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GLIAL CELLS EXPRESS BOTH MINERALOCORTICOID AND GLUCOCORTICOID RECEPTORS

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Summary—In the brain, the action of glucocorticoid steroids is mediated via two intracellular receptors, the mineralocorticoid (MR), or type I receptor, and the glucocorticoid (GR), or type II receptor. These receptors are expressed in many types of neurons and are co-expressed in some neurons such as the hippocampal pyramidal cells. Although glucocorticoids are known to affect gliogenesis and glial cell differentiation, the expression of the GR in different types of glial cells throughout the brain has not been thoroughly studied and the expression of the MR in glia not previously reported. Here we review studies suggesting that both receptors are expressed in astrocytes and oligodendrocytes.

INTRODUCTION

Administration of glucocorticoids to neonatal rodents and birds produces an array of effects on the development of brain morphology, neurochemistry and physiology [1–4]. In some cases, only a brief, transient administration of glucocorticoids during the neonatal period is enough to produce permanent changes in animal behavior [5–8]. While certain neurons in the developing brain are clearly targets for glucocorticoid action, there is abundant evidence suggesting that glucocorticoids also affect gliogenesis and glial cell differentiation.

Glucocorticoids, when given during the period of rapid gliogenesis, inhibit brain growth, the acquisition of DNA, and myelination [9–17]. Conversely, adrenalectomy (ADX) results in increased brain weight and increased myelination [18, 19]. Studies *in vivo*, as well as *in vitro*, have shown that the proliferation of glia is sensitive to the levels of glucocorticoids. Studies using tritiated thymidine have shown that treatment of newborn rats with hydrocortisone markedly depresses the labeling index in the subventricular zone of the lateral ventricle, a glial germinal zone [20]. In addition, the genesis of oligodendrocyes in the optic nerve of the rat is inhibited by glucocorticoid administration [15]. Interestingly, the inhibition of glial proliferation in both the subventricular zone and the optic nerve is reversible following cessation of the glucocorticoid treatment. Inhibition of cell proliferation by glucocorticoids has also been reported in monolayer cultures of rat and human glioma [21, 22].

Glucocorticoids also affect glial maturation. Glutamine synthesase in astrocytes and Müller cells (specialized glia in the neural retina) and glycerol phosphate dehydrogenase in oligodendrocyes are two enzymes known to be induced by glucocorticoids at the transcriptional level [23–28]. Other glial markers such as myelin basic protein, proteolipid protein and 2′,3′-cyclic nucleotide phosphodiesterase are increased by glucocorticoids, but in these cases post-transcriptional mechanisms appear to be involved [24]. Glial fibrillary acidic protein, an astrocyte-specific protein, is down-regulated by glucocorticoids [29]. Glucocorticoids have also been reported to stimulate astrocytic production of angiotensinogen [30], to inhibit astrocyte expression of insulin-like growth factor I mRNA [31] decrease the number of insulin receptors in C6 glioma [32], inhibit interleukin-1-dependent cytokine induction in astrocytoma [33], inhibit glucose transport in hippocampal glia [34] and inhibit acetylcholine-stimulated production of arachidonate in C62B glioma [35]. Interestingly, it has also been suggested that glucocorticoids may play a role in determining whether bipotential glial precursor cells become oligodendrocytes or astrocytes [36].

The action of steroids in all tissues is mediated via intracellular receptors that act

