Distribution of corticosteroid receptors in mature oligodendrocytes and oligodendrocyte progenitors of the adult mouse brain

<table>
<thead>
<tr>
<th>Journal:</th>
<th>Journal of Histochemistry &amp; Cytochemistry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID:</td>
<td>JHC-13-0129.R1</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>Articles</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>n/a</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Matsusue, Yumiko; Nara Medical University, Oral and Maxillofacial Surgery; Nara Medical University, Anatomy and Cell Biology; Hori-Hayashi, Nonko; Nara Medical University, Anatomy and Cell Biology; Kita, Tadaaki; Nara Medical University, Oral and Maxillofacial Surgery; Nishi, Mayumi; Nara Medical University, Anatomy and Cell Biology</td>
</tr>
<tr>
<td>Keywords:</td>
<td>glucocorticoid receptor, oligodendrocyte, oligodendrocyte progenitor, corticosteroid (glucocorticoid), mineralocorticoid receptor, cofactor</td>
</tr>
</tbody>
</table>

Abstract:
The expression of glucocorticoid receptors (GRs) was investigated immunohistochemically in two different lineages of oligodendrocytes, using carbonic anhydrase (CA) II and neuron glial antigen (NG) 2 as markers of mature oligodendrocytes and oligodendrocyte progenitors, respectively. We focused on the gray matter regions, including CA1, CA3 and dentate gyrus of the hippocampus, the primary somatosensory cortex barrel field and the basolateral amygdala, and the white matter regions, including the corpus callosum, external capsule and fimbria of the hippocampus. More than 80% of CAII-immunoreactive (IR) cells and more than 85% of NG2-IR cells expressed GRs in various regions of the brain. In contrast, neither CAII-IR cells nor NG2-IR cells expressed mineralocorticoid receptors (MRs) in the same regions. The intensity of GR expression was drastically reduced in CA II-IR cells and NG2-IR cells in the same regions in adrenalectomized mice. Finally, steroid receptor co-activator (SRC)-1 and p300, both of which are cofactors for GR, were expressed in the gray and white matter regions in NG2-IR cells, but not in CAII-IR cells. These results suggest that the expression of GRs in oligodendrocytes and their progenitor cells mediates several functions in vivo, including differentiation and myelination, as a major target of glucocorticoids and their cofactors.
Running title: Corticosteroid receptors in oligodendrocytes
Distribution of corticosteroid receptors in mature oligodendrocytes and oligodendrocyte progenitors of the adult mouse brain

Yumiko Matsue\textsuperscript{1,2}, Noriko Horii-Hayashi\textsuperscript{3}, Tadaaki Kiriti\textsuperscript{1}, Mayumi Nishi\textsuperscript{2}

\textsuperscript{1} Department of Oral and Maxillofacial Surgery, Nara Medical University
\textsuperscript{2} Department of Anatomy and Cell Biology, Nara Medical University,

Correspondence to Mayumi Nishi
Department of Anatomy and Cell Biology, Faculty of Medicine, Nara Medical University,
840 Shijo-cho, Kashihara, Nara 634-8521, Japan
Tel: +81-744-29-8822
Fax: +81-744-29-4746
E-mail: nmayumi@naramed-u.ac.jp

http://mc manusciptcentral.com/hcs
Abstract

The expression of glucocorticoid receptors (GRs) was investigated immunohistochemically in two different lineages of oligodendrocytes, using carbonic anhydrase (CA) II and neuron glial antigen (NG) 2 as markers of mature oligodendrocytes and oligodendrocyte progenitors, respectively. We focused on the gray matter regions, including CA1, CA3 and the dentate gyrus of the hippocampus, the primary somatosensory cortex barrel field and the basolateral amygdala, and the white matter regions, including the corpus callosum, external capsule and fimbria of the hippocampus. More than 80% of CAII-immunoreactive (IR) cells and more than 85% of NG2-IR cells expressed GRs in various regions of the brain. In contrast, neither CAII-IR cells nor NG2-IR cells expressed mineralocorticoid receptors (MRs) in the same regions. The intensity of GR expression was drastically reduced in CAII-IR cells and NG2-IR cells in the same regions in adrenalectomized mice. Finally, steroid receptor co-activator (SRC)-1 and p300, both of which are cofactors for GR, were expressed in the gray and white matter regions in NG2-IR cells, but not in CAII-IR cells. These results suggest that the expression of GRs in oligodendrocytes and their
progenitor cells mediates several functions \textit{in vivo}, including differentiation and myelination, as a major target of glucocorticoids and their cofactors.

