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The melanocortin ACTH 1-39 promotes protection of oligodendrocytes by astroglia☆

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ABSTRACT

Damage to myelin and oligodendroglia (OL) in multiple sclerosis (MS) results from a wide array of mechanisms including excitotoxicity, neuroinflammation and oxidative stress. We previously showed that ACTH 1-39, a melanocortin, protects OL in mixed glial cultures and enriched OL cultures, inhibiting OL death induced by staurosporine, ionotropic glutamate receptors, quinolinic acid or reactive oxygen species (ROS), but not nitric oxide (NO) or kynurenic acid. OL express melanocortin receptor 4 (MC4R), suggesting a direct protective effect of ACTH 1-39 on OL. However, these results do not rule out the possibility that astroglia (AS) or microglia (MG) also play roles in protection. To investigate this possibility, we prepared conditioned medium (CM) from AS and MG treated with ACTH, then assessed the protective effects of the CM on OL. CM from AS treated with ACTH protected OL from glutamate, NMDA, AMPA, quinolinic acid and ROS but not from kainate, staurosporine, NO or kynurenic acid. CM from MG treated with ACTH did not protect from any of these molecules, nor did CM from AS or MG not treated with ACTH. While protection of OL by ACTH from several toxic molecules involves direct effects on OL, ACTH can also stimulate AS to produce mediators that protect against some molecules but not others. Thus the cellular mechanisms underlying the protective effects of ACTH for OL are complex, varying with the toxic molecules.

1. Introduction

Damage to myelin, oligodendroglia (OL) and neurons in multiple sclerosis (MS) occurs by multiple mechanisms ([19], review). We have established an in vitro approach [3,4] to investigate the efficacy of potential therapeutic agents in preventing death of OL, OL progenitors (OPC) and neurons from cytotoxic agents involved in several of these mechanisms, including excitotoxicity, inflammation-related signals, apoptotic signals and reactive oxygen species (ROS) [5,6,30,31]. One of these potentially protective agents, ACTH 1-39, has been used to treat MS relapses, and was thought to act primarily on melanocortin receptors in the adrenals to stimulate release of corticosteroids. However, neurons, astrocytes (AS), microglia (MG) and oligodendroglia (OL) all express melanocortin receptors [1,6,10,12,42], raising the possibility that melanocortins can act directly on these cells.

We found that ACTH 1-39, a melanocortin, protected OL in mixed glial cultures, inhibiting OL death induced by staurosporine, glutamatergic ionotropic receptors, quinolinic acid (QA) or ROS, but not nitric oxide (NO) or kynurenic acid [6]. We also showed that ACTH 1-39 protected OL in cultures enriched (85–90%) for OL. The OL express melanocortin receptor 4 (MC4R), suggesting a direct effect. While these results support a direct effect of ACTH on OL, they do not rule out the possibility that AS or MG could also play roles in ACTH-mediated protection. To investigate this, we prepared conditioned medium (CM) from AS and MG treated with ACTH 1-39, then assessed the protective effects of the CM on OL treated with the panel of cytotoxic agents.

2. Materials and methods

2.1. Reagents

The ionotropic glutamate receptor ligand α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) was purchased from Tocris (Ellisville MO); the NO donors NOC-12 and NOC-18 were from Calbiochem (LaJolla CA). The other reagents, including adrenocorticotropic hormone 1–39 (ACTH 1-39) and *N*-methyl-D-aspartic acid (NMDA), were from Sigma (St. Louis MO). Cell types were characterized using the following antibodies to phenotypic markers: platelet derived growth factor receptor alpha (PGDFRα) for OPC [36] from Santa Cruz Biotechnology, Dallas TX; galactocerebroside for OL, monoclonal antibody generated from O1 hybridoma cells [43]; anti-glial fibrillary acidic protein (GFAP) (Chemicon International Inc., Temecula CA, M#B360) for AS [37] and the lectin IB4 (Sigma, L9381) for MG [34].

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2.2. Preparation of cultures enriched in oligodendroglia

Cultures were prepared from brains of 2–3 day old rats as previously described [18,29]. For each of the experiments, three forebrains were carried through the dissociation procedure, suspended in 16 ml of Dulbecco's minimal essential medium (DMEM) containing 10% newborn calf serum (NCS) and plated in two T75 flasks. For cultures highly enriched in mature OL, the flasks containing the glial bed layers were subjected to shakeoff every 5–7 days. Cells from the two flasks after the 4th and 5th shakeoffs were plated on a 100 mm uncoated plastic dish for 1 h, and the unattached cells replated on plastic for an additional hour to remove MG. The unattached cells were pelleted, resuspended in 2.5 ml of OL defined medium [6], then 50 μl aliquots were plated on 50 poly-D-lysine coated coverslips and allowed to attach overnight. OL defined medium with 1% newborn calf serum (NCS) was added for the next 24 h, then OL defined medium with 2% NCS [18]. After 6–8 days in OL defined medium with 2% NCS, the enriched OL cultures were treated with ACTH 1-39, the cytotoxic reagents and/or conditioned medium. Untreated cultures with defined medium with 2% NCS served as control. Some of the enriched OL cultures were assessed for composition as described previously [6], using immunocytochemistry with antibodies to cell type specific markers, including galactolipids for OL, glial fibrillary protein for AS, and A2B5 for OPC. Binding of the lectin IB4 was used to detect MG. As found previously in our laboratory, the cultures contained on average 88% differentiated OL, 3% AS, 8% MG and less than 1% OPC.

