interneurons in the LGN (Fitzpatrick et al., 1984; Montero & Zempel, 1985, 1986). Thus, the result of the present study indicates that MARCKS mRNA is not expressed in inhibitory interneurons in the LGN. In contrast, GABA-immunoreactive neurons in the adjacent perigeniculate nucleus expressed MARCKS mRNA (data not shown). In layers II–VI of area 17, MARCKS mRNA was not expressed in GABA-immunoreactive neurons (Figs. 4G–4I). In layer I of area 17, some GABA-immunoreactive neurons that contained weak signals for MARCKS mRNA were observed (data not shown).

The double-label ISH study revealed that all MARCKS mRNA-positive neurons in both the LGN and area 17 expressed GAP-43 mRNA, and vice versa (Fig. 5). Large neurons in layers IVB, V, and VI of area 17, presumed Meynert cells, often contained intense hybridization signals for MARCKS mRNA (arrows in Fig. 5D). However, the signals for GAP-43 mRNA in these neurons were weaker than those in the neighboring neurons (arrows in Fig. 5E).

Effects of monocular deprivation

The level of staining for cytochrome oxidase was lower in layers 2, 3, and 5 than in layers 1, 4, and 6 of the LGN ipsilateral to the TTX-injected eye (Figs. 6B & 6E). This indicates that neuronal activity was reduced in layers 2, 3, and 5, as the result of visual deprivation of the ipsilateral eye. The amount of MARCKS mRNA was also lower in layers 2, 3, and 5 than in layers 1, 4, and 6 (Figs. 6C & 6F), indicating that MARCKS mRNA was down-regulated in the layers deprived of visual input. In the adjacent Nissl-stained section, however, no apparent reduction of staining was observed (Figs. 6A & 6D). In the LGN contralateral to the TTX-injected eye, the level of staining for cytochrome oxidase and the amount of MARCKS mRNA were lower in layers 1, 4, and 6 than in layers 2, 3, and 5, although no apparent reduction of staining was observed in the Nissl-stained section (Fig. 7). These results further confirmed that layers with reduced activity display lower levels of hybridization signals for MARCKS mRNA than do normally active layers. The signals were down-regulated in all layers with reduced activity regardless of the length of the deprivation period: 5 (n = 2), 10 (n = 2), or 30 (n = 1) days (Figs. 8C & 8F). No evidence of cell shrinkage and reduction was detected in the deprived layers of all monkeys (Figs. 8A & 8D). These results were similar throughout the rostral to caudal levels of the coronal sections.

After monocular deprivation for 5, 10, or 30 days, we observed a periodic pattern of staining for cytochrome oxidase in layer IVC of area 17 (Figs. 9C & 9D), indicating reduced neuronal activity of the ocular dominance columns that received visual input from the TTX-injected eye. However, we did not detect such a periodic ocular dominance pattern of staining for MARCKS mRNA after monocular deprivation for each period (Figs. 9E & 9F). Slight unequal distribution of MARCKS mRNA in layer IVC correlated
with the cell density revealed by Nissl-stained section (Figs. 9A & 9B). The hybridization pattern for MARCKS mRNA was identical to those for normal monkeys. These results indicate that, in area 17, the expression of MARCKS mRNA is not affected after monocular deprivation for these periods.

Discussion

Expression of MARCKS in the geniculocortical system of monkey

In the present study, we revealed that MARCKS mRNA was highly expressed in most neurons of the magnocellular, parvocellular, and koniocellular layers in the LGN. GABAergic interneurons in the LGN did not express MARCKS mRNA. Thus, MARCKS mRNA was expressed in excitatory relay neurons in the LGN. The expression of MARCKS proteins is likely to be regulated by mRNA stability (Brooks et al., 1991, 1992; Lindner et al., 1992), and MARCKS protein associates with membrane of the axon terminals and small dendrites (Aderem et al., 1988; Ouimet et al., 1990; Swierczynski & Blackshear, 1995). Taken together with the previous immunohistochemical study showing that immunoreactivity for MARCKS protein is intense in layer IV of the visual area (Ouimet et al., 1990), the present result indicates that MARCKS protein is likely to exist in the geniculocortical axon terminals, most of which are located in layer IV of the visual area. In the LGN, the expression of MARCKS mRNA was down-regulated after monocular deprivation. Since in vivo studies confirmed that the down-regulation of MARCKS mRNA is associated with the down-regulation of MARCKS protein (Brooks et al., 1992), the down-regulation of MARCKS mRNA in excitatory relay neurons of the LGN should result in the down-regulation of MARCKS protein in the geniculocortical axon terminals.

A comparison with GAP-43

We previously investigated gene expression of GAP-43, another major PKC substrate, in the LGN and area 17 (Higo et al., 1999, 2000b). While the differential expression of MARCKS and GAP-43 mRNAs has been observed in some brain areas such as the hippocampus (McNamara & Lenox, 1997; Higo et al., 1998, 2002), the expression of MARCKS mRNA in the LGN and area 17 was similar to those for GAP-43 mRNA. GAP-43 mRNA is expressed in excitatory relay neurons of the LGN in an activity-dependent manner (Higo et al., 2000b). In area 17, intense hybridization signals for GAP-43 mRNA are restricted in layers IV to VI, and the expression is not affected by monocular deprivation (Higo et al., 1999, 2000b). In the present study, we confirmed that the populations of MARCKS mRNA-positive neurons in both the LGN and area 17 were identical to those of GAP-43 mRNA. Our previous studies indicated that the expression pattern of GAP-43 mRNA in the monkey neocortex is also similar to that of MARCKS mRNA (Higo et al., 1999, 2002), but double-label ISH had not yet been performed. The present study provides the first direct evidence for the coexpression of MARCKS and GAP-43 mRNAs in a single neuron. A slightly differential expression of MARCKS...
mRNA and GAP-43 mRNA was observed in the large neurons in layers IVB, V, and VI of area 17, which were presumed to be Meynert cells. Though both MARCKS mRNA and GAP-43 mRNA were expressed in these neurons, intense signals were often observed only for MARCKS mRNA.