**Key words**

Glucocorticoid receptor, oligodendrocyte, oligodendrocyte progenitor, corticosteroid (glucocorticoid), mineralocorticoid receptor, cofactor
Introduction

Glucocorticoids contribute to a wide range of actions in the nervous system, including mediating stress responses, energy metabolism, cell growth and differentiation, and immune and inflammatory responses (Sapolsky et al. 2000; de Kloet et al. 2005). These functions are exerted through two receptor targets, the glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs), which bind the same hormone (primarily cortisol in humans and corticosterone in rodents) and work as ligand-dependent transcription factors to exert classical glucocorticoid actions (Han et al. 2005). In contrast, rapid, non-genomic actions of glucocorticoids have also been reported (Cinfra et al. 1994). The classical hormone actions are regulated by a subset of nuclear proteins called cofactors (coactivators and corepressors), such as steroid receptor coactivator 1 (SRC-1) and p300 (Leo et al. 2000; Wu RC et al. 2005).

Corticosteroids are widely used in the treatment of various kinds of diseases, including neurodegenerative diseases that affect white matter, such as multiple sclerosis (MS) (Myhr and Mellgren. 2009), whereas corticosteroids are ineffective in gray matter injuries, such as head injury and stroke (Sun et al. 2010). Furthermore, recent studies have demonstrated that corticosteroids are involved in various aspects of the regulation of gene expression.

http://mc.manuscriptcentral.com/hcs
of oligodendrocytes, including the proliferation, differentiation, and protection of oligodendrocytes, independent of the treatment of multiple sclerosis. For example, it was recently demonstrated that plasma corticosterone activates SGK1 and induces morphological changes in oligodendrocytes (Miyata et al. 2011). Glucocorticoids also protect oligodendrocytes against excitotoxin (Sun et al. 2010). Thus, it would be interesting to elucidate whether or not the distribution patterns of GR in oligodendrocytes in both white matter and gray matter are different.

Although many studies have reported GR expression in cultured oligodendrocytes (Bohn et al. 1991), very few investigations have reported GR expression in oligodendrocytes in vivo. The primary purpose of the present study was to investigate the expression of GRs and GR cofactors in oligodendrocytes in the brains of adult mice. To this end, we performed three major experiments, as follows. First, the expression of GRs in oligodendrocytes was investigated immunohistochemically in various regions of the brain in adult mice. Since GRs are involved in cell growth and differentiation in the central nervous system (CNS), the expression pattern of GRs in different lineages of oligodendrocytes would be helpful in understanding this role. We selected two markers for oligodendrocytes, carbonic anhydrase (CA) II and neuron glial antigen (NG) 2. CAII
is one of seven CA isozymes that are expressed in CNS and is considered as a marker of adult oligodendrocytes (Ghandour et al. 1980). In contrast, NG2 is an integral membrane chondroitin sulfate proteoglycan, which is expressed on oligodendrocyte progenitors (Nishiyama et al. 1996). We analyzed the gray matter regions, including CA1, CA3 and the dentate gyrus in the hippocampus, primary somatosensory cortex barrel field and basolateral amygdala, and on the white matter regions, including the corpus callosum, external capsule and fimbria of the hippocampus. The expression of another corticosteroid receptor, MR, was also investigated in oligodendrocytes in the same regions of the adult brain using the same oligodendrocyte markers. Next, the effect of reduction of corticosterone (CORT) levels on the expression of GRs in CAII-immunoreactive (IR) oligodendrocytes and NG2-IR oligodendrocyte progenitors was examined in adult mice that had undergone bilateral adrenalectomy to determine whether GRs expressed in oligodendrocytes are functional and responsive to its ligand, CORT. Finally, the expression of steroid receptor co-activator (SRC)-1 and p300 was investigated immunohistochemically in CAII-IR oligodendrocytes and NG2-IR oligodendrocyte progenitors, along with GR expression. The transcriptional activity of GRs is enhanced by the recruitment of one of the transcriptional co-activators of the
p160 family (SRCs), with a docking platform for secondary co-activators, such as the
cAMP-response element binding protein (CREB)-binding protein (CBP), or its close
homologue p300. While glucocorticoids have been reported to promote myelination
through SRC-1 or p300 in Schwann cells, it is not known whether SRC-1 and p300 are
localized in oligodendrocytes (Désarnaud et al. 2000). Therefore, clarification of SRC-1
and p300 expression in oligodendrocytes would help to determine whether GR function
is regulated by these cofactors.

The results demonstrate for the first time that GRs are expressed in different
lineages of oligodendrocytes, mature CAII-IR oligodendrocytes and NG2-IR
oligodendrocyte progenitors in vivo, and suggest that GRs mediate several important
functions, including promotion of myelination, as a major target of glucocorticoids.

Material and Method

Animals

Adult male C57BL/6 mice (8-10 weeks old) were purchased from Japan SLC
(Hamamatsu, Japan) and housed in groups (4 animals /case) in a room with a 12 hr
light-dark cycle with the light turned on at 8:00 AM. Food pellets and drinking water
were provided *ad libitum*. The protocols for this study were approved by the Animal Care Committee of Nara Medical University in accordance with the policies established by the NIH for the care and use of laboratory animals.