2.3. Preparation of astroglial cultures

OL and MG were removed from mixed glial cultures [29] by shake-off from one T75 flask (cells from 1½ forebrains, as above), leaving behind the bed layer of highly enriched AS. The AS cultures were treated with 0.05% trypsin, pelleted, washed with DMEM containing 10% NCS, then plated on poly-lysine coated flasks. The AS cultures were maintained in 10% NCS in DMEM, then used after 1 week to produce AS conditioned medium (CM).

2.4. Preparation of microglial cultures

Cells obtained from shake-off of two T75 flasks of mixed glial cultures (16 ml of 10% NCS in DMEM, cells from 3 rat forebrains, as above) were plated on uncoated plastic dishes for 2 h and non-adherent cells removed. The adherent cells were maintained in 10% NCS in DMEM for one day, then used to prepare CM. The cells were 87% MG based on binding of the lectin IB4.

2.5. Preparation of conditioned medium

Purified AS or MG were incubated for 24 h with either DMEM or with DMEM containing 200 nM ACTH 1-39. Supernatants were removed, and cultures were washed with DMEM to remove ACTH in the medium; DMEM was added for an additional 24 h, then collected to give conditioned medium (CM) from AS or MG pretreated with ACTH, designated as AS CM ACTH or MG CM ACTH, respectively. Controls consisted of medium from AS and MG treated as above except not exposed to ACTH, AS CM and MG CM, respectively. Supernatants were stored at −20 °C until used.

2.6. Cell treatment and measurement of cell death

For analysis of the effects of ACTH 1-39 or CM on OL death, purified OL cultures were incubated with ACTH 1-39 at 200 nM or the various CM for 30 min before addition of the toxic agents. Cell death was assessed after 1 day using trypan blue uptake as the indicator of cell death as described in our previous studies [3,4]. Trypan blue is considered a preferred method for measurement of total cell death [35] compared to terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), which measures only apoptosis, or live/dead fluorescent assays, which may not detect permeable dead cells with degraded DNA, thus underestimating cell death [46]. Differentiated OL were identified by their characteristic morphology, that is, rounded or oval birefringent cells with multiple lacy branching processes, and in some cases by immunostaining with antibodies to galactolipids.

The % of trypan blue + OL was determined by counting between 100 and 200 OL in 4–6 random fields on two coverslips per experiment with 3 or more independent experiments for each condition. Values for duplicate coverslips agreed within 10% of one another.

The concentration of ACTH was selected based on the concentration giving optimal protection against the toxic agents [6]. Concentrations of the cytotoxic agents were selected to give 60–75% OL death after 1 day, with no death of AS or MG ([6]; see legends in graphs). All experiments were repeated three times with duplicate coverslips in each experiment. Results were analyzed by one-way ANOVA with Tukey post-test ($p < 0.05$).

3. Results

3.1. Astrocyte conditioned medium and microglia conditioned medium do not affect oligodendroglial viability at 24 h

We first prepared CM from untreated AS cultures and from AS cultures treated with 200 nM ACTH 1-39 for 24 h, washed to remove ACTH 1-39, then incubated for another 24 h in DMEM as detailed in Methods. In initial experiments, we found no difference in OL viability in the presence of OL defined medium with 2% NCS or AS CM (prepared in DMEM with no serum). After 24 h, OL death under each condition varied between 1 and 4% (data not shown). Similar results for OL viability were obtained with MG CM. In subsequent experiments, controls in each experiment consisted of OL in defined medium with 2% NCS.

3.2. Astrocytes treated with ACTH secrete factors that protect OL from cytotoxic insults

We previously showed that ACTH can protect OL from a variety of cytotoxic insults [6]. To investigate whether ACTH stimulated AS to produce factors protective to OL, we compared AS CM ACTH to ACTH for protection against excitotoxic, inflammation-related, oxidative and nitrosylation mediated OL death.

With regard to excitoxicity, glutamate can act via ionotropic glutamate receptors on OL to induce cell death. As shown in Fig. 1, in agreement with our previous results [6], ACTH 1-39 at 200 nM protected OL from killing by 100 μM glutamate, reducing OL death at 24 h from 52% to 28% ($p < 0.05$). Glutamate was slightly more toxic to OL in the presence of AS CM compared to control medium, although the differences were not significant (58% vs. 52%, AS CM + glutamate vs. control medium + glutamate, ns). However, AS CM ACTH (from AS cultures pretreated for 24 h with ACTH then washed to remove ACTH before collection of CM) protected OL against glutamate compared to AS CM itself (46% vs. 59%, AS CM ACTH + glutamate vs. AS CM + glutamate, $p < 0.05$). Under these culture conditions, ACTH at 200 nM was more effective than AS CM ACTH in protecting OL from glutamate (28% vs. 46%, ACTH + glutamate vs. AS CM ACTH + glutamate, $p < 0.05$).

When OL death was induced with the ionotropic glutamate ligand NMDA at 2 mM, both ACTH 1–39 in control medium and AS CM ACTH were protective under these conditions (Fig. 1). In these experiments, ACTH at 200 nM was slightly more effective than AS CM ACTH in protecting OL from NMDA (30% vs. 37%, ACTH + NMDA vs. AS CM ACTH + NMDA, $p < 0.05$) compared to control medium + NMDA (55% OL death). Again, AS CM from AS not pretreated with ACTH was not protective for OL treated with NMDA, similar to control medium (57% vs. 55%, AS CM + NMDA vs. control medium + NMDA, ns).

