Experimental Neurology 273 (2015) 161–167

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Melanocortin receptor agonist ACTH 1–39 protects rat forebrain neurons from apoptotic, excitotoxic and inflammation-related damage

Robert P. Lisak *[,1], Liljana Nedelkoska, Beverly Bealmear, Joyce A. Benjamins [1]

Department of Neurology, Wayne State University School of Medicine, 8D University Health Center, 4201 St. Antoine St., Detroit, MI 48201, USA

ARTICLE INFO

Article history:
Received 20 May 2015
Received in revised form 12 August 2015
Accepted 14 August 2015
Available online 20 August 2015

Keywords:
ACTH
Amyotrophic lateral sclerosis
Corticosteroids
Glutamate
Melanocortin receptors
Multiple sclerosis
Neuroinflammation
Neurons
Protection

ABSTRACT

Patients with relapsing–remitting multiple sclerosis (RRMS) are commonly treated with high doses of intravenous corticosteroids (CS). ACTH 1–39, a member of the melanocortin family, stimulates production of CS by the adrenals, but melanocortin receptors are also found in the central nervous system (CNS) and on immune cells. ACTH is produced within the CNS and may have direct protective effects on glia and neurons independent of CS. We previously reported that ACTH 1–39 protected oligodendroglia (OL) and their progenitors (OPC) from a panel of excitotoxic and inflammation-related agents. Neurons are the most vulnerable cells in the CNS. They are terminally differentiated, and sensitive to inflammatory and excitotoxic insults. For potential therapeutic protection of gray matter, it is important to investigate the direct effects of ACTH on neurons. Cultures highly enriched in neurons were isolated from 2–3 day old rat brain. After 4–7 days in culture, the neurons were treated for 24 h with selected toxic agents with or without ACTH 1–39. ACTH 1–39 protected neurons from death induced by staurosporine, glutamate, NMDA, AMPA, kainate, quinolinic acid, reactive oxygen species and, to a modest extent, from rapidly released NO, but did not protect against kynurenic acid or slowly released nitric oxide. Our results show that ACTH 1–39 protects neurons *in vitro* from several apoptotic, excitotoxic and inflammation-related insults.

1. Introduction

Relapses of multiple sclerosis (MS) are frequently treated with corticosteroids (CS), which shorten duration of the relapses. In some instances patients are treated with ACTH in a gel formulation (Simsarian et al., 2011). In several studies ACTH has proven equivalent to CS for treatment of relapses (Barnes et al., 1985; Milanese et al., 1989; Thompson et al., 1989). It has been assumed that the therapeutic effects of ACTH in MS are mediated by endogenous CS. ACTH is a melanocortin peptide and binds with varying avidity to melanocortin receptors (MCRs) which are widely distributed throughout the body including in the central nervous system (CNS) (Catania et al., 2004b, 2010; Catania, 2008; Arnason et al., 2013). Within the CNS, MCRs have been shown to be expressed on astrocytes (AS) (Selkirk et al., 2007), microglia (MG) (Lipton et al., 1998) and neurons (Catania et al., 2004b, 2010; Catania, 2008; Mountjoy, 2010). Recently we demonstrated that melanocortin 4 receptors (MC4R) are expressed by OL *in vitro* (Benjamins et al., 2013). This raises the possibility that ACTH might exert a therapeutic effect independent of stimulating endogenous CS production and secretion by signaling through MC2R in the adrenal glands (Catania et al., 2004b, 2010; Ross et al., 2013; Montero-Melendez, 2015). ACTH has been shown to be superior to CS in treatment of infantile spasms with hypsarrhythmia (Mytinger et al., 2012; Shumiloff et al., 2013; Pavone et al., 2014), suggesting that ACTH has a therapeutic effect that is not simply the result of stimulating increase in levels of endogenous CS.

We previously reported that ACTH was capable of protecting OL (Benjamins et al., 2013) and their precursors (OPC) (Benjamins et al., 2014) from *in vitro* cytotoxic cell death induced by several mediators of excitotoxic, oxidative and inflammation-related damage. We also showed that ACTH stimulated OPC proliferation and induced OL to elaborate larger and more dense membrane sheets (Benjamins et al., 2014). It is increasingly clear that damage to axons/neurons is important in the pathogenesis of MS in all stages of the disease (Ferguson et al., 1997; Trapp et al., 1998; Dutta and Trapp, 2007; Lassmann, 2013; Mahad et al., 2015). While some axonal and neuronal loss may result from changes in function in demyelinated axons (Dutta and Trapp, 2011; Criste et al., 2014), some damage and neuronal loss may be caused by direct effects of mediators of excitotoxicity and inflammation (Lisak et al., 2012; Lassmann, 2014a, 2014b). It is important to

* Corresponding author.
E-mail address: rlisak@med.wayne.edu (R.P. Lisak).
[1] Both contributed equally to this manuscript.

http://dx.doi.org/10.1016/j.expneurol.2015.08.012
0014-4886/

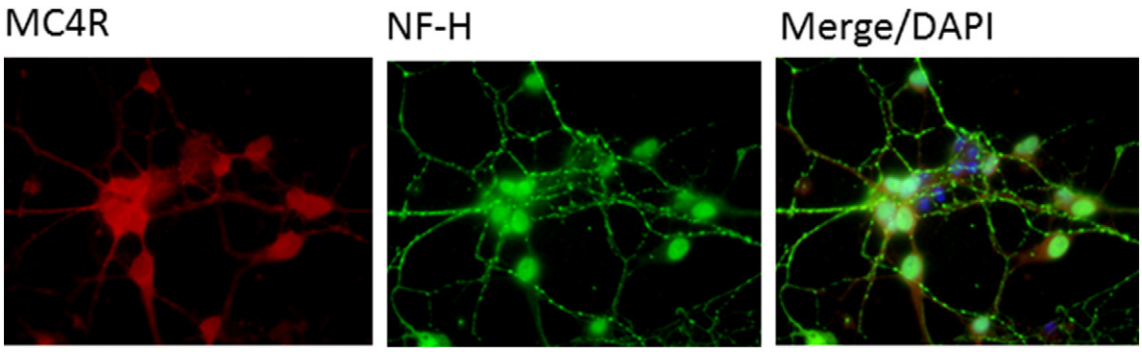

Fig. 1. Neurons in culture express melanocortin 4 receptors. Purified neuronal cultures were prepared from brains of 2 day old rats, and immunostained after one week in culture. Expression of MC4R in neurons was investigated by fixing cells for 15 min in 4% paraformaldehyde, permeabilizing with 0.05% Triton X-100 for 20 min, immunostaining for MC4R, then double label immunostaining for NF-H. Melanocortin 4 receptor, MC4R (red); neurofilament H, NF-H (green); DAPI, nuclear stain (blue).

determine if ACTH could also provide protection to neurons, the most vulnerable cells within the CNS, from the same set of toxic molecules.