**Preparation of the brain sections**

Mice were deeply anesthetized in the morning (9:30-11:30 AM) by intraperitoneal injection of pentobarbital sodium salt solution (Somnopencyl 0.1ml/10g bodyweight; Kyoritsu Seiyaku Corporation, Tokyo, Japan) and then perfused intracardially with ice-cold phosphate-buffer saline (PBS) followed by 4% paraformaldehyde (PFA), pH7.4. After perfusion, the brains were removed and fixed in 4% PFA at 4°C overnight. Coronal sections were cut (50μm sections) with a linear slicer (Pro. 7, DOSAKA EM CO. (D.K.S.), Kyoto, Japan).

**Adrenalectomy and corticosterone (CORT) assay**

Six mice underwent bilateral adrenalectomy in the morning (9:30-11:30 AM) and another six mice underwent sham-operation for adrenalectomy. After the mice were anesthetized with isoflurane (Escain; Mylan Inc., Pittsburgh, USA), the skin of the right
side of their flank was opened, the muscles dissected and the adrenal gland removed.

The left adrenal gland was removed through the same skin incision. After adrenalectomy, the mice were provided drinking water supplemented with 0.9% saline.

Three days after adrenalectomy, the animals were sacrificed under pentobarbital anesthesia. Blood samples were collected by intracardiac puncture and stored in heparinized tubes. Plasma was obtained by centrifugation (2,000g for 10min at 4°C) and stored at -80°C until the day for subsequent assay. The plasma concentrations of CORT, the rodent glucocorticoid, were measured using an EIA kit (YK240, Yanaihara Institute, Inc., Shizuoka, Japan) to confirm resection of the adrenal glands. After the blood samples were obtained, the animals were perfused with ice-cold PBS followed by PFA. The brains were removed and fixed with PFA. Coronal sections (50μm thick) were prepared as described above.

**Immunohistochemistry**

Free-floating coronal sections were washed with PBS, immersed in 25mM glycine in PBS for 20 min, and permeabilized with 0.3% Triton-X 100 in PBS (PBS-T) for 15 min. After blocking in PBS-T supplemented with 5% normal goat serum (NGS) or 5%
normal horse serum (NHS) at room temperature for 2 hours, rabbit polyclonal antibody
against rat GR (1:1000; provided by Prof. M. Kawata [Morimoto et al. 1996]) and the
mouse monoclonal antibody against rat MR (1:200; provided by Prof. C. E.
Gomez-Sanchez [Gomez-Sanchez CE et al. 2006]) were used to detect GR- and
MR-immunoreactivities, respectively. A sheep polyclonal antibody against human
Carbonic Anhydrase II antibody (CAII, ab8953) (1:1000; Abcam, UK) was used to
identify mature oligodendrocytes, and a polyclonal guinea pig antibody against rat NG2
(1:200; provided by Dr. W. B. Stallcup, [Ozerdem et al. 2001; Stallcup et al. 2002;
Kucharova et al. 2011]) was used to identify oligodendrocyte progenitors. The
antibodies used to examine the cofactors in the oligodendrocytes included a polyclonal
rabbit antibody against human SRC-1 (M-341) (1:500; Santa Cruz Biotechnology,
USA), and a monoclonal mouse antibody against rat p300 (NM-11; 1:250; Calbiochem,
Germany). Sections were incubated with primary antibodies (diluted in PBS-T) with 1%
NHS at 4°C overnight. After washing, sections were incubated with either
AlexaFlour-488 labeled donkey-anti-rabbit, AlexaFlour-488 labeled goat-anti-rabbit
IgG, AlexaFlour-488 labeled donkey-anti-mouse IgG, AlexaFlour-488 labeled
goose-anti-mouse IgG or AlexaFlour-546 labeled donkey-anti-sheep and AlexaFlour-546
labeled goat-anti-guinea pig (all secondary antibodies 1:1000, Molecular Probes, USA)
in PBS-T with 1% NHS for 2h at room temperature. After washing with PBS for 15min,
sections were mounted and shielded with Vectashield® mounting media (Vector
Laboratories, Burlingame, CA) containing 4′, 6-diamino-2-phenylindole (DAPI), and
stored in the dark until further analysis. Observation and capturing of images were
carried out using a FluoView 1000 confocal microscope (Olympus, Tokyo, Japan).