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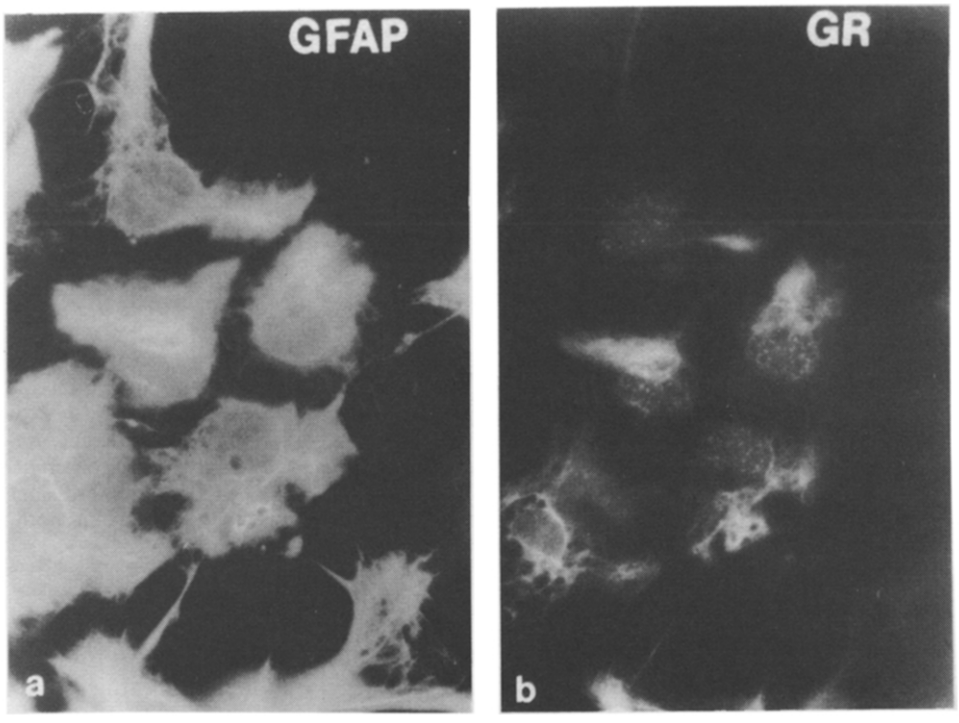

Fig. 1. GR expression in type 1 astrocytes in a mixed population of rat cerebellar glial cells treated with 10^{-6} M dexamethasone 30 min prior to fixation. (a) Immunofluorescence for GFAP; (b) immunofluorescence for GR showing nuclear localization in same cells as shown in (a). Reproduced by permission of Wiley-Liss Inc.

in the nucleus to regulate gene transcription. In the brain, glucocorticoids are the endogenous ligands for two receptors, the MR which has a high affinity for corticosterone, and the GR which has a four-fold lower affinity for corticosterone and prefers the synthetic glucocorticoid, dexamethasone [37]. The GR is more widely distributed throughout the brain than the MR [38, 39]. Both receptors are expressed in many types of neurons, however, the expression and distribution of these receptors in glial cells has not been thoroughly studied. Macromolecules with physicochemical characteristics similar to those of the GR in pituitary and liver have

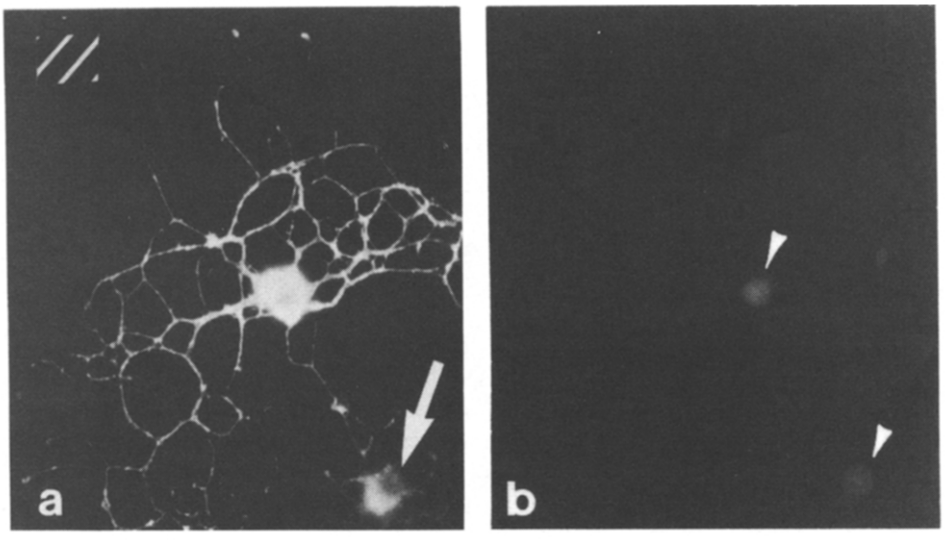

Fig. 2. GR expression in mature and immature (arrow) oligodendrocytes prepared from rat cerebral cortex. (a) Immunofluorescence for CNP; (b) immunofluorescence for GR showing nuclear localization (arrowheads) in same cells as shown in (a). Reproduced by permission of Wiley-Liss Inc.