2. Materials and methods

Cultures highly enriched in neurons were isolated from 2–3 day old rat brain (Eide and McMurray, 2005), with minor modifications including the addition of 1 μg/ml nerve growth factor (Gibco Lifetech, Grand Island, NY, NGF 2.5S) (Lisak et al., 2011). Cell types were characterized using the following antibodies to phenotypic markers: anti-phosphorylated neurofilament H (NF-H) (Covance, Berkley, CA, SMI-31P) (Sternberger and Sternberger, 1983) and anti-NeuN (Chemicon International, Bellerica, MA, MAB377) for neurons (Mullen et al., 1992), anti-glial fibrillary acidic protein (GFAP) (Chemicon International Inc., Temecula, CA, M#B360) for AS (Raff et al., 1978) and the lectin IB4 (Sigma, St. Louis, MO, L9381) for MG (Nedelkoska and Benjamins, 1998). Expression of MC4R in neurons was investigated by fixing cells for 15 min in 4% paraformaldehyde, permeabilizing with 0.05% Triton X-100 for 20 min. then double label immunostaining with antibodies to MC4R and NF-H. Rabbit polyclonal antibody to

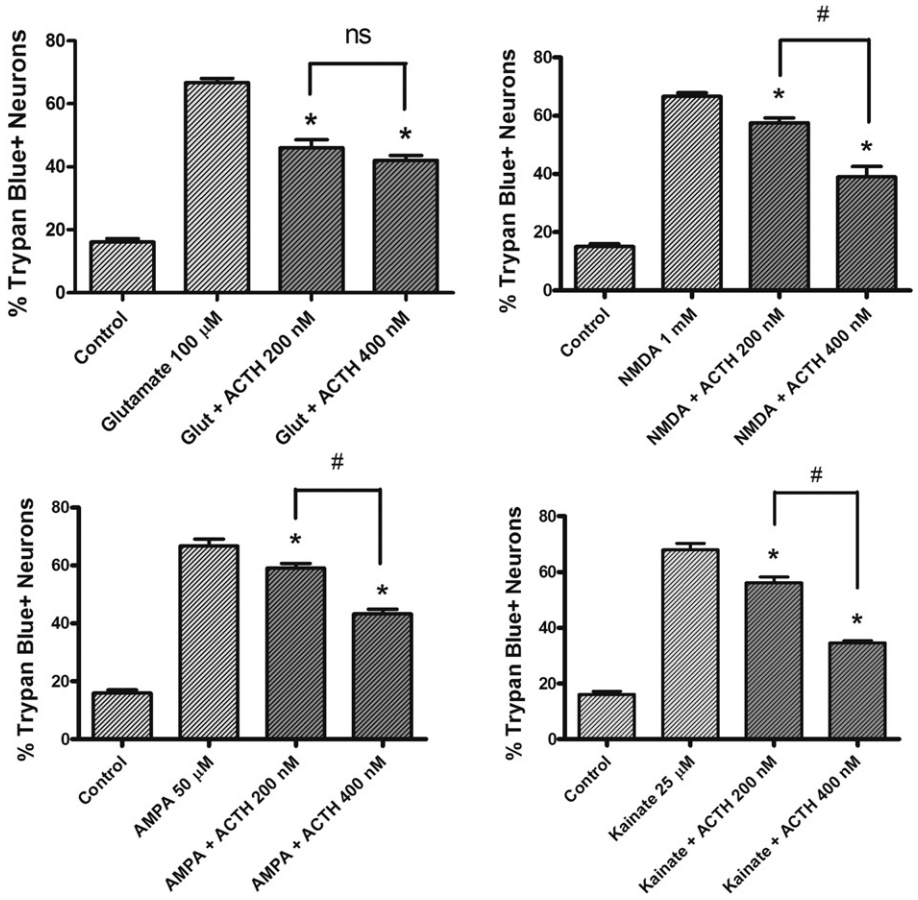

Fig. 2. ACTH protects neurons against glutamate-mediated toxicity. Neuronal cultures were untreated (control) or treated for 30 min with 200 nM or 400 nM ACTH 1–39 before addition of glutamate or agonists of glutamate receptors. After 24 h, cell death was measured in neurons by uptake of trypan blue. (A) Addition of 100 μM glutamate to activate glutamate receptors. (B) Addition of 1 mM NMDA to activate ionotropic NMDA glutamate receptors. (C) Addition of 50 μM AMPA to activate ionotropic AMPA glutamate receptors. (D) Addition of 25 μM kainate to activate high affinity glutamate receptors. Results represent means +/− S.E.M. from 3 or more experiments with duplicate coverslips in each experiment. Glutamate receptor agonist *vs.* ACTH + agonist, *$p < 0.002$, +$p < 0.02$; #200 nM vs 400 nM ACTH; $p < 0.001$.