Quantitative analysis

To quantify the percentages of GR- and MR-immunoreactivity in oligodendrocytes
and to measure the fluorescent intensity of GR-immunoreactivity in oligodendrocytes
from sham- and adrenalectomy-operated mice, brain sections were double-labeled with
GR and CAII or GR and NG2, all of which were counterstained with DAPI. Sections at
1.4-1.7 mm posterior to the Bregma according to the mouse brain atlas (Franklin and
Paxinos, 2008) were used for all analyses. Analyzed regions were illustrated in Figure 1.
Fluorescent images were captured using a confocal laser microscope (Olympus
Fluoview 1000) with the 40×objective lens. To count GR-IR cells in oligodendrocytes,
four images per region were obtained per mouse and the ratio of GR-IR cells in CAII-
or NG2-IR cells was calculated. The total number of CAII-IR or NG2-IR positive cells
counted in each brain region ranged from 35 to 453 cells with an average of 116 cells.
The data was expressed as the mean value ± S. D. (n = 4 mice). For signal intensity
analyses, images of all samples were captured under the same microscopic conditions
and four images per region were analyzed per mouse using the FV10-ASW software
(Olympus). Briefly, cell bodies of the oligodendrocytes were outlined by means of
CAII- or NG2-IR and the average fluorescent intensity of GR was measured within the
outlined areas. The mean value of the sham-operated group (n = 6) in each region is
considered as 100% and the relative GR intensity in the adrenalectomy group (n = 6) is
shown.

Statistical analysis

Statistical analyses were performed by t-test using JMP8 software (SAS institute Japan,
Tokyo, Japan) and P-values < 0.05 were considered statistically significant.
Results

GR expression in CAII-IR cells in the gray and white matter

Expression of GRs in CAII-IR oligodendrocytes in the gray and white matter regions was examined by double immunofluorescent staining. We focused on CA1, CA3 and the dentate gyrus of the hippocampus, the primary somatosensory cortex barrel field and the basolateral amygdala in the gray matter regions and the corpus callosum, external capsule and fimbria of the hippocampus in the white matter regions.

GRs were expressed broadly in various areas of the gray matter regions. The immunostaining pattern for GRs and CAII was somewhat different in each region. Since a recent paper demonstrated that different populations of oligodendrocytes are differentially distributed in various layers of the hippocampus (Vinet et al. 2010), we analyzed the differential distribution patterns of GR in oligodendrocytes in the hippocampal subregions. In the CA1 region, GRs were expressed in many cells with strong intensity, but CAII-IR cells were sparse in this region (Fig. 2). In contrast, while the number of CAII-IR cells per field was slightly greater in the CA3 and dentate gyrus regions, the intensity of GR expression was weak in these regions (Fig. 2), which was similar to the previous studies of neurons (Sarabdjitsingh et al. 2009). Most of the
CAII-IR cells expressed GRs in the pyramidal cell layers of CA1 and CA3 and in the hilus of the dentate gyrus region. The expression of GRs in CAII-IR cells was observed in small nuclei with relatively strong intensity. In the primary somatosensory cortex barrel field and basolateral amygdala, most of the CAII-IR cells also expressed GRs (Fig. 2). In the basolateral amygdala, the intensity of GR immunoreactivity was weaker than that in other gray matter regions (Fig. 2). The ratio of double IR cells of GRs and CAII per total CAII-IR cells was calculated in each region. More than 80% of CAII-IR cells expressed GRs in the gray matter regions examined (Table 1). In the white matter regions, GRs were primarily expressed in small nuclei of the cells in the corpus callosum, external capsule and the fimbria of the hippocampus (Fig. 3). The number of CAII-IR cells per field was greater in the fimbria of the hippocampus compared with the corpus callosum and external capsule. More than 85% of CAII-IR cells expressed GRs in the white matter regions examined (Table 1).

GR expression in NG2-IR oligodendrocyte progenitors in the gray and white matter regions

Expression of GRs in NG2-IR in oligodendrocyte progenitors of the gray and
white matter regions was examined by double immunofluorescent staining. We
examined the same brain regions as those of CAII-IR oligodendrocytes. NG2-IR cells
were observed in the pyramidal cell layers of the hippocampus, CA1 and CA3 and hilus
of dentate gyrus regions in the gray matter regions (Fig. 4). In the primary
somatosensory cortex barrel field and basolateral amygdala, the majority of NG2-IR
cells were large cells. Almost all NG2-IR cells expressed GRs in the gray matter regions
(Table 1). In the white matter regions, NG2-IR cells exhibited small nuclei in the corpus
callosum, external capsule and the fimbria of the hippocampus (Fig. 5). GRs were
expressed in more than 95% of NG2-IR cells (Table 1).