been identified in optic nerve suggesting that glia express the GR [40, 41]. In addition, high-affinity binding sites for dexamethasone with characteristics of the type II GR have been identified in cultures of Schwann cells, astrocytes, oligodendrocytes and C6 glioma [42–44]. Immunocytochemical studies also support the expression of GR in most, if not all, glial cells [44, 45].

IMMUNOCYTOCHEMICAL STUDIES OF GR IN GLIA

Immunocytochemistry using monoclonal antibodies raised against the GR purified from rat liver suggest that all classes of glial cells, as well as bipotential glial precursor cells, C6 glioma and Schwannoma express GRs [44, 45]. Studies mapping GR by immunocytochemistry or uptake of tritated dexamethasone have suggested that small, non-neuronal cells in white matter presumed to be oligodendrocytes express the GR [39, 46]. Similarly, *in situ* hybridization studies have demonstrated low levels of mRNA for GR over small cells in white matter [47]. In our studies, we have studied the expression of GRs in glial cultures prepared from rat cerebral cortex or cerebellum where it was convenient to identify co-expression of GR immunoreactivity (IR) and glial cell markers in single cells. These studies showed that *in vitro* GRs are expressed in astrocytes stained for glial fibrillary acidic protein (GFAP; Fig. 1) and in oligodendrocytes stained for 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNP; Fig. 2; [45]). In addition, immature or intermediate cell types are immunoreactive for GR [45]. In glial cells grown in the absence of glucocorticoids, the cytoplasm is stained while nuclei are devoid of GR IR. Addition of glucocorticoids to the medium results in heavy nuclear staining (Fig. 3), suggesting that, as in neurons [39, 48, 49], the GR in glial cells requires ligand for nuclear translocation.

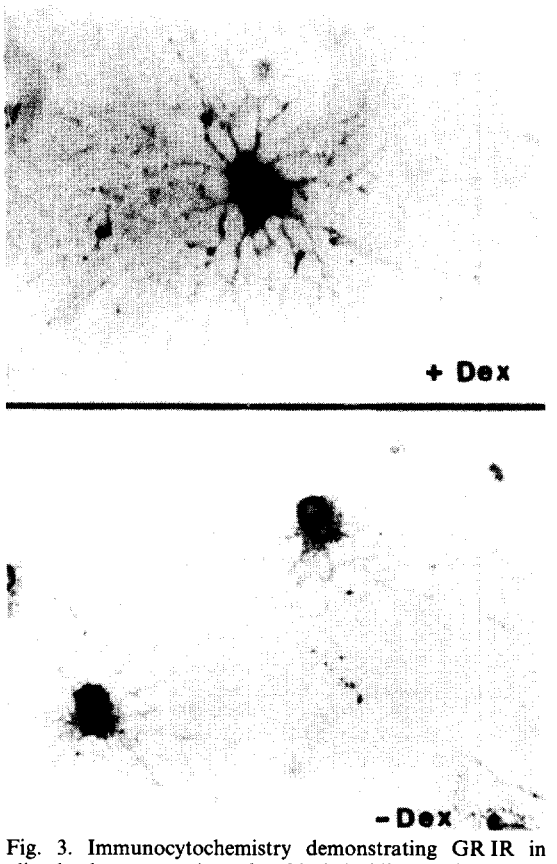

Fig. 3. Immunocytochemistry demonstrating GR IR in oligodendrocytes using the biotin/avidin method with diaminobenzidine as chromagen showing nuclear and cytoplasmic localization of the GR in the presence of dexamethasone (10^{-6} M) and the lack of nuclear staining in the absence of dexamethasone.