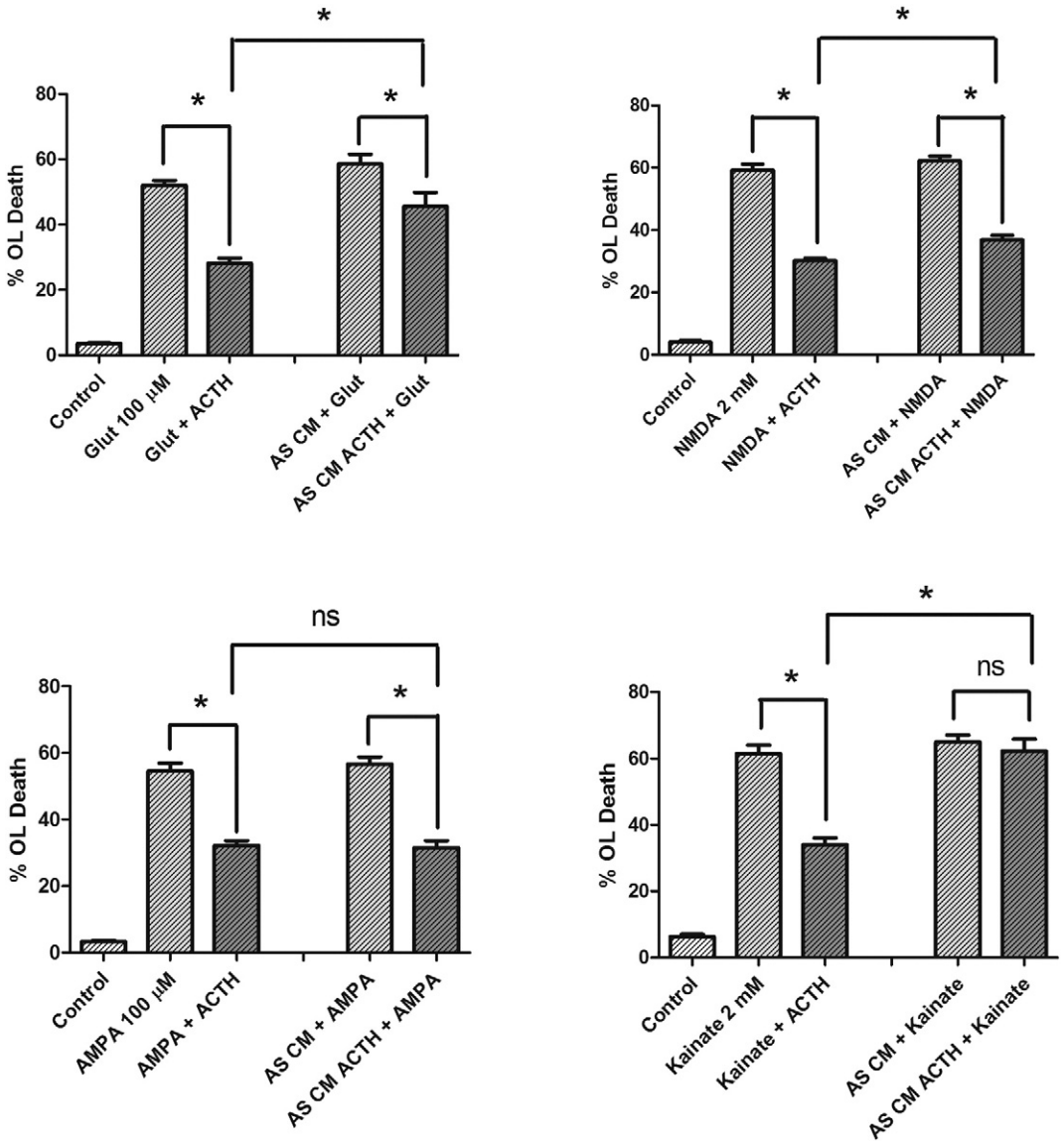

Fig. 1. Conditioned medium from astrocytes pretreated with ACTH protects OL from glutamate, NMDA and AMPA but not kainate. Purified OL were incubated for 24 h with medium alone (control), or with glutamate (100 μM), NMDA (2 mM), AMPA (100 μM) or kainate (2 mM) in the presence of: (a) DMEM alone; (b) 200 nM ACTH; (c) conditioned medium from AS incubated with DMEM alone (AS CM); or (d) conditioned medium from AS pretreated with ACTH (AS CM ACTH). Cell death was determined by uptake of trypan blue. Results represent means ± S.E.M. from 3 experiments with 2 duplicates in each experiment. *$p < 0.05$; ns, not significant.

In the next series of experiments, when OL death was induced with the ionotropic glutamate ligand AMPA at 100 μM, both ACTH 1-39 and AS CM ACTH protected OL (Fig. 1). In this case, ACTH 1-39 in control medium and AS CM ACTH were equally protective (32% vs. 31%, ACTH + AMPA vs. AS CM ACTH + AMPA) compared to control medium + AMPA (59% OL death). Again, AS CM was not protective for OL treated with AMPA, similar to control medium (62% vs. 59%, AS CM + AMPA vs. control medium + AMPA).

However, while ACTH 1-39 in control medium protected OL against 2 mM kainate, AS CM ACTH was not protective (34% vs. 62%, ACTH + kainate vs. AS CM ACTH + kainate, $p < 0.05$), compared to control medium + kainate (61% OL death). Again, AS CM was not protective for OL treated with kainate, similar to kainate in control medium (65% vs. 61%, AS CM + kainate vs. control medium + kainate). The lack of protection by AS CM ACTH against kainate could account for the low level of protection seen for AS CM ACTH against glutamate, which binds to kainate receptors as well as NMDA and AMPA receptors.

