The Identification and Characterization of Oligodendrocyte Thromboxane A<sub>2</sub> Receptors*

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Samuel C. Blackman‡, Glyn Dawson§, Kostas Antonakis¶, and Guy C. Le Breton‡‡

From the ‡Department of Pharmacology, University of Illinois at Chicago, Chicago, Illinois 60612, the §Department of Biochemistry, University of Chicago School of Medicine, Chicago, Illinois 60637, and the ¶Institut de Recherches Scientifiques Sur Le Cancer, 94801 Villejuif, France

The presence of functional thromboxane A<sub>2</sub> receptors in neonatal rat oligodendrocytes and human oligodendroglialoma cells was investigated using immunochemistry, ligand affinity chromatography, radioligand binding analysis, immunoblot analysis, and calcium mobilization studies. Immunochemical studies revealed the presence of receptor protein on both oligodendrocytes and human oligodendroglialoma cells. Ligand affinity chromatography allowed for the purification of a protein with an electrophoretic mobility (55 kDa) indistinguishable from human platelet thromboxane A<sub>2</sub> receptors. This affinity purified protein was immunoreactive against a polyclonal anti-thromboxane A<sub>2</sub> receptor antibody. Intact human oligodendroglialoma cells specifically bound <sup>3</sup>H]SQ29,548 with a K<sub>D</sub> of 4 nM and were found to have approximately 3500 binding sites per cell. Human oligodendroglialoma cells also demonstrated calcium mobilization in response to receptor activation with U46619. These results demonstrate the presence of a functional thromboxane A<sub>2</sub> receptor in oligodendrocytes and are consistent with previous observations indicating a high density of thromboxane A<sub>2</sub> receptors in myelinated brain and spinal cord fiber tracts.

Thromboxane A<sub>2</sub> (TXA<sub>2</sub>) is a bioactive arachidonic acid metabolite with thrombotic, vasospastic, and bronchospastic properties (1–3). It has been implicated in a variety of physiological and pathophysiological states (4). The receptor for TXA<sub>2</sub> has been purified (5, 6), cloned, and sequenced, and the structure of the gene indicates that this receptor is a member of the seven transmembrane domain, G-protein-coupled receptor superfamily. The receptor protein has been located in a variety of tissues including blood platelets (7), vascular smooth muscle (8, 9), vascular endothelium (10), placenta (11), and kidney (12) and appears to be directly involved in the regulation of intracellular calcium levels (13). In this regard, previous studies have provided evidence that the TXA<sub>2</sub> receptor protein is coupled to at least one G-protein, i.e. G<sub>a</sub> in platelets (14, 15) and astrocytes (16), and appears to modulate phospholipase C-β activity resulting in activation of the inositol triphosphate second messenger system (17, 18).

Although highly specific agonists and antagonists have proven to be of significant value in characterizing TXA<sub>2</sub> receptors in a number of different cell lines, little information is currently available concerning their existence or function in the central nervous system. To a large extent, this has been due to the presence of high lipid concentrations in central nervous system tissue. Thus, nonspecific binding has obscured the demonstration of receptor binding in solubilized brain, due to the lipophilic nature of TXA<sub>2</sub> receptor ligands. In addition, the heterogeneity of cell types in the central nervous system has complicated attempts at receptor localization at a cellular level.

Based on these limitations, previous studies have employed cultured cell systems in an effort to investigate the presence of TXA<sub>2</sub> receptors in the central nervous system. In these experiments, it was demonstrated that cultured rabbit astrocytes were capable of specifically binding the TXA<sub>2</sub> ligand ONO NT-126 (20). These results, therefore, provided evidence that at least one cellular component of the central nervous system possesses TXA<sub>2</sub> receptor activity. However, since astrocytes are ubiquitously distributed in the brain, it has not been possible to ascribe a specific or unique function for TXA<sub>2</sub> in the various brain structures.