IMMUNOCYTOCHEMICAL STUDIES OF MR IN GLIA

Since an antibody has not yet been raised against MR purified from tissue, the localization of the MR in the brain using immunocytochemistry has not been described. However, expression of the MR in the brain has been studied using *in situ* hybridization to localize mRNA coding for MR and *in vitro* binding using radiolabeled steroids [37, 38, 50, 51]. These studies suggest that GR and MR are differentially expressed in the brain. However, both receptors are highly expressed in the limbic system and are co-expressed in pyramidal and granule neurons in the hippocampus [51]. Recently, an antibody has been raised against a 16 amino acid synthetic peptide from the hinge region between the DNA and steroid binding domains of MR [52]. Using this antibody, we have studied the expression of MR IR in mixed glial–neuronal cultures of embryonic day 18 hippocampus. The majority of neurons in these cultures are heavily stained for MR IR (unpublished data). Many, but not all, astrocytes in these cultures are also stained for MR IR (Fig. 4). In the presence of corticosterone (10^{-6} M), the nuclei are heavily stained, however, some staining is still observed over the cytoplasm suggesting that not all MRs in astrocytes translocate to the nucleus in the presence of ligand. Although oligodendrocytes are rare in

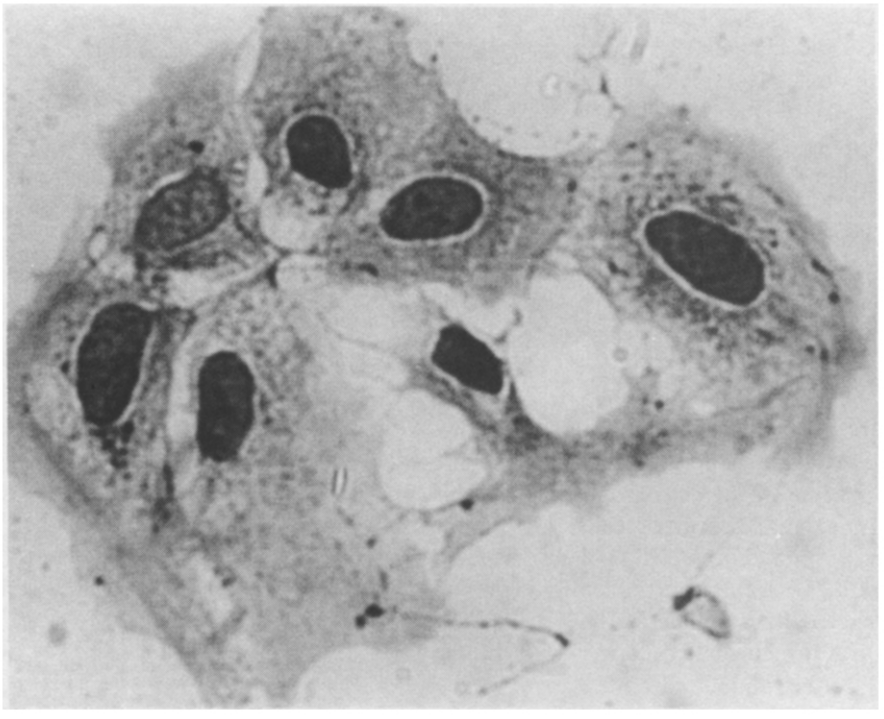

Fig. 4. Immunocytochemistry using the biotin/avidin method with diaminobenzidine as chromagen demonstrating MR IR in type 1 astrocytes in hippocampal dissociates prepared from embryonic day 18 rat, grown for 5 days and treated with corticosterone (10^{-6} M) 30 min prior to fixation. Note heavy nuclear staining and lighter cytoplasmic staining.

hippocampal cultures, the few oligodendrocytes we have observed are also stained for MR IR (Fig. 5).