To assess the effects of AS CM ACTH on OL apoptosis, we utilized staurosporine, a classic inducer of apoptosis that acts via inhibition of several kinases with generation of ROS [7,23,27,39]. Unlike ACTH 1-39 in control medium, AS CM ACTH was not protective against OL death induced by staurosporine (32% vs. 67%, ACTH + staurosporine vs. AS CM ACTH + staurosporine, $p < 0.05$), compared to control medium + staurosporine (62% OL death) (Fig. 2). In the case of quinolinic acid, an inflammation-related molecule in the indoleamine pathway, ACTH 1-39 and AS CM ACTH were equally protective (36% vs. 37%, ACTH + quinolinic vs. AS CM ACTH + quinolinic), compared to control medium + quinolinic acid (62% OL death) (Fig. 3). In comparison, when OL were exposed to ROS generated from H_2O_2, both ACTH 1-39 and AS CM ACTH were protective, with AS CM ACTH less protective (37% vs. 52%, ACTH + H_2O_2 vs. AS CM ACTH + H_2O_2, $p < 0.05$), compared to control medium + H_2O_2 (65% OL death) (Fig. 4). In all of these cases, the conditioned medium from AS (AS CM) not pretreated with ACTH was not itself toxic to OL, but also was not protective against staurosporine, quinolinic acid or ROS.

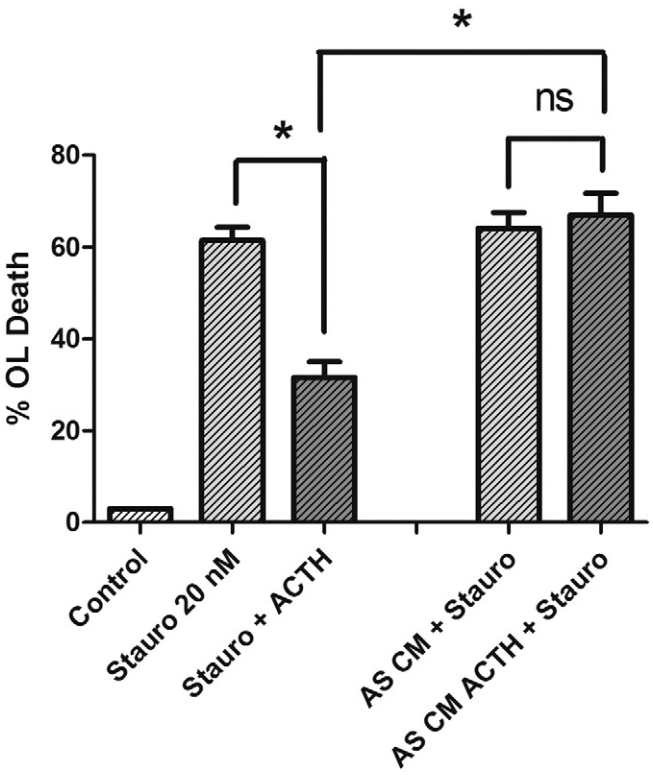

Fig. 2. Conditioned medium from astrocytes pretreated with ACTH does not protect OL from staurosporine. Purified OL were incubated for 24 h with medium alone (control), or with staurosporine (20 nM) in the presence of: (a) DMEM alone; (b) 200 nM ACTH; (c) conditioned medium from AS incubated with DMEM alone (AS CM); or (d) conditioned medium from AS pretreated with ACTH (AS CM ACTH). Cell death was determined by uptake of trypan blue. Values represent means ± S.E.M. from 3 experiments, with duplicates in each experiment; *$p < 0.05$; ns, not significant.

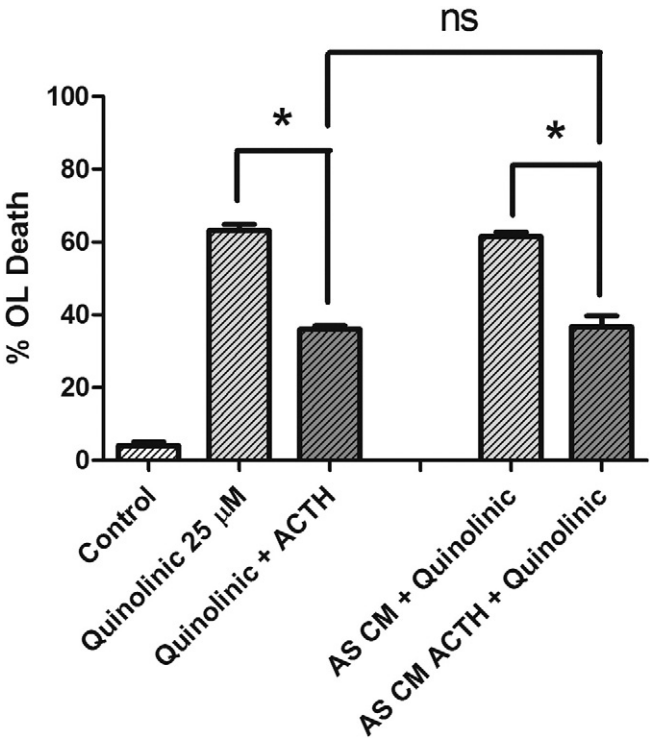

Fig. 3. Conditioned medium from astrocytes pretreated with ACTH protects OL from quinolinic acid. Purified OL were incubated for 24 h with medium alone (control), or with quinolinic acid (25 μM) in the presence of: (a) DMEM alone; (b) 200 nM ACTH; (c) conditioned medium from AS incubated with DMEM alone (AS CM); or (d) conditioned medium from AS pretreated with ACTH (AS CM ACTH). Cell death was determined by uptake of trypan blue. Values represent means ± S.E.M. from 3 experiments, with duplicates in each experiment; *$p < 0.05$; ns, not significant.