On the other hand, a separate approach has involved the use of TXA<sub>2</sub> receptor antibodies. These experiments provided documentation that TXA<sub>2</sub> receptors are in fact localized in discrete brain regions (21). Specifically, polyclonal antibodies against the complete receptor protein (TxAb), as well as against two decapeptide sequences (P<sub>1</sub>Ab and P<sub>2</sub>Ab) of the receptor, revealed a high density of TXA<sub>2</sub> receptors in myelinated fiber tracts, notably in the striatum, spinal cord, and optic tract (Fig. 1). Given that astrocytes and vascular endothelial cells both possess TXA<sub>2</sub> receptors (10, 20), the preferential labeling of myelinated fiber tracts indicates the existence of a highly concentrated pool of TXA<sub>2</sub> receptors separate from either astrocytes or vascular endothelial cells. One possible source of this high density labeling pattern would be oligodendrocytes, the cell type exclusively responsible for myelin synthesis in the central nervous system.

To determine whether or not oligodendrocytes possess TXA<sub>2</sub> receptors, the present experiments were undertaken using cul-
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Experimental Procedures

Materials

Pregnant female Sprague-Dawley rats were obtained from Sasco (Madison, WI). Human oligodendroglial cells were a generous gift from Dr. Glyn Dawson, Department of Biochemistry, University of Chicago. Poly-l-lysine, bovine pancreatic insulin, human apo-transferrin, cytosine-β-arabinofuranside, deoxyribonuclease I (DNase I), sodium selenite, fura-2/AM, Pluronic F-127, CHAPS, polyclonal anti-glial fibrillary acidic protein (anti-GFAP), and anti-galactocerebroside (anti-GalCer) antibodies were purchased from Sigma. Mouse monoclonal anti-myelin basic protein (anti-MBP) was purchased from Boehringer Mannheim GmbH (Mannheim, Germany). Triton X-100-solubilized BM13.177 (24) was a generous gift from Dr. K. Stegmeier, Boehringer Ingelheim (Briarcliff Manor, NY). All other tissue culture plasticware was from BD Biosciences (San Jose, CA). Poly-L-lysine (30- and 100-micron pore) was purchased from Tetko, Inc. [3H]SQ29,548 was obtained from NEN Life Science Products. SQ29,548 and the color developing reagent was from DuPont (Wilmington, Delaware). Fura-2/AM was purchased from Molecular Probes (Eugene, OR). FITC, Alexa 488, and Alexa 555 conjugates were purchased from Molecular Probes. FITC-stained cells were visualized using a Jenval microscope with appropriate fluorescence optics. Preabsorbtion of P2Ab was accomplished by overnight incubation of the antibody with 0.1 μM P2 peptide (HAALFEWHAV) at 4 °C. Appropriate control experiments were performed by staining control slides in the absence of a primary antibody, secondary antibody or chromagen.

Membrane Solubilization

Solubilized oligodendrocytes (for immunoblot analysis and ligand affinity chromatography) were prepared by aspirating the culture medium and incubating the cells with serum-free DMEM for 15 min at 37 °C. Cells were then sequentially washed with warm serum-free DMEM (2 × 5 ml) with ice-cold 25/5 buffer (25 mM Tris, 5 mM MgCl₂, pH 7.4). Three ml of solubilizing buffer (50 mM Tris-HCl, 5 mM MgCl₂, 2 mM CHAPS) supplemented with protease inhibitors (100 μM EDTA, 1.0 mM leupeptin, 1.0 mM phenylmethylsulfonyl fluoride) were added to each tissue culture dish, and the cells were incubated at 4 °C with constant rocking. Unlysed cells and cellular debris were removed by centrifugation. Protein concentration in pooled solubilization fractions was determined according to the method of Bradford (28). HOG cells were solubilized according to a previously described method (6).