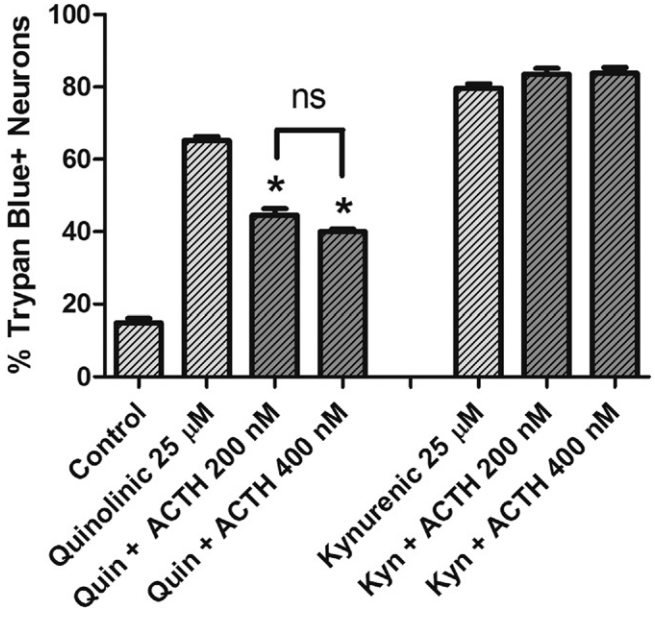

Fig. 3. ACTH protects neurons against quinolinic acid but not kynurenic acid. Neuronal cultures were untreated or treated for 30 min with 200 nM or 400 nM ACTH 1–39 before addition of 25 μM kynurenic acid or 25 μM quinolinic acid. After 24 h, cell death was measured in neurons by uptake of trypan blue. Results represent means +/− S.E.M. from 3 experiments with duplicate coverslips in each experiment. *Quinolinic acid *vs.* ACTH + quinolinic acid, $p < 0.001$.

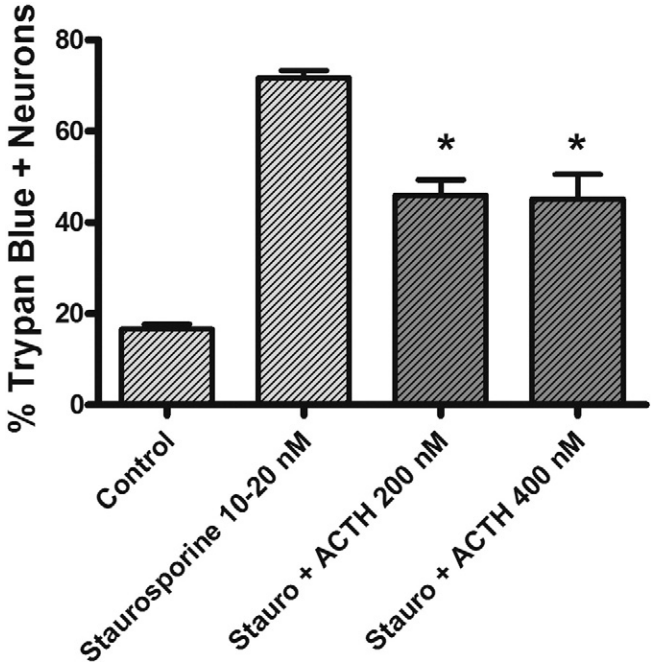

Fig. 5. ACTH protects neurons against death induced by staurosporine. Purified neuronal cultures were untreated or treated for 30 min with 200 nM or 400 nM ACTH 1–39 before addition of 10 nm or 20 nM staurosporine. After 24 h, cell death was measured in neurons by uptake of trypan blue. Results represent means +/− S.E.M. from 3 experiments with duplicate coverslips in each experiment. * Staurosporine *vs.* ACTH + staurosporine, $p < 0.001$.

MC4R was purchased from Cayman Chemicals (Ann Arbor, MI, 10006355).

Neurons, immunostained for NeuN or NF-H represented 85–90% of the cells, with less than 10% AS and 4% MG as determined by double label immunofluorescence. After 4–7 days in culture, the neurons were treated for 1 day with selected toxic agents with or without 200 or 400 nM ACTH. Concentrations of toxic agents were chosen to result in 65–80% neuronal cell death. Untreated neurons served as controls. Neuronal death was assessed by trypan blue uptake (Lisak et al., 2011; Benjamins et al., 2013). Neuronal apoptosis was measured using the Apoptag *in situ* apoptosis kit (EMD Millipore, Billerica, MA, #S7165) to visualize terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), as we previously described for glia (Skoff et al., 1998; Benjamins et al., 2014), followed by immunolabeling of neurons with antibodies to NF-H.

Three or more independent experiments were performed for each condition; data were analyzed by the unpaired Student's *t*-test.