In our previous study, we assessed the protective effects of ACTH 1-39 against two other agents toxic to OL, kynurenic acid and NO; ACTH 1-39 was not protective against either agent. However, we tested the effects of AS CM and AS CM ACTH on OL treated with kynurenic acid (25 μM), NOC-12 (rapidly released NO) and NOC-18 (slowly released NO); AS CM ACTH also showed no protective effects against these cytotoxic agents (data not shown).

3.3. Microglia treated with ACTH do not secrete factors that protect OL from cytotoxic insults

All of the above experiments were repeated using conditioned medium from MG without or with ACTH 1-39 pretreatment. As noted above, MG CM alone was not toxic to OL. Neither MG CM nor MG CM ACTH was protective against any of the toxic agents, in contrast to the protective effects of AS CM ACTH. Both the AS CM ACTH and MG CM ACTH were prepared from cells that were washed with DMEM after treatment of AS or MG with 200 nM ACTH. Even if 5% of the ACTH, or 10 nM, remained after washing the cells, we previously showed that ACTH at 50 nM was only slightly protective against several of the toxic agents. Further, the MG CM ACTH prepared in the same way was not protective against any of the toxic agents [6]. Thus it is unlikely that the protective effects seen with AS CM ACTH are due to residual ACTH in the conditioned medium. In preliminary experiments to characterize the nature of the protective factor(s), we found that centrifugation at 15,000 g did not alter the ability of AS CM ACTH to protect OL against quinolinic acid. Further, the protective factor(s) were almost entirely retained by a 300 kD cutoff membrane (Pall Corp., Ann Arbor MI; data not shown).

4. Discussion

Analysis of molecular changes in both white matter and cortical lesions in MS provide evidence for CNS damage related to inflammation and oxidative stress [19,21,28,45]. We have used rat glial cultures to investigate protective strategies against some of the mechanisms underlying OL damage in MS lesions. We previously reported that ACTH 1-39 protected purified OL from death induced by glutamate excitotoxicity (glutamate, NMDA, AMPA or kainate), staurosporine, quinolinic acid and ROS but not from NO or kynurenic acid [6]. In the present study, we show that ACTH 1-39 can induce AS to produce factors that protect OL against some of these cytotoxic agents. As summarized in Table 1, both ACTH 1-39 and AS CM ACTH protected purified OL from cell death induced by glutamate, NMDA, AMPA, quinolinic acid and ROS. While ACTH protected OL from kainate and staurosporine, AS CM ACTH was not protective against these two cytotoxic agents. Neither ACTH nor AS CM ACTH protected against kynurenic acid or NO. The protection of OL by ACTH from several toxic molecules involves direct effects on OL. However, ACTH also stimulated AS to produce mediators that protect against some of these molecules but not others. These results indicate that ACTH can act on AS to induce secretion of factors that protect OL. Thus in these cases, ACTH can act indirectly via an astroglial response, as well as directly on OL, based on our results with highly purified OL. For staurosporine and kainate, the lack of protection by the conditioned medium from ACTH-pretreated AS supports the conclusion that ACTH can protect OL directly against those agents.

MG ACTH CM did not protect from any of these molecules, nor did medium from control AS or MG not treated with ACTH. Thus ACTH-stimulated MG do not provide protection through secretion of mediators under these experimental conditions. MG are known to secrete both protective and damaging soluble factors in response to other agents and in response to growth factors and cytokines ([8,10,17],

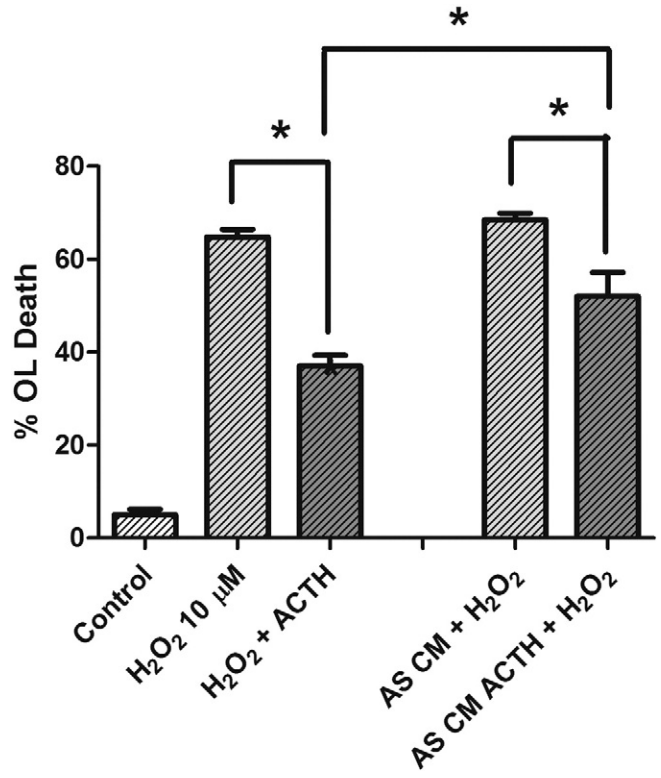

Fig. 4. Conditioned medium from astrocytes pretreated with ACTH protects OL from reactive oxygen species. Purified OL were incubated for 24 h with medium alone (control), or with H_2O_2 (10 μM) in the presence of: (a) DMEM alone; (b) 200 nM ACTH; (c) conditioned medium from AS incubated with DMEM alone (AS CM); or (d) conditioned medium from AS pretreated with ACTH (AS CM ACTH). Cell death was determined by uptake of trypan blue. Values represent means ± S.E.M. from 3 experiments, with duplicates in each experiment; * $p < 0.05$.

reviews). Possible reasons for the lack of effects of MG CM in our system could include the state of activation of the MG, low concentration of factors produced, or requirement for direct physical cell–cell interactions.