Relationship between activity-dependent expression of PKC substrates and modifications of geniculocortical synapses

From both our present and previous studies (Higo et al., 2000b), we suggest that both of the major PKC substrates, MARCKS and GAP-43, are expressed in the geniculocortical axon terminals in an activity-dependent manner. Several lines of evidence have related the expression of MARCKS and GAP-43 mRNAs with structural synaptic reorganization in the adult nervous system (Van der Zee et al., 1989; Tetzlaff et al., 1991; Linda et al., 1992; Levin & Dunn-Meynell, 1993; Chong et al., 1994; Aigner et al., 1995; Holtmaat et al., 1995; Bendotti et al., 1997; McNamara et al., 2000; McNamara & Lenox, 2000). Both MARCKS and GAP-43 regulate the dynamics of the actin cytoskeleton at the synaptic membrane (Laux et al., 2000). Previous studies reported that the α-subunit of CAMKII is up-regulated and the β-subunit of CAMKII is down-regulated in the deprived ocular dominance column of area 17 in adults (Hendry & Kennedy, 1986; Benson et al., 1991; Tighilet et al., 1998). CAMKII is known to be involved in the structural changes of dendrites (Wu & Cline, 1998). The fact that visual input regulates the expression of both presynaptic MARCKS and GAP-43 and postsynaptic α- and β-subunits of CAMKII supports the idea that some types of structural changes of the geniculocortical synapses, such as alterations of synaptic number and morphology, may be driven by visual input even in the adult monkey. In the adult monkey, monocular deprivation does not cause extensive rearrangement of geniculocortical axons (LeVay et al., 1980). Nevertheless, physiological expansion of ocular dominance columns serving the normal eye of the monocularly deprived monkey has been observed by single-unit recording (Monkey No. 150 of LeVay et al., 1980). We suggest that the activity-dependent competition among adult geniculocortical synapses may account for this phenomenon.

Relationship to the length of the deprivation period

The present and previous studies found that when monocular visual input is deprived for 5 to 30 days, both MARCKS mRNA and GAP-43 mRNA are down-regulated in the LGN (Higo et al., 2000b).
Regulation of MARCKS mRNA in the monkey LGN and V1

Fig. 9. Surface parallel sections through area 17 of a monkey that had been monocularly deprived for 30 days (A, C, E). The relative optical densities in each section were also shown in pseudocolor representation (B, D, F). A, B: Nissl-stained section. C, D: Cytochrome oxidase-stained section. E, F: Localization of MARCKS mRNA. Arrows indicate the same blood vessels. In area 17, no deprivation effect was observed for MARCKS mRNA. Scale bar = 500 μm.

We did not detect a temporal difference between the expression of MARCKS and GAP-43 mRNAs. In contrast, previous studies reported differential temporal expression patterns of MARCKS and GAP-43 mRNAs during regeneration following neuronal lesion (McNamara et al., 2000; McNamara & Lenox, 2000). Thus, the activity-dependent expression of MARCKS and GAP-43 mRNAs in the monkey geniculocortical system is regulated by a different mechanism from that in the regeneration process.

In the monkey LGN, GABA and glutamate decarboxylate are also down-regulated by deprivation of visual input (Hendry, 1991). However, the down-regulation occurs only after a deprivation period of at least 3 weeks. The relatively rapid down-regulation of both MARCKS and GAP-43 mRNAs is similar to that of glutamate. When monocular visual input is deprived for 5 days or longer, the glutamate immunoreactivity was reduced in deprived geniculocortical axon terminals (Carder & Hendry, 1994). Rapid down-regulation is also observed in the expression of GABA_A receptor subunits and GABA_B receptor, which are expressed in excitatory relay neurons of the LGN (Huntsman et al., 1995; Hendry & Miller, 1996; Munoz et al., 1998). Thus, the expression of molecules in excitatory relay neurons may be down-regulated by monocular deprivation more rapidly than that in inhibitory interneurons of the LGN.

Acknowledgments

We are grateful to Dr. K. Kawano and Dr. S. Yamane for valuable discussion and continuous encouragement during this study, and Mr. T. Takasu and Ms. A. Kameyama for excellent technical assistance. We thank Dr. D. Stumpo for providing cDNA clone. This work was supported by the National Institute of Advanced Industrial Science and Technology of METI, the Japan Society for the Promotion of Science, and the Cooperation Research Program of Primate Research Institute, Kyoto University.

References


Hendry, S.H. & Kennedy, M.B. (1986). Immunoreactivity for a calmodulin-
dependent protein kinase is selectively increased in macaque striate cortex after monocular deprivation. Proceedings of the National Academy of Sciences of the U.S.A. 83, 1536–1541.


Lund, J.S. (1973). Organization of neurons in the visual cortex, area 17, of the monkey (Macaca mulatta). Journal of Comparative Neurology 147, 455–496.