Immunocytochemistry

Oligodendrocytes or HOG cells were plated onto Nunc 4- or 8-well polystyrene chamber slides that had been pretreated with poly-l-lysine (0.1 mg/ml). Cells were thoroughly washed with warm, serum-free DMEM and then fixed using 2% paraformaldehyde in phosphate-buffered saline for 1 h at 4 °C. Unreacted aldehyde groups were blocked with NH₄Cl in Tris-buffered saline (TBS, 30 mM Tris, 120 mM NaCl, pH 7.4), and nonspecific antibody binding sites were blocked with 3% normal goat serum in DMEM. For the detection of intracellular epitopes, cells were permeabilized with 0.5% Triton X-100 for 3 min, followed by treatment with paraformaldehyde, NH₄Cl, and normal goat serum. Cells were then incubated at room temperature with the appropriate dilutions of primary antibody (TxAb, P₂Ab, anti-MBP, or anti-GalCer) or a pre-immune IgG control for 30 min. After washing with TBS, cells were then incubated with the appropriate secondary antibody (1:200). Both primary and secondary antibodies were diluted in 3% normal goat serum in TBS. Fluorescently labeled secondary antibodies were then stained using a metal-enhanced DAB kit (Pierce) and mounted in glycerol. FITC-stained cells were visualized using a Jenval microscope with the appropriate fluorescence optics. Preabsorption of P₂Ab was accomplished by overnight incubation of the antibody with 0.1 μM peptide (HAALFEWHAV) at 4 °C. Appropriate control experiments were performed by staining cells in the absence of a primary antibody, secondary antibody, or chromagen.
Oligodendrocyte Thromboxane A$_2$ Receptors

Solubilized OLG or HOG cell membranes or ligand affinity purified TXA$_2$ receptors were subjected to SDS-PAGE on 10% polyacrylamide gels, under nonreduced conditions, according to the method of Laemmli (29). For immunoblot analysis of TXA$_2$ receptors, the proteins were transferred onto nitrocellulose membranes according to the method of Towbin (30). After transfer, nonspecific antibody binding sites were blocked with 3% gelatin in TBS for 2 h and then incubated with the appropriate primary antibody, diluted in 1% gelatin/TBS, overnight at room temperature. Bound antibody was detected by the Vectastain ABC kit method using a biotinylated goat anti-rabbit IgG (H + L) or goat anti-mouse IgG (H + L), diluted 1:500 in 1% gelatin/TBS, as the secondary antibody. Color bands were developed using 4-chloro-1-naphthol. Silver staining of polyacrylamide gels was performed according to the method of Morrissey (31).

Radioligand Binding Assay

Binding of [³H]SQ29,548 to intact HOG cells was evaluated using a vacuum filtration assay. Confluent layers of HOG cells were incubated for 30 min in warm, serum-free DMEM and then harvested using gentle trypsinization and mechanical dissociation with a stream of warm DMEM delivered through a Pasteur pipette. The monolayer was dissociated into a single cell suspension by gentle trituration. Maintenance of cell integrity, cell counts, and extent of dissociation were monitored by light microscopy and assessment of trypan blue uptake. Cells were diluted to a concentration of 1 × 10$^7$ cells/ml and aliquoted into glass tubes (0.5 ml/tube). Following a 5-min incubation at room temperature with either buffer (total binding) or 20 μM unlabeled SQ29,548 (nonspecific binding), the cells were incubated for 20 min at room temperature with various concentrations of [³H]SQ29,548. At the end of the incubation period, the cells were vacuum filtered through GF/C glass fiber filters, and the filters were rapidly washed (2 × 5 ml) with ice-cold 25/5 buffer. The receptor-bound radioligand was measured by liquid scintillation spectrometry. Specific binding was calculated as the difference between binding measured in the absence and in the presence of 20 μM unlabeled SQ29,548. Scatchard analysis of radioligand binding data was performed using the EBDA and LIGAND software packages (32).