AS express both MC1R and MC4R [42] and MG are known to express MC1R, MC3R, MC4R and MC5R [10]; see [1,9,12], reviews). These receptors differ in their affinities for the various MC peptides [13,40,41]. We showed that OL express MC4R [6], but expression of other MCR subtypes in OL has not been investigated.

MC peptides promote downregulation of inflammatory responses in both AS and MG ([12,13], reviews). The possible roles of MCR in AS was first investigated by Zohar and Salomon [47,48]; several MC peptides were shown to act via G proteins to upregulate cAMP levels and induce morphologic alterations. In response to ACTH 1-24, AS showed decreased expression of ciliary neuronotrophic factor, with no effects on brain derived neuronotrophic factor (BDNF), nerve growth factor or neurotrophin 3 [26]. However, in another study, α-melanocyte stimulating hormone (α-MSH) induced expression of both message and protein for BDNF [11,38]. An analog of α-MSH stimulated the release of the anti-inflammatory cytokine TGF-β from AS [10]. Of interest, Dreyfus and co-workers have shown that glutamate induces release of BDNF from cultured AS via metabotropic glutamate receptors [2,24]. Their in vivo experiments showed that BDNF from AS promotes myelin protein synthesis following demyelination induced by cuprizone [20]. However, so far, definitive evidence that increased BDNF is effective in decreasing symptoms in MS is lacking.

AS can promote OPC differentiation and myelination, enhancing the potential for remyelination ([14,32], reviews). An early study showed that over several days in culture, unstimulated AS decreased apoptosis of OL prepared from mature mouse brain. The effect was not seen with AS conditioned medium or several neurotrophic factors, but involved interaction of α6β1 integrin on OLs with laminin on AS [16]. In our study, we observed very low levels of death in OL prepared from newborn rat brain; we also found that conditioned medium from AS not pretreated with ACTH 1-39 did not protect OL from death induced by any of the exogenous cytotoxic agents we used.

Several lines of evidence suggest that AS may be key mediators in MS progression. The roles of AS in MS pathogenesis have been investigated by analyzing changes in gene expression in AS in normal appearing gray matter from MS patients compared to control tissue [45]. The analysis showed reduced expression of AS-enriched genes in the AS-neuron lactate cycle and the glutamate-glutamine cycle, along with upregulation of toll-like receptor/IL-1β related genes, indicating immune-related signaling. Experiments in culture and in vivo supported the conclusion that immune activation induces AS metabolic changes that could lead to damage of both neurons and OL. A recent analysis of direct effects of peripheral immune cells on human OPC survival and differentiation also examined the indirect effects of conditioned medium from AS treated with supernatants from proinflammatory Th1/Th17 and M1-polarized myeloid cells [33]. Those supernatants decreased OPC differentiation via AS-derived CXCL10. Conditioned medium from AS treated with supernatants from Th2 cells, M2 macrophages or MG had no effect on OPC differentiation. The protective or toxic effects of the AS conditioned media were not examined. In our study, we show that ACTH can stimulate AS in culture to produce mediators that protect OL against some excitotoxic and immune-related molecules but not others. We have not yet examined the effects of the AS CM ACTH on differentiation or protection of OPCs. We do not know at this point whether the protective factors released by AS are contained in large molecular complexes or in extracellular vesicles such as exosomes [15,25], which are known to be released by numerous cell types including AS [22,44]. As found for other agents in MS lesions and model systems, the cellular mechanisms underlying both the direct and indirect protective effects of ACTH for OL are complex, varying with the specific type of insult.

Table 1
Conditioned medium from astrocytes treated with ACTH protects against NMDA, AMPA, quinolinic acid and H_2O_2.

Agent	Agent + ACTH Direct*	Agent + AS CM ACTH Indirect
Glutamate	Protects	Protects
NMDA	Protects	Protects
AMPA	Protects	Protects
Kainate	Protects	No protection
Staurosporine	Protects	No protection
Quinolinic acid	Protects	Protects
Kynurenic acid	No protection	No protection
H_2O_2	Protects	Protects
NOC-12	No protection	No protection
NOC-18	No protection	No protection

* [6].

References

[1] B.G. Arnason, R. Berkovich, A. Catania, R.P. Lisak, M. Zaidi, Mechanisms of action of adrenocorticotropic hormone and other melanocortins relevant to the clinical management of patients with multiple sclerosis, Mult. Scler. 19 (2013) 130–136.
[2] I.P. Bagayogo, C.F. Dreyfus, Regulated release of BDNF by cortical oligodendrocytes is mediated through metabotropic glutamate receptors and the PLC pathway, ASN Neuro 1 (1) (2009), e00001.
[3] J.A. Benjamins, L. Nedelkoska, Cyclic GMP-dependent pathways protect differentiated oligodendrocytes from multiple types of injury, Neurochem. Res. 32 (2007) 321–329.
[4] J.A. Benjamins, L. Nedelkoska, R.P. Lisak, J.H. Hannigan, R.J. Sokol, Cytokines reduce toxic effects of ethanol on oligodendroglia, Neurochem. Res. 36 (2011) 1677–1686.
[5] J.A. Benjamins, L. Nedelkoska, R.P. Lisak, Adrenocorticotropin hormone 1-39 promotes proliferation and differentiation of oligodendroglial progenitor cells and protects from excitotoxic and inflammation-related damage, J. Neurosci. Res. 92 (2014) 1243–1251.
[6] J.A. Benjamins, L. Nedelkoska, B. Bealmear, R.P. Lisak, ACTH protects mature oligodendroglia from excitotoxic and inflammation-related damage in vitro, Glia 61 (2013) 1206–1217.