Absence of MR expression in the CAII-IR or NG2-IR cells in the gray and white
matter regions

Expression of MRs in CAII-IR or NG2-IR cells in the gray and white matter
regions was examined by double staining. MRs were ubiquitously expressed in CA1,
CA3 and dentate gyrus in the gray matter region (Fig. 6, 7). Most of the CAII-IR or
NG2-IR cells were negative for MRs in these regions. MRs were also expressed in the
corpus callosum, external capsule and the fimbria of the hippocampus in the white

http://mc.manuscriptcentral.com/hcs
matter region (data not shown). Similarly, very few of the CAI-II-IR or NG2-IR cells expressed MRs in these regions (data not shown).

Effects of serum CORT on GR distribution in CAI-II-IR and NG2-IR cells

In adrenalectomized mice, plasma levels of CORT decreased to about 20% of those in sham-operated mice (data not shown). This result indicates that plasma levels of CORT were successfully reduced by adrenalectomy. GR immunoreactivity was remarkably reduced in almost all brain regions in the adrenalectomy group. The reduction of GR immunoreactivity was also evident in both CAI-II-IR and NG2-IR cells in the regions examined (Fig. 8, 9). We showed the representative regions, CA1, primary somatosensory cortex, and corpus callosum of the gray and white matter regions. The relative intensity of GR immunoreactivity in CAI-II-IR or NG2-IR cells was significantly reduced in adrenalectomized mice compared with sham-operated mice (Fig. 8, 9).

Expression of GR cofactors in CAI-II-IR and NG2-IR cells

Expression of the GR cofactors, SRC-1 and p300, was examined in CAI-II-IR and
NG2-IR cells. In the CAII-IR cells, neither SRC-1 nor p300 was expressed in the gray
and white matter regions examined (Fig. 10, 11). On the other hand, both SRC-1 and
p300 were expressed in NG2-IR cells in both the gray matter (Fig. 12) and white matter
regions, showing more intensely and clearly in the corpus callosum and external capsule
(Fig. 13). Because NG2-IR cells express both SRC-1 and p300, we investigated
colocalization of GRs with these cofactors in NG2-IR cells. We observed that
co-expression of GR and p300 in NG2-IR oligodendrocyte progenitors by triple
immunostaining (data not shown).
Discussion

Previous studies have demonstrated that GRs are expressed in various regions of the adult brain (Cintra et al. 1994; Usku et al. 2005). We confirmed that the distribution and staining pattern of GR immunoreactivity in the present study were consistent with those of previous studies using the same GR antibody (Morimoto et al. 1996). Many in vitro studies have demonstrated expression of GRs in cultured oligodendrocytes and that GRs are involved in promoting myelination (Vielkind et al. 1990; Chan et al. 1998).

Moreover, a recent in vivo study of the peripheral nervous system demonstrated that Schwann cells expressed GRs and that glucocorticoids enhanced the rate of myelin formation through GRs (Morisaki et al. 2010). In the present study, we investigated GR expression in two different lineages of oligodendrocytes using immunohistochemistry.

Characterization of a number of specific biochemical markers has increased our knowledge regarding the stages of oligodendrocyte maturation, both in vitro and in vivo (Baumann and Pham-Dinh. 2001). We found that GRs were expressed in mature CAII-IR oligodendrocytes in various brain regions of adult mice including both gray and white matter. These findings suggest that signal transduction through GRs in oligodendrocytes plays an important role in myelination of the adult brain in vivo.
Furthermore, we observed that GRs were expressed in NG2-IR oligodendrocyte progenitors in various brain regions of adult mice, including both gray and white matter. Nishiyama and colleagues demonstrated that NG2-IR cells existed uniformly throughout the mature CNS, in both the gray and white matter, and that NG2-IR cells were antigenically oligodendrocyte progenitors that were differentiating into oligodendrocytes in vivo (Nishiyama et al. 1999). Careful examination of the relationship between NG2-IR cells and microglia showed no overlap between the two populations of cells. Thus, NG2 should be considered as one of the most reliable and widely used markers for oligodendrocyte progenitors in the CNS (Nishiyama et al. 1999; Polito et al. 2005; Kucharova et al. 2010). Our results, demonstrating GR expression in oligodendrocyte progenitors in the adult brain, suggest that GRs are involved in the differentiation processes of oligodendrocytes. These findings are consistent with the role of GRs in regulating cell growth and differentiation in the CNS (Chan et al. 1998; Alonso. 2000; Schröter et al. 2009). It has been reported that NG2-IR oligodendrocyte progenitors are also present in MS lesions (Chang et al. 2000). The reasons for the absence of remyelination in MS lesions remain unclear and it remains controversial as to whether remyelinating cells are derived from pre-existing mature
oligodendrocytes and/or from proliferating progenitor cells (Wood et al. 1991; Armstrong et al. 1992). Our findings of GR expression in more than 80% of mature CAIL-IR oligodendrocytes and in more than 85% of NG2-IR oligodendrocyte progenitors demonstrate that GRs could be one of the possible targets for therapies intended to enhance remyelination in MS patients.