Ligand Affinity Chromatography

TXA$_2$ receptors were purified by the method of Kim et al. (6). Specifically, CHAPS-solubilized OLG or HOG cell membranes in buffer A (20 mM Tris, 10 mM CHAPS, 20% (v/v) glycerol, 550 mM KCl, 0.2 mM EGTA, 0.5 mg/ml asolectin) were incubated overnight at 4 °C with an affinity matrix consisting of the specific TXA$_2$ receptor ligand SQ31,491 immobilized to Affi-Gel 102 (Bio-Rad). Unbound proteins were eluted as flow-through, and the affinity column was washed with 14 ml of buffer B. TXA$_2$ receptors were eluted with buffer containing 50 mM BM13.177 at a flow rate of 1 ml/8 min. Following elution of the first 1-ml fraction, flow was stopped for 30 min and restarted to elute the subsequent 1-ml fractions. Receptor content was assessed by immunoblot analysis utilizing TxAb (1:500) as a probe.

Measurement of Intracellular Ca$^{2+}$ Mobilization

Cytosolic calcium levels were measured in HOG cells using the method of Lum and co-workers (33). HOG cells were grown on poly-L-lysine treated, 25-mm diameter glass coverslips in DMEM (phenol red-free) supplemented with 10% FBS until cells were >75% confluent. Cells were serum-starved for 2 days in DMEM supplemented with 0.2% lactalbumin enzyme hydrolysate and then loaded with 2 μM fura-2/AM for 45 min at room temperature. Pluronic F-127 (0.5% (w/v)) was employed as a dispersing agent for the AM ester. Following a 2 × wash with Hanks' buffered salt solution, coverslips were placed in a Sykes-

oligodendrocytes (OLG) as shown. Cells were labeled with (A) anti-myelin basic protein IgG (anti-MBP, 1:50) or (B) anti-myelin acidic protein IgG (anti-GFAP, 1:50). Immunocytocchemical staining was visualized using metal-enhanced DAB as a substrate. HOG cells were (C) immunofluorescently labeled with anti-GalCer IgG (1:100). Demonstration of myelin basic protein isoforms in solubilized neonatal rat brain oligodendrocytes by immunoblot analysis (D) using anti-MBP (1:500) IgG. Lane 1, MWM, molecular weight marker; lane 2, MBP, purified rabbit myelin basic protein; lane 3, OLG, solubilized neonatal rat brain oligodendrocytes; lane 4, HOG, solubilized human oligodendrogliaoma cells. Results are representative of experiments repeated at least 3 times.

FIG. 2. Immunocytochemical identification of oligodendrocyte-specific antigens on OLG and HOG cells (see “Experimental Procedures” for details). Primary cultures of neonatal rat brain
Moore chamber (Bellco, Vineland, NJ). The chamber was placed onto the stage of an inverted Nikon Diaphot microscope that was equipped with quartz optics and coupled to a Deltascan microspectrofluorometer system (PTI, Inc., Princeton, NJ). An optically isolated group of 3–4 cells was illuminated by a 75-watt xenon arc lamp at alternating excitation wavelengths of 340 and 380 nm. The emitted light was passed through an interference filter at 510 nm and collected via a photomultiplier. Fluorescence intensity was measured at 10 points/s. Background autofluorescence (in the absence of fura-2) was determined at the beginning of each experiment and was subtracted automatically during data collection. At the end of each experiment, 10 μM ionomycin was added to obtain fluorescence of Ca<sup>2+</sup>-saturated fura-2, and 0.1 M EGTA was added to obtain fluorescence of free fura-2. The fluorescence ratio of excitation wavelengths 340 and 380 nm (R<sub>340/380</sub>) were computed using the PTI software. Statistical analysis was performed using the Student's t test.