[7] R. Bertrand, E. Solary, P. O'Connor, K.W. Kohn, Y. Pommier, Induction of a common pathway of apoptosis by staurosporine, Exp. Cell Res. 211 (1994) 314–321.
[8] J.F. Bogie, P. Stinissen, J.J. Hendriks, Macrophage subsets and microglia in multiple sclerosis, Acta Neuropathol. 128 (2014) 191–213 Review.
[9] T. Brzoska, T.A. Luger, C. Maaser, C. Abels, M. Böhm, Alpha-melanocyte-stimulating hormone and related tripeptides: biochemistry, antiinflammatory and protective effects in vitro and in vivo, and future perspectives for the treatment of immune-mediated inflammatory diseases, Endocr. Rev. 29 (2008 Aug) 581–602 Review.
[10] L. Carniglia, D. Durand, C. Caruso, M. Lasaga, Effect of NDP-α-MSH on PPAR-γ and -β expression and anti-inflammatory cytokine release in rat astrocytes and microglia, PLoS One 8 (2013), e57313.
[11] C. Caruso, L. Carniglia, D. Durand, P.V. Gonzalez, T.N. Scimonelli, M. Lasaga, Melanocortin 4 receptor activation induces brain-derived neurotrophic factor expression in rat astrocytes through cyclic AMP-protein kinase A pathway, Mol. Cell. Endocrinol. 348 (2012) 47–54.
[12] A. Catania, Neuroprotective actions of melanocortins: a therapeutic opportunity, Trends Neurosci. 31 (2008) 353–360.
[13] A. Catania, S. Gatti, G. Colombo, J.M. Lipton, Targeting melanocortin receptors as a novel strategy to control inflammation, Pharmacol. Rev. 56 (2004 Mar) 1–29 (Review).
[14] D. Clemente, M.C. Ortega, C. Melero-Jerez, F. de Castro, The effect of glia–glia interactions on oligodendrocyte precursor cell biology during development and in demyelinating diseases, Front. Cell. Neurosci. 7 (2013) 1–15 (Review).
[15] M. Colombo, G. Raposo, C. Thery, Biogenesis, secretion, and intercellular interactions of exosomes andother extracellular vesicles, Annu. Rev. Cell Dev. Biol. 30 (2014) 255–289.
[16] S.M. Corley, U. Ladiwala, A. Besson, V.W. Yong, Astrocytes attenuate oligodendrocyte death in vitro through an alpha(6) integrin–laminin-dependent mechanism, Glia 36 (2001) 281–294.
[17] R. Delgado, A. Carlin, L. Airaghi, M.T. Demitri, L. Meda, D. Galimberti, P. Baron, J.M. Lipton, A. Catania, Melanocortin peptides inhibit production of proinflammatory cytokines and nitric oxide by activated microglia, J. Leukoc. Biol. 63 (1998) 740–745.
[18] C.A. Dyer, J.A. Benjamins, Redistribution and internalization of antibodies to galactocerebroside by oligodendroglia, J. Neurosci. 8 (1988) 883–891.
[19] M.A. Friese, B. Schattling, L. Fugger, Mechanisms of neurodegeneration and axonal dysfunction in multiple sclerosis, Nat. Rev. Neurol. 10 (2014) 225–238 Review.
[20] C.G. Fulmer, M.W. VonDran, A.A. Stillman, Y. Huang, B.L. Hempstead, C.F. Dreyfus, Astrocyte-derived BDNF supports myelin protein synthesis after cuprizone-induced demyelination, J. Neurosci. 34 (2014) 8186–8196.
[21] R.E. Gonsette, Neurodegeneration in multiple sclerosis: the role of oxidative stress and excitotoxicity, J. Neurol. Sci. 274 (2008) 48–53 (Review).
[22] R.D. Gosselin, P. Meylan, I. Decosterd, Extracellular microvesicles from astrocytes contain functional glutamate transporters: regulation by protein kinase C and cell activation, Front. Cell. Neurosci. 7 (2013) 251.
[23] J.M. Herbert, E. Seban, J.P. Maffrand, Characterization of specific binding sites for [3 H]-staurosporine on various protein kinases, Biochem. Biophys. Res. Commun. 171 (1990) 189–195.
[24] Y.Y. Jean, L.D. Lercher, C.F. Dreyfus, Glutamate elicits release of BDNF from basal forebrain astrocytes in a process dependent on metabotropic receptors and the PLC pathway, Neuron Glia Biol. 4 (2008) 35–42.
[25] I. Kawikova, P.W. Askenase, Diagnostic and therapeutic potentials of exosomes in CNS diseases, Brain Res. 1617 (2015) 63–71 (Review).
[26] M. Kokubo, K. Asai, N. Yamamoto, M. Aoyama, M. Morikawa, H. Togari, Y. Wada, T. Kato, ACTH(1–24) down-regulates expression of ciliary neurotrophic factor mRNA in cultured rat astrocytes, Pediatr. Res. 52 (2002) 950–957.
[27] A.J. Krohn, E. Preis, J.H. Prehn, Staurosporine-induced apoptosis of cultured rat hippocampal neurons involves caspase-1-like proteases as upstream initiators and increased production of superoxide as a main downstream effector, J. Neurosci. 18 (1998) 8186–8197.
[28] H. Lassmann, Mechanisms of white matter damage in multiple sclerosis, Glia 62 (2014) 1816–1830 (Review).