In contrast to the pattern of GR distribution in oligodendrocytes in the adult mouse brain, the other corticosteroid receptors, MRs, were not expressed in either CAIL-IR cells or NG2-IR cells. In some regions of the brain, such as the hippocampal and cortex regions, GRs and MRs are co-expressed in the same neurons and exert various functions, including stress responses (Han et al. 2005). However, it remains to be determined whether or not oligodendrocytes co-express GRs and MRs. We did not observe any MR expression in either oligodendrocytes or oligodendrocyte progenitors in the adult mouse brain. These results indicate that MRs may not be involved in the myelination processes and differentiation of oligodendrocytes. In contrast, it should be noted that MRs are expressed in other types of glial cells, astrocytes and microglia after ischemic insult (Hwang et al. 2006; Oyamada et al. 2008).

Next, we confirmed whether the distribution pattern of GRs in oligodendrocytes
was reflective of the hormonal milieu. In the present study, adrenalectomy significantly reduced the plasma CORT level to about 20% of the basal level. Consequently, the intensity of GR expression in CAII-IR oligodendrocytes and NG2-IR oligodendrocyte progenitors was reduced significantly compared with sham-operated mice. Our finding is consistent with those of previous studies, in which there was a marked decrease of GR immunoreactivity in neurons (Ozawa et al. 1999; Usuku et al. 2005) and astrocytes (Gonzalez Deniselle MC et al. 1996), especially in the nuclear region, after adrenalectomy. These results suggest that GR expression is reflective to CORT levels in CAII-IR oligodendrocytes and NG2-IR oligodendrocyte progenitors. In the absence of CORT, GRs cannot translocate into the nucleus. However, the possibility also exists that the down-regulation of GR synthesis is induced by decreases in plasma CORT.

Finally, we investigated the expression of steroid hormone cofactors in mature oligodendrocytes and oligodendrocyte progenitors of the adult mouse brain. We focused on SRC-1 and p300, because the transcriptional activity of GRs is enhanced by the recruitment of one of the transcriptional co-activators of p160 family (SRCs), with docking platforms for secondary co-activators, such as CBP, or its close homologue p300 (Fonte et al. 2007). Previous in vitro studies have shown that SRC-1 is a
co-activator for GR in Schwann cells and astrocytes (Grenier et al. 2004 and 2006), whereas CBP/p300 is a repressor of GR in Schwann cells and in astrocytes (Fonte et al. 2007). There has also been a report of the co-expression of SRC-1 and p300 in the adult rat brain (Ogawa et al. 2001). We found that there was no expression of SRC-1 or p300 in mature CAII-IR oligodendrocytes. In contrast, both cofactors were expressed in NG2-IR oligodendrocyte progenitors, with more intense expression in the white matter regions, such as the corpus callosum and external capsule. These results suggest that mature oligodendrocytes in the adult mouse brain do not use SRC-1 or p300, but use some other transcriptional cofactors. Furthermore, we confirmed that p300 was co-expressed with GRs in NG2-IR oligodendrocyte progenitors, whereas we have not yet successfully accomplished the triple immunostaining of GR, SRC-1 and NG2. Although further study is needed, these results may lead to the speculation that oligodendrocytes and their progenitor cells are regulated through GRs and their cofactors in region specific manners. Future studies are necessary to elucidate what cofactors are required for GR action in CAII-IR mature oligodendrocytes.

In conclusion, the present study demonstrated for the first time that GRs are expressed in different cell lineages of oligodendrocytes, i.e., the CAII-IR cell, a typical
adult-type, mature oligodendrocyte, and the NG2-IR cell, an oligodendrocyte progenitor, in various brain regions of adult mice, including both gray and white matter. Using adrenalectomy in mice, we confirmed that the GRs expressed in these oligodendrocytes and oligodendrocyte progenitors are reactive to CORT. Furthermore, cofactors for GR, SRC-1 and p300, are expressed in NG2-IR oligodendrocyte progenitors, more specifically, at high levels in the white matter regions, but are not expressed in mature CAl2-IR oligodendrocytes.

Acknowledgments

We are grateful to Dr. Katsuhiko Ono for valuable advice. This work was supported by a Grant-in-Aid for Scientific Research (B) from the Japan Society for the Promotion of Science to MN.