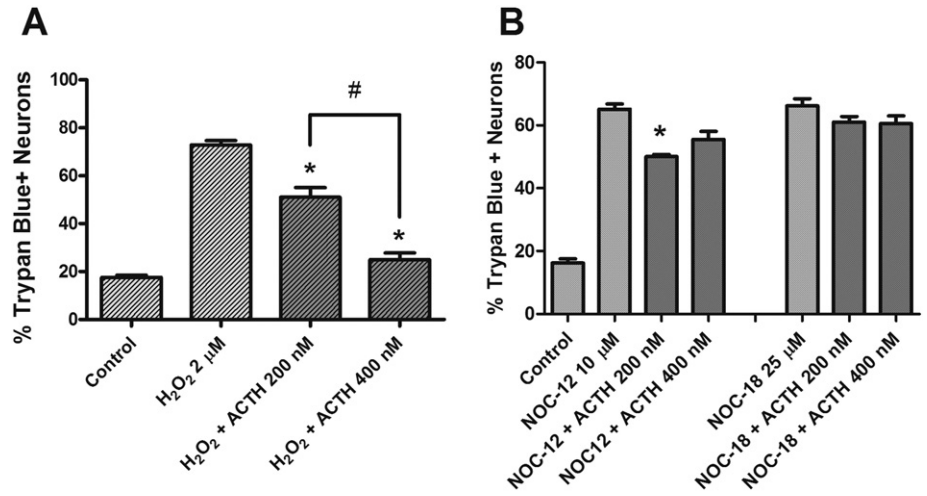

Fig. 4. ACTH protects neurons against reactive oxygen species, with little or no protection from NO. (A) Cultures were exposed to 2 μM. H_2O_2 or treated for 30 min with 200 or 400 nM ACTH 1–39 before addition of H_2O_2 to generate ROS. After 24 h, cell death was measured in neurons by uptake of trypan blue. *H_2O_2 *vs.* ACTH + H_2O_2, $p < 0.001$; #200 nM vs 400 nM ACTH, $p < 0.002$. (B) Except for modest protection against NOC-12, ACTH did not protect neurons against rapid or slow release of NO, generated from NOC-12 or NOC-18 by hydrolysis. Neurons were treated for 30 min with 20 nM ACTH before addition of NOC-12, which releases NO with a half-life of 5.5 h, or NOC-18, which releases NO with a half-life of 20 h. After 24 h, cell death was measured in neurons by uptake of trypan blue. Results represent means +/− S.E.M. from 3 with duplicate coverslips in each experiment. *NOC-12 *vs.* ACTH + NOC-12, $p < 0.001$; +NOC-12 *vs.* ACTH + NOC-12, $p < 0.03$.

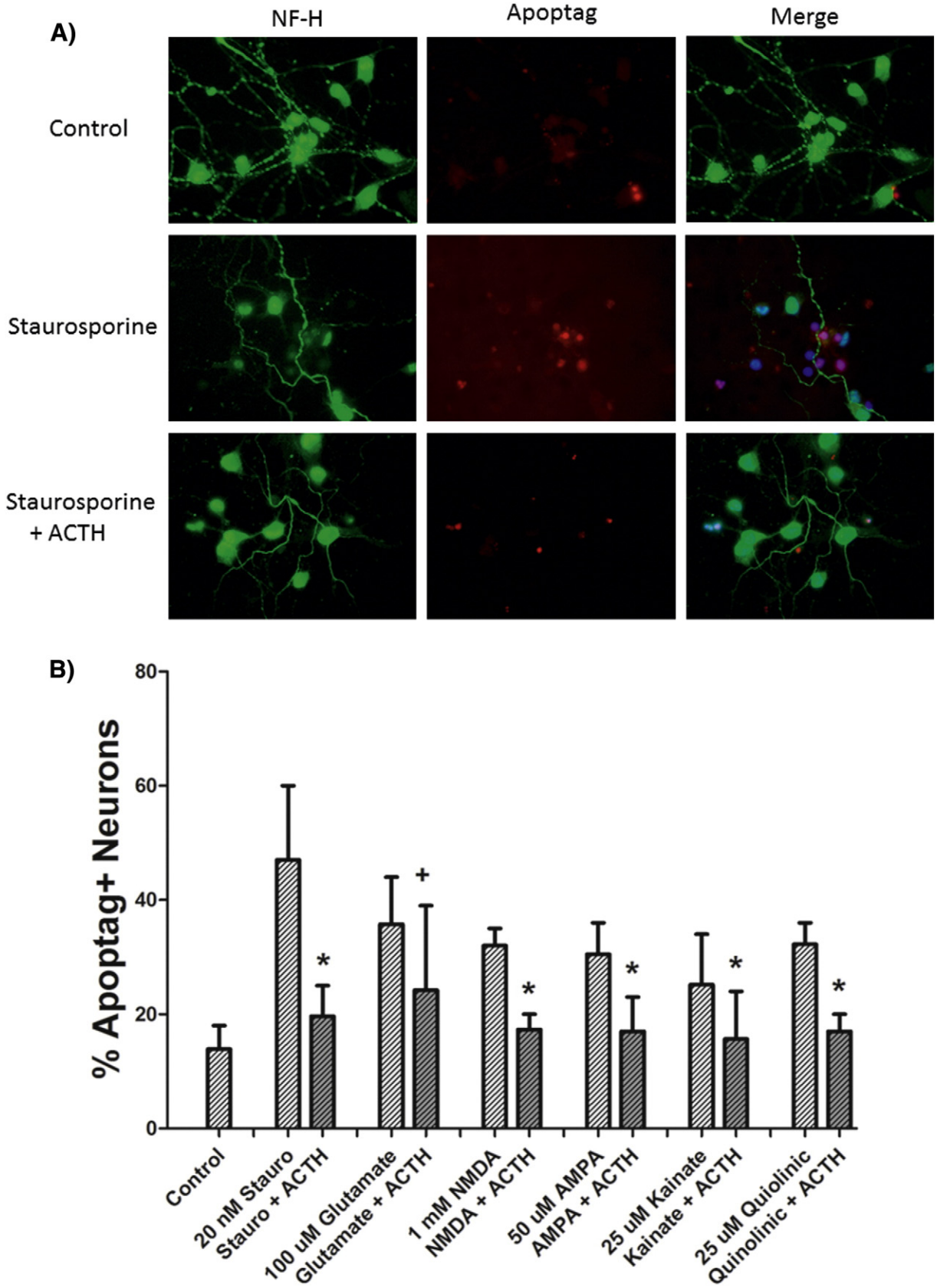

Fig. 6. Apoptosis contributes to neuronal death initiated by staurosporine, glutamate, NMDA, AMPA, kainate and quinolinic acid, and is decreased by ACTH. Neurons were treated for 24 h with 20 nM staurosporine, 100 μM glutamate, 1 mM NMDA, 50 μM AMPA, 25 μM kainate, or 25 μM quinolinic acid, with or without 400 nM ACTH. A. Apoptosis was assessed by Apoptag staining; neurons were immunostained with antibodies to NF-H. B. Values indicate the percent of NF-H + cells that were positive for Apoptag staining at this time point. Results represent means +/− S.E.M. from 3 or more experiments with 1–2 coverslips in each experiment. * Agent *vs.* ACTH + agent, $p < 0.02$; + agent *vs.* ACTH + agent, $p < 0.05$.

3. Results

3.1. Expression of melanocortin receptors by neurons in culture

As we previously demonstrated for OL (Benjamins et al., 2013), we found that MC4R receptors were expressed by neurons under our culture conditions (Fig. 1). These results are consistent with those reported by others for neurons *in vitro* and *in vivo* (Catania, 2008; Mountjoy, 2010).