RESULTS

Morphologically, cultured oligodendrocytes demonstrated a complex network of cytoplasmic processes, as well as numerous intra-process connections characteristic of this cell type (Fig. 2, A and B). Identification of cells as differentiated oligodendrocytes was accomplished by immunohistochemical labeling of subcultured cells with anti-myelin basic protein (anti-MBP), an OLG specific marker (Fig. 2A). Solubilized oligodendrocytes from primary culture were also found to immunoreact with anti-MBP, producing an immunoreactive protein band at approximately 21.5 kDa (Fig. 2D). The degree of purity of OLG cultures was further established by the absence of labeling with anti-glial fibrillary acidic protein (anti-GFAP), an astro-
cyte-specific protein (Fig. 2B). Human oligodendroglialoma (HOG) cells appeared flat and epithelioid in culture, demonstrating short, narrow cytoplasmic processes. These cells, which have been shown to possess some OLG-specific proteins, were immunoreactive against an anti-GalCer antibody (Fig. 2C).

To determine whether cultured OLG and/or HOG cells contained TXA2 receptors, immunohistochemical studies were undertaken using antibodies against the purified receptor (TxAb).

Both cell types revealed a marked staining of TXA2 receptors in fixed, Triton X-100-permeabilized cells (Fig. 3, A and D). Unpermeabilized HOG cells demonstrated labeling that was restricted to the plasma membrane (Fig. 3C). On the other hand, no staining was observed when using pre-immune IgG in either cell type (Fig. 3, B and E).

Additional evidence for the existence of TXA2 receptors in HOG cells was obtained using a different polyclonal antibody (P2Ab), raised against a decapeptide sequence from the first extracellular loop of the receptor protein (residues 89–98). As can be seen in Fig. 4, A and B, incubation of HOG cells with P2Ab resulted in a positive staining pattern that was abolished by preabsorption of the antibody with the peptide antigen (P2). Collectively, these results indicate that both OLG and HOG cells possess TXA2 receptors.

Expression of TXA2 receptors was further established using SDS-PAGE and immunoblot analysis. Specifically, HOG and OLG cell membrane proteins were extracted by treating washed cells with CHAPS at 4 °C. Unlysed cells were removed by centrifugation, and soluble proteins were resolved by electrophoresis and blotted onto nitrocellulose. A human platelet solubilized membrane preparation (SMP) was immunoblotted in parallel as a positive control. It was found (Fig. 5, lanes 2–4) that TxAb (diluted 1:500) not only immunoreacted with TXA2 receptor protein in platelet SMP but also revealed positive immunoreactivity against the 55-kDa receptor protein in solubilized OLG cells and HOG cell membranes. This immunoreactivity was not observed when probing either cell type with the pre-immune IgG (Fig. 5, lanes 6–9). To confirm that the positive immunoreactivity seen in solubilized OLG and HOG cells was due to expression of the receptor in the immature or transformed phenotypes, respectively, immunoblot analysis was performed on a Triton X-100 soluble membrane fraction prepared from adult ovine oligodendrocytes (Fig. 5, lane 5). This experiment demonstrated a single immunoreactive band present at 55 kDa.

To purify the TXA2 receptor present on oligodendrocytes, affinity chromatography was utilized. In this experiment, solubilized OLG proteins were incubated with an SQ31,491 ligand affinity column previously shown to purify TXA2 receptors from platelets (6). The column was then eluted with the receptor antagonist (BM13.177), and SDS-PAGE analysis of the wash and the elution fractions was performed. It can be seen (Fig. 6A) that while the final wash fraction (W4, lane 4) was essentially devoid of protein, competition with BM13.177 resulted in the elution of a major protein band at 55 kDa (lanes 5 and 6). Evidence that this 55-kDa protein represented the TXA2 receptor was obtained from immunoblot analysis which revealed
positive reactivity with TxAb (Fig. 6B). Taken together, these results represent the first purification of TXA₂ receptors from oligodendrocytes and provide evidence that this receptor protein has a molecular mass in the range of 55 kDa.