Statement of conflicts of interest

The authors report that there are no conflicts of interest.
References


http://mc.manuscriptcentral.com/hcs


26

http://mc.manuscriptcentral.com/hcs


http://mc.manuscriptcentral.com/hcs


Kucharova K, Chang Y, Boor A, Yong VW, Stallicup WB. 2011. Reduced inflammation accompanies diminished myelin damage and repair in the NG2 null mouse spinal cord. J Neuroinflammation 8:158

Kucharova K, Stallcup WB. 2010. The NG2 proteoglycan promotes oligodendrocyte progenitor proliferation and developmental myelination. Neuroscience 166:185-194


http://mc.manuscriptcentral.com/hcs


http://mc.manuscriptcentral.com/hcs


http://mc.manuscriptcentral.com/hcs


http://mc.manuscriptcentral.com/hcs


Figure Legends

Fig. 1: Brain regions

The shaded regions were analyzed in all experiments. Sections at 1.4-1.7 mm posterior to the Bregma according to the mouse brain atlas (Franklin and Paxinos. 2008).

For abbreviations, see Table 1.

Fig. 2: Double immunohistochemical labeling of GR and CAII in the gray matter regions

Brain slices were double immunofluorescently stained for GR (green) and CAII (red). The staining in CA1 (a-c), CA3 (d-f), dentate gyrus (g-i), primary somatosensory cortex barrel field (j-l) and the basolateral amygdala (m-o) are shown. The cells indicated by arrows in the merged images indicate co-localization of CAII and GR (inset: magnified view). Scale bar: 50μm.

Fig. 3: Double immunohistochemical labeling of GR and CAII in the white matter regions
Brain slices were double immunofluorescently stained for GR (green) and CAlI (red). The staining in the corpus callosum (a-c), external capsule (d-f) and fimbria of the hippocampus (g-i) are shown. The cells indicated by arrows in the merged images indicate co-localization of CAlI and GR (inset: magnified view). Scale bar: 50μm.

Fig. 4: Double immunohistochemical labeling of GR and NG2 in the gray matter regions

Brain slices were double immunofluorescently stained for GR (green) and NG2 (red). The staining in CAI (a-c), CA3 (d-f), dentate gyrus (g-i), primary somatosensory cortex barrel field (j-l) and the basolateral amygdala (m-o) are shown. The cells indicated by arrows in the merged images indicate co-localization of GR and NG2.

Scale bar: 50μm.

Fig. 5: Double immunohistochemical labeling of GR and NG2 in the white matter regions

Brain slices were double immunofluorescently stained for GR (green) and NG2 (red). The staining in the corpus callosum (a-c), external capsule (d-f) and the fimbria of...
the hippocampus (g-i) are shown. The cells indicated by arrows in the merged images indicate co-localization of GR and NG2. Scale bar: 25μm.

Fig. 6: Double immunohistochemical labeling of MR and CA II in the gray matter regions

Brain slices were double immunofluorescently stained for MR (green) and CA II (red). The staining in CA1 (a-c), CA3 (d-f) and the dentate gyrus (g-i) are shown.

CAII-IR cells indicated by arrowheads in the merged images do not express MRs. Scale bar: 50μm.

Fig. 7: Double immunohistochemical labeling of MR and NG2 in the gray matter regions

Brain slices were double immunofluorescently stained for MR (green) and NG2 (red). The staining in CA1 (a-c), CA3 (d-f) and the dentate gyrus (g-i) are shown.

NG2-IR cells indicated by arrowheads in the merged images do not express MRs. Scale bar: 50μm.
Fig. 8: Effects of adrenalectomy on GR immunohistochemical labeling in CAII positive cells

Brain slices were double immunofluorescently stained for GR (green) and CAII (red). For comparison, the staining in CA1 (a-c), primary somatosensory cortex (g-i) and the corpus callosum (m-o) after sham operation are shown. The staining in CA1 (d-f), primary somatosensory cortex (j-l), and corpus callosum (p-r) after adrenalectomy are shown. GR immunoreactivity in the nucleus was very weak in the CAII-IR cells (arrows). Scale bar is 50μm.

The relative intensity of GR-immunoreactivity in CAII-IR cells in adrenalectomized mice (n=6) compared with that in sham-operated mice (n=6) was shown (s). The intensity of GR immunoreactivity in CAII-IR cells was notably reduced in adrenalectomized mice compared with that in sham-operated mice. The difference was statistically significant (*p<0.05). Data are expressed as the mean ± S. D.

Fig. 9: Effects of adrenalectomy on GR immunohistochemical labeling in NG2 positive cells

Brain slices were double immunofluorescently stained for GR (green) and NG2
(red). For comparison, the staining in CA1 (a-c), primary somatosensory cortex (g-i) and the corpus callosum (m-o) after sham operation are shown. The staining in CA1 (d-f), primary somatosensory cortex (j-l), and the corpus callosum (p-r) after adrenalectomy are shown. GR immunoreactivity in the nucleus was very weak in NG2-IR cells (arrows). Scale bar: 50μm.

The relative intensity of GR-immunoreactivity in CAII-IR cells in adrenalectomized mice (n=6) against that in sham-operated mice (n=6) was shown (s). The intensity of GR immunoreactivity in NG2-IR cells was remarkably reduced in adrenalectomized mice compared with that in sham-operated mice. The difference was statistically significant (*p<0.05). Data are expressed as the mean ± S.D.