3.2. Neuroprotective effects of ACTH against excitotoxic, inflammation related and oxidative death

ACTH at concentrations of 100–400 nM had no toxic effect on neurons (data not shown), while ACTH provided protection from excitotoxic neuronal death induced by glutamate (100 μM), NMDA (1 mM), AMPA (50 μM), and kainate (25 μM) (Fig. 2). It is of interest that 200 nM ACTH provided modest protection from NMDA, AMPA or kainate, while ACTH at 400 nM provided substantial protection in each case. Neuronal death in untreated cultures was consistently between 15–20%, on average 17%.

With regard to inflammation related damage, quinolinic acid is a neurotoxic product of the indoleamine dioxygenase/kynurenine pathway, upregulated during neuroinflammation. ACTH at either 200 or 400 nM protected neurons from quinolinic acid (25 μM) (Fig. 3). However, ACTH did not protect neurons from death induced by kynurenic acid (25 μM).

There was also protection by ACTH from cell death induced by 2 μM H_2O_2, which gives rise to reactive oxygen species (ROS), with significantly more protection at 400 nM ACTH compared to 200 nM (Fig. 4A). ACTH gave modest protection against rapid release of nitric oxide (NO) by NOC-12 but not slow release by NOC-18 (Fig. 4B).

As shown in Fig. 5, ACTH (200 or 400 nM) protected neurons from cytotoxic effects of staurosporine (10–20 nM), a classic inducer of cell death *via* apoptosis. ACTH reduced cell death from 80% to 55% ($p < 0.01$).

Both apoptotic and non-apoptotic mechanisms can contribute to neuronal death, depending on the nature, severity and duration of the insult. While the trypan blue assay measured the percent of live *versus* dead neurons present after 24 h of insult, we further assessed the percent of apoptotic neurons present in cultures at 24 h after treatment with staurosporine, glutamate, NMDA, AMPA, kainate or quinolinic acid, using the Apoptag assay and NF-H immunostaining to measure apoptosis in neurons; immunostaining for the staurosporine-treated cells is shown in Fig. 6A. We asked whether ACTH protected against apoptotic cell death. In each case, as expected at a given time point, the percent of TUNEL+ cells was less than the total number of dead cells (positive for trypan blue uptake). However, for all six toxic agents, ACTH significantly reduced the percent of apoptotic cells compared to cultures not treated with ACTH (Fig. 6B).

4. Discussion

Our study demonstrates that ACTH 1–39 protects neurons *in vitro* from several excitotoxic and inflammation-related insults (summarized in Table 1). Only a few early studies examined the direct effects of melanocortins on neurons in culture. Melanocortins have been shown to protect neurons *in vitro* against cisplatin toxicity (Bar et al., 1993a, 1993b), and α-MSH promotes neurite outgrowth in culture (Joosten et al., 1996). An MSH analog protected an immortalized neuronal cell line from apoptosis due to serum deprivation (Chai et al., 2006). Other protective effects of melanocortin mediated AS and MG have been reported, as well as protection of AS and inhibition of toxic effects of activated MG (Catania et al., 2004a, 2010). A number of studies have investigated the protective effects of melanocortins *in vivo* (reviewed in Catania, 2008). As examples, melanocortins are neuroprotective in animal models of ischemia (Spaccapelo et al., 2013), traumatic brain injury (Schaible et al., 2013) and Alzheimer's disease (Giuliani et al., 2014), as well as in peripheral nerve development, protection and repair (Strand et al., 1993; Gispen et al., 1994; Ter Laak et al., 2003). While the mechanisms underlying these effects could arise from peripheral actions such as release of CS and peripheral anti-inflammatory effects, some of the protection could arise from direct protection on neurons within the CNS or PNS.

We show that ACTH protects neurons from the excitotoxic effects of glutamate as well as NMDA, AMPA and kainate, as found for both OL (Benjamins et al., 2013) and OPC (Benjamins et al., 2014) (summarized in Table 2). Neurons, as expected, were more sensitive to killing by NMDA, AMPA and kainate than OL or OPC. Further, a higher concentration of ACTH, 400 nM, was required to provide substantial protection to neurons from NMDA, AMPA and kainate, compared to 200 nM ACTH for OL and OPC (Benjamins et al., 2013, 2014). There was increased protection with the higher concentration of ACTH against cytotoxicity induced by NMDA, AMPA and kainate, but no increased protection from glutamate itself. Excitotoxic damage to neurons is an important mechanism of damage to axons and neurons in several experimental and clinical disorders of the CNS (Werner et al., 2001; Matute, 2011; Mehta et al., 2013). Agents that reduce production of glutamate or block NMDA receptors have had only limited success as treatments for amyotrophic lateral sclerosis (ALS) and Alzheimer's disease respectively (Hu et al., 2012; Miller et al., 2012; Rive et al., 2013; Zemek et al., 2014). It is possible that treatments that inhibit *in vitro* neuronal cytotoxicity mediated through all of the ionotropic glutamate receptors might, in theory, have better therapeutic effects in CNS disorders in which glutamate excitotoxicity plays an important pathogenic role.

As shown in Fig. 3, ACTH inhibited neuronal death induced by quinolinic acid, a kynurenine pathway metabolite of tryptophan associated with inflammation (Sas et al., 2007; Vamos et al., 2009; Stone and Darlington, 2013) that can produce NMDA excitotoxic damage or oxidative damage dependent on or independent of NMDA receptors (Perez-De La Cruz et al., 2012). As was the case with OL and OPC, ACTH did not protect neurons from kynurenic acid, the product of an earlier step in the kynurenine pathway (Vamos et al., 2009; Moroni et al., 2012). Quinolinic acid is a weak agonist for NMDA receptors, particularly NR2A and NR2B subunits, while kynurenic acid has multiple potential actions, including antagonist at the glycine site of NMDA receptors, antagonist of cholinergic α7 nicotinic receptors, agonist of GPR35 G protein coupled receptors and agonist of aryl hydrocarbon receptors (Moroni et al., 2012). Both *in vivo* and *in vitro*, kynurenic acid is generally considered to be neuroprotective (Klein et al., 2013; Bohar et al., 2015). However, kynurenic acid can also be toxic during brain development or at mM concentrations in cultured cell lines (Turski et al., 2014; Bohar et al., 2015); the mechanisms are not known, but in our primary culture system at μM levels could involve antagonism of cholinergic receptors or mitochondrial dysfunction in developing neurons. Of interest, kynurenic acid levels are decreased in CSF of MS patients during remission, and increased in both plasma and CSF during relapse, possibly reflecting peripheral inflammatory alterations (Bohar et al., 2015).