Because oligodendrocytes synthesize large quantities of lipids and proteolipids, which result in increased nonspecific binding of lipophilic TXA₂ receptor ligands, the next series of experiments was undertaken to evaluate the capability of intact HOG cells to specifically bind the TXA₂ antagonist [³H]SQ29,548. Using a modification of the protocol employed in detecting TXA₂ binding sites in astrocytoma cells (19), these experiments revealed a single class of high affinity binding sites, with a dissociation constant \( (K_d) \) of 4 ± 0.2 nM and a maximum binding of 34 ± 4 pm (Fig. 7). The calculated maximum binding \( (B_{\text{max}}) \) of 34 pm corresponds to approximately 3500 binding sites per HOG cell. This \( K_d \) of 4 nM is comparable to that obtained for the TXA₂ receptor in human platelets \( (K_d = 7.3 \text{ nM}) \) (34) and 1321N1 human astrocytoma cells \( (K_d = 10.9 \text{ nM}) \) (19).

The final series of experiments investigated the ability of a TXA₂ receptor antagonist to modulate intracellular calcium levels in HOG cells. Cells grown on glass coverslips were loaded with fura-2/AM and exposed to various concentrations of U46619 in the presence or absence of the receptor antagonist, SQ29,548. It was found that concentrations of U46619 as low as 300 nM were capable of eliciting intracellular Ca²⁺ mobilization as visualized by an increase in the fluorescence ratio of excitation wavelengths 340 and 380 nm (R340/380). The time course of the U46619-induced calcium mobilization revealed a biphasic response (Fig. 8A). A challenge with 300 nM U46619 caused an initial rapid increase in calcium, which peaked by 19 ± 4 s. The peak rise in Ca²⁺ \( (224 ± 15\% \text{ over base line}, n = 3, p = 0.0002) \) was followed by a second phase of slow decrease to half-maximal \( (t_{1/2}) \) within 30 s and a prolonged plateau phase at a higher R340/380. Pretreatment of HOG cells with 100 nM SQ29,548 for 3 min prior to agonist stimulation completely ablated the U46619-mediated response (Fig. 8B).

DISCUSSION
Recent evidence suggests the existence of thromboxane A₂ receptors in cells of the central nervous system. In this connection, radioligand binding assays have revealed the presence of TXA₂ binding sites in cultured rabbit astrocytes (35), and electrophysiological experiments have demonstrated a TXA₂ response in CA1 hippocampal neurons (36). In addition, association of TXA₂ receptor protein with myelinated tracts in adult rat brain and spinal cord slices has been provided using immunohistochemical techniques (21). Based on this latter finding, the present series of experiments were undertaken to investigate the presence of the thromboxane A₂ receptor in oligodendrocytes, the cell type responsible for central nervous system myelination.

In the initial studies, cultured OLG and HOG cells were immunocytochemically probed with antibodies directed against the purified TXA₂ receptor (TxAb) and against a decapetide segment from a putative extracellular receptor loop (P₁₂Ab). These experiments provided evidence for specific receptor labeling on the plasma membranes of both cell types and receptor labeling of non-nuclear intracellular sites in permeabilized HOG cells. The molecular size of the labeled protein was next established by SDS-PAGE of CHAPS-solubilized OLG and HOG cells. The observed electrophoretic mobility of this protein (55 kDa) was indistinguishable from that of the TXA₂ receptor purified from platelets.

The functional ability of this 55-kDa protein to bind TXA₂ receptor ligand was investigated using affinity chromatography. In these studies, solubilized oligodendrocytes were incubated with a ligand affinity matrix. Elution of the column matrix resulted in the selective purification of receptor protein having an apparent molecular mass of 55 kDa.

Separate experiments evaluated the ability of intact HOG cells to bind a TXA₂ receptor antagonist ([³H]SQ29,548). The
results demonstrated that HOG cells exhibit approximately 3500 binding sites per cell with a $K_D$ of approximately 4 nM. These values are comparable to those observed in human platelets (i.e. $B_{max} = 2000$ and $K_D = 5$ nM).