Fig. 10: Double immunohistochemical labeling of SRC-1 and CAII in the gray matter regions

Brain slices were double immunofluorescently stained for SRC-1 (green) and CAII (red). The staining in CA1 (a-c), CA3 (d-f) and the dentate gyrus (g-i) are shown. CAII-IR cells indicated by arrowheads in the merged images do not express SRC-1 in these brain regions. Scale bar: 50μm.
Fig. 11: Double immunohistochemical labeling of p300 and CAII in the gray matter regions

Brain slices were double immunofluorescently stained for p300 (green) and CAII (red). The staining in CA1 (a-c), CA3 (d-f) and dentate gyrus (g-i) are shown. CAII-IR cells indicated by arrowheads in the merged images do not express p300 in these brain regions. Scale bar: 50μm.

Fig. 12: Double immunohistochemical labeling of SRC-1 and NG2 in the gray and white matter regions

Brain slices were double immunofluorescently stained for SRC-1 (green) and NG2 (red). The staining in CA1 (a-c), CA3 (d-f) and the dentate gyrus (g-i) in the gray matter region, and the corpus callosum (j-l) and external capsule (m-o) in the white matter region are shown. The cells indicated by arrows in the merged images indicate co-localization of SRC-1 and NG2. Expression of SRC-1 in NG2-IR cells in the white matter regions is stronger than that in the gray matter regions. Scale bar: 50μm.
Fig. 13: Double immunohistochemical labeling of p300 and NG2 in the gray and white matter regions

Brain slices were double immunofluorescently stained for p300 (green) and NG2 (red). The staining in CA1 (a-c), CA3 (d-f) and the dentate gyrus (g-i) in the gray matter region, and the corpus callosum (j-l) and external capsule (m-o) in the white matter region are shown. The cells indicated by arrows in the merged images indicate co-localization of p300 and NG2. Expression of p300 was stronger in the NG2-IR cells in the white matter region compared with the gray matter region. Scale bar: 50μm.
Figure 1

209x297mm (300 x 300 DPI)

http://mc.manuscriptcentral.com/hcs
Figure 2

![Image of Figure 2](http://mc.manuscriptcentral.com/hcs)

209x297mm (300 x 300 DPI)
Figure 4

209x297mm (300 x 300 DPI)

http://mc.manuscriptcentral.com/hcs
Figure 5

209x297mm (300 x 300 DPI)

http://mc.manuscriptcentral.com/hcs
Figure 6

209x297mm (300 x 300 DPI)

http://mc.manuscriptcentral.com/hcs
Figure 7

209x297mm (300 x 300 DPI)

http://mc.manuscriptcentral.com/hcs
Figure 8

http://mc.manuscriptcentral.com/hcs
Figure 9

209x297mm (300 x 300 DPI)

http://mc.manuscriptcentral.com/hcs
Figure 11

209x297mm (300 x 300 DPI)

http://mc.manuscriptcentral.com/hcs
Figure 12

Figure 12 shows a series of micrographs labeled as SRC-1, NG2, and Merge. The images depict various sections labeled CA1, CA3, DG, and CC, each showing distinct patterns and structures highlighted with arrows. The micrographs are labeled with letters from a to f, indicating different conditions or stages.

209x297mm (300 x 300 DPI)

http://mc.manuscriptcentral.com/hcs
Table 1: GR expression in CAII-IR or NG2-IR cells

<table>
<thead>
<tr>
<th>Brain regions</th>
<th>GR and CAII double IR cells per total CAII-IR cells (%)</th>
<th>GR and NG2 double IR cells per total NG2-IR cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gray matter regions</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippocampus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA1</td>
<td>83 ± 11</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>CA3</td>
<td>81 ± 5</td>
<td>99 ± 1</td>
</tr>
<tr>
<td>Dentate gyrus (DG)</td>
<td>91 ± 3</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>Primary somatosensory cortex barrel field (Cx)</td>
<td>90 ± 2</td>
<td>99 ± 2</td>
</tr>
<tr>
<td>Basolateral amygdala (BLA)</td>
<td>87 ± 4</td>
<td>100 ± 0</td>
</tr>
<tr>
<td><strong>White matter regions</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corpus callosum (cc)</td>
<td>91 ± 1</td>
<td>96 ± 3</td>
</tr>
<tr>
<td>External capsule (ec)</td>
<td>86 ± 2</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>Fimbria hippocampus (fi)</td>
<td>91 ± 2</td>
<td>100 ± 0</td>
</tr>
</tbody>
</table>

CAII-IR or NG2-IR cells in each brain region were counted in 4 mice. Data are expressed as the mean value ± S. D. (n = 4 mice).