Of interest, ACTH also protected neurons from ROS generated by incubation with H_2O_2 (Fig. 4). There was protection from rapid generation of NO but not slower release, the opposite of what we found with the effect of ACTH on OPC (Benjamins et al., 2014). For mature OL,

Table 1
Summary of protective effects of ACTH on neurons.

Protection		No protection
Glutamate	Quinolinic acid	Kynurenic acid
NMDA	H_2O_2 (ROS)	NOC-18 (slow NO release)
AMPA	NOC-12 (rapid NO release)	
Kainate	Staurosporine	

Neurons were treated for 24 h with the toxic agents in the absence or presence of 400 nM ACTH. Neuronal death was measured by trypan blue uptake.

Table 2
Comparison of protective effects of ACTH 1–39 on neurons, oligodendroglia and oligodendroglial progenitors.

Agent	OL[a]	OPC[b]	Neurons
Glutamate	+	+	+
NMDA	+	+	+
AMPA	+	+	+
Kainate	+	+	+
Quinolinic acid	+	+	+
Kynurenic acid	np	np	np
H_2O_2	+	+	+
NOC-12 (rapid NO release)	np	np	+
NOC-18 (slow NO release)	np	+	np
Staurosporine	+	+	+

np = no protection.
[a] Benjamins et al. (2013).
[b] Benjamins et al. (2014).

ACTH did not provide protection from either rapid or slow NO release (Benjamins et al., 2013). These differences may reflect differences between the cell types, for example, in the kinetics and pathways of signaling used by each cell type to respond to reactive nitrogen species, or differences in the damaging effects of nitrosylation of key receptors or enzymes (Boullerne and Benjamins, 2006). Staurosporine is widely used to study apoptosis and induces apoptosis *via* mechanisms including inhibition of protein kinases and generation of ROS (Bertrand et al., 1994; Krohn et al., 1998). ACTH inhibited total staurosporine-induced neuronal death as measured by uptake of trypan blue at 24 h (Fig. 5), and reduced the percentage of apoptotic neurons as assessed by Apoptag staining (Fig. 6A, B).

Both MC3R and MC4R have been reported to be expressed by neurons (Catania, 2008; Mountjoy, 2010). MC3R message is found in the hypothalamus and limbic system, while MC4R message is found in the cortex, thalamus, hypothalamus, brain stem and spinal cord (reviewed in Catania, 2008). We confirmed the expression of MC4R in our neuronal cultures (Fig. 1). Our neuronal cultures are derived from forebrain of newborn rats. We have not yet analyzed if other MCRs are expressed on the neurons in these cultures, nor do we know which receptor subtypes are important in mediating the direct neuroprotective effects of ACTH in our *in vitro* model.

Our cultures contain a very small percentage of AS and MG and it is certainly possible that these cells provide some protective effects involving direct cell to cell contact *in vitro* as well as *in vivo*. Astroglial uptake of excessive glutamate is an important protective function of astrocytes (Anderson and Swanson, 2000) as have been demonstrated to express MC1R and MC4R, and MG are known to express MC1R (Catania, 2008; Catania et al., 2010). Studies to determine if AS and/or MG contribute to the neuroprotective effects seen in this study will be important for future studies. It should be emphasized that neurons are reported to express MCRs including MC4R, as confirmed in our system.

In conclusion, ACTH, a relatively small protein that produced within the CNS as well as by the adrenals and other tissues, has potential as a protective agent for neurons as well as for OL and OPC. The protective effects of ACTH for OL, OPC and neurons show striking similarities, the only difference being the effects of ACTH protection for slow *versus* rapid NO generation (Table 2). ACTH and related melanocortins such as α-MSH have been shown to have therapeutic benefits in several animal models of inflammatory-mediated CNS and peripheral nervous system diseases (Moyer et al., 1950; Catania, 2008; Taylor and Kitaichi, 2008; Cusick et al., 2014; Montero-Melendez, 2015). How much of the protection results from anti-inflammatory effects in the periphery or within the CNS, and how much results from direct effects on neurons, OL and OPC within the brain is not well understood. Finally, whether such beneficial effects can be demonstrated *in vivo* in human diseases is not yet known.

Acknowledgments

This study is supported by an Investigator Initiated Research Award (RPL) from the Mallinckrodt Pharmaceuticals Autoimmune and Rare Diseases Division (formerly Questcor Pharmaceuticals) and the Parker Webber Chair in Neurology (DMC Foundation/WSU) (RPL).