[29] R.P. Lisak, J.A. Benjamins, B. Bealmear, B. Yao, S. Land, L. Nedelkoska, D. Skundric, Differential effects of Th1, monocyte/macrophage and Th2 cytokine mixtures on early gene expression for immune-related molecules by central nervous system mixed glial cell cultures, Mult. Scler. 12 (2006) 149–168.
[30] R.P. Lisak, L. Nedelkoska, J.A. Benjamins, Effects of dextromethorphan on glial cell function: proliferation, maturation, and protection from cytotoxic molecules, Glia 62 (5) (2014) 751–762.
[31] R.P. Lisak, L. Nedelkoska, B. Bealmear, J.A. Benjamins, Melanocortin receptor agonist ACTH 1-39 protects rat forebrain neurons from apoptotic, excitotoxic and inflammation-related damage. Mult Scler J, 6th Trienial meeting of the Americas Committee on Treatment and Research in Multiple Sclerosis/European Committee on Treatment and Research in Multiple Sclerosis, Boston, MA, September 9–12, 2014, Poster 714, 2014.
[32] C.S. Moore, S.L. Abdullah, A. Brown, A. Arulpragasam, S.J. Crocker, How factors secreted from astrocytes impact myelin repair, J. Neurosci. Res. 89 (2011) 13–21 (Review).
[33] C.S. Moore, Q.L. Cui, N.M. Warsi, B.A. Durafourt, N. Zorko, D.R. Owen, J.P. Antel, A. Bar-Or, Direct and indirect effects of immune and central nervous system-resident cells on human oligodendrocyte progenitor cell differentiation, J. Immunol. 194 (2015) 761–772.
[34] L. Nedelkoska, J.A. Benjamins, Binding of cholera toxin B subunit: a surface marker for murine microglia but not oligodendrocytes or astrocytes, J. Neurosci. Res. 53 (1998) 605–612.
[35] F. Piccinini, A. Tesei, G. Paganelli, W. Zoli, A. Bevilacqua, Improving reliability of live/dead cell counting through automated image mosaicing, Comput. Methods Prog. Biomed. 117 (2014) 448–463.
[36] N.P. Pringle, H.S. Mudhar, E.J. Collarini, W.D. Richardson, PDGF receptors in the rat CNS: during late neurogenesis, PDGF alpha-receptor expression appears to be restricted to glial cells of the oligodendrocyte lineage, Development 115 (1992) 535–551.
[37] M.C. Raff, R. Mirsky, K.L. Fields, R.P. Lisak, S.H. Dorfman, D.H. Silberberg, N.A. Gregson, S. Leibowitz, M.C. Kennedy, Galactocerebroside is a specific cell-surface antigenic marker for oligodendrocytes in culture, Nature 274 (1978) 813–816.
[38] D. Ramírez, J. Saba, L. Carniglia, D. Durand, M. Lasaga, C. Caruso, Melanocortin 4 receptor activates ERK-cFos pathway to increase brain-derived neurotrophic factor expression in rat astrocytes and hypothalamus, Mol. Cell. Endocrinol. 411 (2015) 28–37.
[39] U.T. Rüegg, G.M. Burgess, Staurosporine, K-252 and UCN-01: potent but nonspecific inhibitors of protein kinases, Trends Pharmacol. Sci. 10 (1989) 218–220 (Review).
[40] H.B. Schiöth, R. Muceniece, J.E. Wikberg, V. Chhajlani, Characterization of melanocortin receptor subtypes by radioligand binding analysis, Eur. J. Pharmacol. 288 (1995) 311–317.
[41] H.B. Schiöth, R. Muceniece, J.E. Wikberg, Characterisation of the melanocortin 4 receptor by radioligand binding, Pharmacol. Toxicol. 79 (1996) 161–165.
[42] J.V. Selkirk, L.M. Nottebaum, J. Lee, W. Yang, A.C. Foster, S.M. Lechner, Identification of differential melanocortin 4 receptor agonist profiles at natively expressed receptors in rat cortical astrocytes and recombinantly expressed receptors in human embryonic kidney cells, Neuropharmacology 52 (2007) 459–466.
[43] I. Sommer, M. Schachner, Monoclonal antibodies (O1 to O4) to oligodendrocyte cell surfaces: an immunocytological study in the central nervous system, Dev. Biol. 83 (1981) 311–327.
[44] G. Wang, M. Dinkins, Q. He, G. Zhu, C. Poirier, A. Campbell, M. Mayer-Proschel, E. Bieberich, Astrocytes secrete exosomes enriched with proapoptotic ceramide and prostate apoptosis response 4 (PAR-4): potential mechanism of apoptosis induction in Alzheimer disease (AD), J. Biol. Chem. 287 (2012) 21384–21395.
[45] T. Zeis, I. Allaman, M. Gentner, K. Schroder, J. Tschopp, P.J. Magistretti, N. Schaeren-Wiemers, Metabolic gene expression changes in astrocytes in Multiple Sclerosis cerebral cortex are indicative of immune-mediated signaling, Brain Behav. Immun. 48 (2015) 313–325.
[46] Zhou S, Cui Z, Urban J. Dead cell counts during serum cultivation are underestimated by the fluorescent live/dead assay. Biotechnol. J. May 2011;6:513–8.
[47] M. Zohar, Y. Salomon, Melanocortins stimulate proliferation and induce morphological changes in cultured rat astrocytes by distinct transducing mechanisms, Brain Res. 576 (1992) 49–58.
[48] M. Zohar, Y. Salomon, Mechanism of action of melanocortin peptides. Possible role in astrocyte regulation, J. Mol. Neurosci. 4 (1993) 55–62 (Review).