Finally, experiments were conducted to investigate the functional significance of TXA$_2$ receptor activation in these cells. Since the TXA$_2$ receptor is known to be linked to increases in platelet (37) and astrocyte (55) cytosolic calcium, it was determined whether receptor activation leads to a similar effect in HOG cells. Utilizing fura-2 as an indicator of changes in intracellular calcium concentration, HOG cells were shown to respond to the TXA$_2$ mimetic U46619, and this effect could be blocked by pretreatment of cells with the TXA$_2$ receptor antagonist SQ29,548.

Collectively, these findings demonstrate that oligodendrocytes possess functional TXA$_2$ receptors. However, in order for TXA$_2$ to play a role in the modulation of OLG cell function or development, a source for this eicosanoid must be made available under physiological and/or pathological conditions. In this respect, oligodendrocytes may have access to TXA$_2$ in at least three possible manners. First, although the identification of an exact in vivo source remains controversial, TXA$_2$ has been shown to be generated by astrocytes (38), microglia (39), and vascular endothelial cells (10) in culture. With regard to astrocytes, a ubiquitously distributed glial cell, activation of phospholipase $A_2$ and production of TXA$_2$ by thromboxane synthase occurs in response to stimulation of cells by mediators such as platelet-activating factor (40) and ATP (41).

A second possible source of TXA$_2$ may be as a consequence of central nervous system injury. In this case, compromise of the blood-brain barrier, either by mechanical trauma or by cerebrovascular insult, could potentially result in exposure of oligodendrocytes and myelin-ensheathed neurons to TXA$_2$ which was produced by activated platelets or other blood system components (42). In addition, a dramatic increase in the generation of arachidonic acid metabolites and reactive lipid mediators, with subsequent breakdown of the blood-brain barrier, has been shown to occur following post-hypoxic or post-anoxic injury and reperfusion (43). In this regard, oligodendrocyte damage has been shown to occur following ischemic neuronal damage and the breakdown of the blood-brain barrier, and an apoptosis-like process has been implicated in ischemic oligodendrocytic death (44).

Finally, inflammatory processes within the brain parenchyma, which trigger the activation of microglia, can lead to the production of TXA$_2$ (39). In addition, the cytokine interleukin-1$\beta$ has been shown to elicit TXA$_2$ release from astrocytes (45). Thus, a variety of physiological and pathophysiological conditions could lead to the exposure of oligodendrocytes to TXA$_2$.

With regard to a possible role for TXA$_2$ in this cell type, it should be noted that oligodendrocytes are a highly specialized and terminally differentiated cell type, solely responsible for central nervous system myelination. Oligodendrocytes originate from a bipotential precursor cell (O-2A) (46) and undergo a complex differentiation process that directs the cells to synthesize large quantities of the protein, glycoprotein, and proteolipid components of myelin sheaths (47). At present, relatively little information is available concerning the endogenous or exogenous regulation of myelin protein synthesis and maintenance. However, recent evidence suggests that changes in intracellular calcium may modulate myelin basic protein levels.
calcium mobilization and diacylglycerol generation. Furthermore, activation of protein kinase C by diacylglycerol in oligodendrocytes has been shown to lead to phosphorylation of myelin basic protein (50, 51). Since it has been postulated that phosphorylation of MBP may act as a myelinoergic signal, TXA₂ receptor activation could play a role in elaboration and/or maintenance of myelin membranes.

In summary, the present results provide the first demonstration of functional thromboxane A₂ receptors on neonatal rat oligodendrocytes and human oligodendroglialoma cells. These findings, coupled with the high density expression of the receptor in myelinated tracts in situ, raise the possibility that thromboxane may play an important role in the regulation of oligodendrocyte function. Clearly, additional experiments are required to explore this interesting possibility.

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