References

Anderson, C.M., Swanson, R.A., 2000. Astrocyte glutamate transport: review of properties, regulation, and physiological functions. Glia 32, 1–14.
Arnason, B.G., Berkovich, R., Catania, A., Lisak, R.P., Zaidi, M., 2013. Mechanisms of action of adrenocorticotropic hormone and other melanocortins relevant to the clinical management of patients with multiple sclerosis. Mult. Scler. 19, 130–136.
Bar, P.R., Hol, E.M., Gispen, W.H., 1993a. Trophic effects of melanocortins on neuronal cells in culture. Ann. N. Y. Acad. Sci. 692, 284–286.
Bar, P.R., Mandys, V., Turecek, R., Gispen, W.H., 1993b. Alpha-melanocyte-stimulating hormone has protective properties against the toxic effect of cisplatin on cultured dorsal root ganglia. Ann. N. Y. Acad. Sci. 680, 649–651.
Barnes, M.P., Bateman, D.E., Cleland, P.G., Dick, D.J., Walls, T.J., Newman, P.K., Saunders, M., Tilley, P.J., 1985. Intravenous methylprednisolone for multiple sclerosis in relapse. J. Neurol. Neurosurg. Psychiatry 48, 157–159.
Benjamins, J.A., Nedelkoska, L., Bealmear, B., Lisak, R.P., 2013. ACTH protects mature oligodendroglia from excitotoxic and inflammation-related damage in vitro. Glia 61, 1206–1217.
Benjamins, J.A., Nedelkoska, L., Lisak, R.P., 2014. Adrenocorticotropin hormone 1–39 promotes proliferation and differentiation of oligodendroglial progenitor cells and protects from excitotoxic and inflammation-related damage. J. Neurosci. Res. 92, 1243–1251.
Bertrand, R., Solary, E., O'Connor, P., Kohn, K.W., Pommier, Y., 1994. Induction of a common pathway of apoptosis by staurosporine. Exp. Cell Res. 211, 314–321.
Bohar, Z., Toldi, J., Fulop, F., Vecsei, L., 2015. Changing the face of kynurenines and neurotoxicity: therapeutic considerations. Int. J. Mol. Sci. 16, 9772–9793.
Boullerne, A.I., Benjamins, J.A., 2006. Nitric oxide synthase expression and nitric oxide toxicity in oligodendrocytes. Antioxid. Redox Signal. 8, 967–980.
Catania, A., 2008. Neuroprotective actions of melanocortins: a therapeutic opportunity. Trends Neurosci. 31, 353–360.
Catania, A., Colombo, G., Carlin, A., Garofalo, L., Gatti, S., Buffa, R., Carboni, N., Rosso, L., Santambrogio, L., Cantalamessa, L., Lipton, J.M., 2004a. Autocrine inhibitory influences of alpha-melanocyte-stimulating hormone in malignant pleural mesothelioma. J. Leukoc. Biol. 75, 253–259.
Catania, A., Gatti, S., Colombo, G., Lipton, J.M., 2004b. Targeting melanocortin receptors as a novel strategy to control inflammation. Pharmacol. Rev. 56, 1–29.
Catania, A., Lonati, C., Sordi, A., Carlin, A., Leonardi, P., Gatti, S., 2010. The melanocortin system in control of inflammation. Sci. World J. 10, 1840–1853.
Chai, B., Li, J.Y., Zhang, W., Newman, E., Ammori, J., Mulholland, M.W., 2006. Melanocortin-4 receptor-mediated inhibition of apoptosis in immortalized hypothalamic neurons via mitogen-activated protein kinase. Peptides 27, 2846–2857.
Criste, G., Trapp, B., Dutta, R., 2014. Axonal loss in multiple sclerosis: causes and mechanisms. Handb. Clin. Neurol. 122, 101–113.
Cusick, M.F., Libbey, J.E., Oh, L., Jordan, S., Fujinami, R.S., 2014. Acthar gel treatment suppresses acute exacerbations in a murine model of relapsing–remitting multiple sclerosis. Autoimmunity 1–9.
Dutta, R., Trapp, B.D., 2007. Pathogenesis of axonal and neuronal damage in multiple sclerosis. Neurology 68, S22–S31 (discussion S43–54).
Dutta, R., Trapp, B.D., 2011. Mechanisms of neuronal dysfunction and degeneration in multiple sclerosis. Prog. Neurobiol. 93, 1–12.
Eide, L., McMurray, C.T., 2005. Culture of adult mouse neurons. Biotechniques 38, 99–104.
Ferguson, B., Matyszak, M.K., Esiri, M.M., Perry, V.H., 1997. Axonal damage in acute multiple sclerosis lesions. Brain 120, 393–399.
Gispen, W.H., Verhaagen, J., Bar, D., 1994. ACTH/MSH-derived peptides and peripheral nerve plasticity: neuropathies, neuroprotection and repair. Prog. Brain Res. 100, 223–229.
Giuliani, D., Bitto, A., Galantucci, M., Zaffe, D., Ottani, A., Irrera, N., Neri, L., Cavallini, G.M., Altavilla, D., Botticelli, A.R., Squadrito, F., Guarini, S., 2014. Melanocortins protect against progression of Alzheimer's disease in triple-transgenic mice by targeting multiple pathophysiological pathways. Neurobiol. Aging 35, 537–547.
Hu, N.W., Ondrejcak, T., Rowan, M.J., 2012. Glutamate receptors in preclinical research on Alzheimer's disease: update on recent advances. Pharmacol. Biochem. Behav. 100, 855–862.
Joosten, E.A., Verhaagh, S., Martin, D., Robe, P., Franzen, R., Hooiveld, M., Doornbos, R., Bar, P.R., Moonen, G., 1996. Alpha-MSH stimulates neurite outgrowth of neonatal rat corticospinal neurons in vitro. Brain Res. 736, 91–98.
Klein, C., Patte-Mensah, C., Taleb, O., Bourguignon, J.J., Schmitt, M., Bihel, F., Maitre, M., Mensah-Nyagan, A.G., 2013. The neuroprotector kynurenic acid increases neuronal cell survival through neprilysin induction. Neuropharmacology 70, 254–260.
Krohn, A.J., Preis, E., Prehn, J.H., 1998. 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, 8186–8197.
Lassmann, H., 2013. Pathology and disease mechanisms in different stages of multiple sclerosis. J. Neurol. Sci. 333, 1–4.
Lassmann, H., 2014a. Mechanisms of white matter damage in multiple sclerosis. Glia 62, 1816–1830.