DISCUSSION

Glial cells are a major target for glucocorticoid action in the nervous system. Not only do all major classes of glia express the GR, but some astrocytes and oligodendrocytes also express the MR. Furthermore, immature, undifferentiated glial cells express the GR suggesting that glucocorticoids may act through these receptors early in brain development to affect gliogenesis, and glial differentiation and

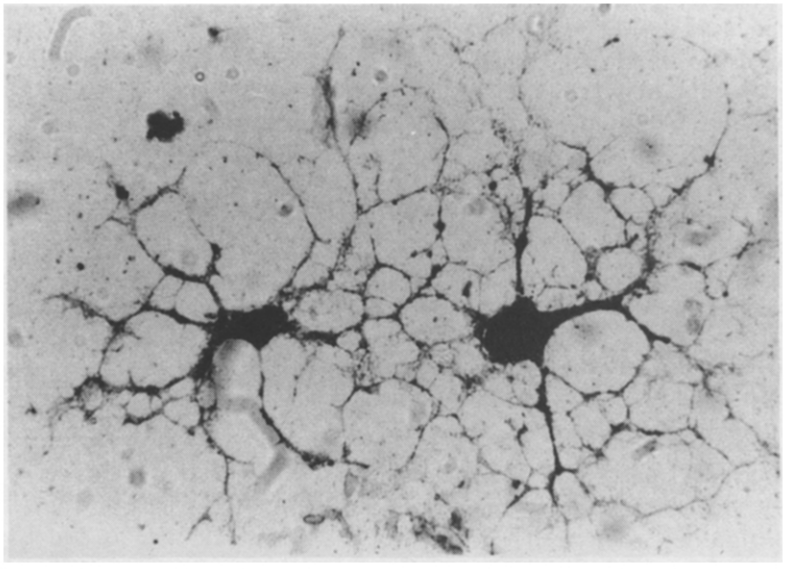

Fig. 5. Immunocytochemistry using the biotin/avidin method with diaminobenzidine as chromagen and nickel enhancement demonstrating MR IR in oligodendrocytes in hippocampal cultures as described in Fig. 4.

perhaps also glial lineage. Although our studies demonstrating the expression of the GR in glial cells have been performed *in vitro*, these receptors are likely to be also expressed *in vivo* since both binding and *in situ* hybridization studies suggest this to be the case [39–42]. In the case of MR expression, it is not clear whether the antigen recognized in the immunocytochemical studies is the same as the MR expressed in the kidney and neurons since binding data for a receptor in glia that has a higher affinity for corticosterone than dexamethasone has not yet been reported. Since binding studies were performed with cerebral astrocytes and the immunocytochemical studies with hippocampal astrocytes, there may be a regional difference in expression of MR or the glial MR IR protein may have different characteristics than the neuronal MR. This issue remains to be explored in future studies.

A number of glial proteins are known to be affected by glucocorticoids, including glutamine synthetase [23, 25–28], glycerol phosphate dehydrogenease [23], glial fibrillary acidic protein [29], angiotensinogen [30], myelin basic protein [24], proteolipid protein [24], and insulin-like growth factor [31]. Glucocorticoids have also been shown to inhibit glucose transport and utilization [34], to stimulate sulfated lipid formation in glia [36], and to affect the pattern of proteins secreted from astrocytes and C6 glioma [53, 54]. These latter observations suggest that glucocorticoids may affect the neuronal milieu indirectly by affecting astrocytes. In some cases, the effects on glial proteins have been shown to be mediated by the GR, but not the MR, and to occur either directly at the level of mRNA or indirectly through a mechanism requiring protein synthesis. Since some glia apparently express both receptors, MR and GR may mediate glucocorticoid effects on different genes or act antagonistically as reported for effects of glucocorticoids on excitability of hippocampal CA1 neurons [55]. Alternatively, as have been suggested for neurons [38], the expression of two receptors in individual glial cells may be necessary to accommodate the large fluctuations in glucocorticoids that occur between resting and stress states. Furthermore, the function of MR in glia is at present completely unknown. It is also not known why the MR appears to be expressed heterogeneously in astrocytes. All of these intriguing issues promise to lead to interesting studies in the future.

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