Lassmann, H., 2014b. Multiple sclerosis: lessons from molecular neuropathology. Exp. Neurol. 262PA, 2–7.
Lipton, J.M., Catania, A., Delgado, R., 1998. Peptide modulation of inflammatory processes within the brain. Neuroimmunomodulation 5, 178–183.
Lisak, R.P., Nedelkoska, L., Studzinski, D., Bealmear, B., Xu, W., Benjamins, J.A., 2011. Cytokines regulate neuronal gene expression: differential effects of Th1, Th2 and monocyte/macrophage cytokines. J. Neuroimmunol. 238, 19–33.
Lisak, R.P., Benjamins, J.A., Nedelkoska, L., Barger, J.L., Ragheb, S., Fan, B., Ouamara, N., Johnson, T.A., Rajasekharan, S., Bar-Or, A., 2012. Secretory products of multiple sclerosis B cells are cytotoxic to oligodendroglia in vitro. J. Neuroimmunol. 246, 85–95.
Mahad, D.H., Trapp, B.D., Lassmann, H., 2015. Pathological mechanisms in progressive multiple sclerosis. Lancet Neurol. 14, 183–193.
Matute, C., 2011. Glutamate and ATP signalling in white matter pathology. J. Anat. 219, 53–64.
Mehta, A., Prabhakar, M., Kumar, P., Deshmukh, R., Sharma, P.L., 2013. Excitotoxicity: bridge to various triggers in neurodegenerative disorders. Eur. J. Pharmacol. 698, 6–18.
Milanese, C., La Mantia, L., Salmaggi, A., Campi, A., Eoli, M., Scaioli, V., Nespolo, A., Corridori, F., 1989. Double-blind randomized trial of ACTH versus dexamethasone versus methylprednisolone in multiple sclerosis bouts. Clinical, cerebrospinal fluid and neurophysiological results. Eur. Neurol. 29, 10–14.
Miller, R.G., Mitchell, J.D., Moore, D.H., 2012. Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). Cochrane Database Syst. Rev. 3, CD001447.
Montero-Melendez, T., 2015. ACTH: the forgotten therapy. Semin. Immunol. 27, 216–226.
Moroni, F., Cozzi, A., Sili, M., Mannaioni, G., 2012. Kynurenic acid: a metabolite with multiple actions and multiple targets in brain and periphery. J. Neural Transm. 119, 133–139.
Mountjoy, K.G., 2010. Distribution and function of melanocortin receptors within the brain. Adv. Exp. Med. Biol. 681, 29–48.
Moyer, A.W., Jervis, G.A., Black, J., Koprowski, H., Cox, H.R., 1950. Action of adrenocorticotropic hormone (ACTH) in experimental allergic encephalomyelitis of the guinea pig. Proc. Soc. Exp. Biol. Med. 75, 387–390.
Mullen, R.J., Buck, C.R., Smith, A.M., 1992. NeuN, a neuronal specific nuclear protein in vertebrates. Development 116, 201–211.
Mytinger, J.R., Joshi, S., Pediatric Epilepsy Research Consortium, S.o.I.S., 2012. The current evaluation and treatment of infantile spasms among members of the Child Neurology Society. J. Child Neurol. 27, 1289–1294.
Nedelkoska, L., Benjamins, J.A., 1998. Binding of cholera toxin B subunit: a surface marker for murine microglia but not oligodendrocytes or astrocytes. J. Neurosci. Res. 53, 605–612.
Pavone, P., Striano, P., Falsaperla, R., Pavone, L., Ruggieri, M., 2014. Infantile spasms syndrome, West syndrome and related phenotypes: what we know in 2013. Brain Dev. 36, 739–751.
Perez-De La Cruz, V., Carrillo-Mora, P., Santamaria, A., 2012. Quinolinic acid, an endogenous molecule combining excitotoxicity, oxidative stress and other toxic mechanisms. Int. J. Tryptophan Res. 5, 1–8.
Raff, M.C., Mirsky, R., Fields, K.L., Lisak, R.P., Dorfman, S.H., Silberberg, D.H., Gregson, N.A., Leibowitz, S., Kennedy, M.C., 1978. Galactocerebroside is a specific cell-surface antigenic marker for oligodendrocytes in culture. Nature 274, 813–816.
Rive, B., Gauthier, S., Costello, S., Marre, C., Francois, C., 2013. Synthesis and comparison of the meta-analyses evaluating the efficacy of memantine in moderate to severe stages of Alzheimer's disease. CNS Drugs 27, 573–582.
Ross, A.P., Ben-Zacharia, A., Harris, C., Smrtka, J., 2013. Multiple sclerosis, relapses, and the mechanism of action of adrenocorticotropic hormone. Front. Neurol. 4, 21.
Sas, K., Robotka, H., Toldi, J., Vecsei, L., 2007. Mitochondria, metabolic disturbances, oxidative stress and the kynurenine system, with focus on neurodegenerative disorders. J. Neurol. Sci. 257, 221–239.
Schaible, E.V., Steinstrasser, A., Jahn-Eimermacher, A., Luh, C., Sebastiani, A., Kornes, F., Pieter, D., Schafer, M.K., Engelhard, K., Thal, S.C., 2013. Single administration of tripeptide alpha-MSH(11–13) attenuates brain damage by reduced inflammation and apoptosis after experimental traumatic brain injury in mice. PLoS One 8, e71056.
Selkirk, J.V., Nottebaum, L.M., Lee, J., Yang, W., Foster, A.C., Lechner, S.M., 2007. 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, 459–466.
Shumiloff, N.A., Lam, W.M., Manasco, K.B., 2013. Adrenocorticotropic hormone for the treatment of West Syndrome in children. Ann. Pharmacother. 47, 744–754.
Simsarian, J.P., Saunders, C., Smith, D.M., 2011. Five-day regimen of intramuscular or subcutaneous self-administered adrenocorticotropic hormone gel for acute exacerbations of multiple sclerosis: a prospective, randomized, open-label pilot trial. Drug Des. Devel. Ther. 5, 381–389.
Skoff, A.M., Lisak, R.P., Bealmear, B., Benjamins, J.A., 1998. TNF-alpha and TGF-beta act synergistically to kill Schwann cells. J. Neurosci. Res. 53, 747–756.
Spaccapelo, L., Galantucci, M., Neri, L., Contri, M., Pizzala, R., D'Amico, R., Ottani, A., Sandrini, M., Zaffe, D., Giuliani, D., Guarini, S., 2013. Up-regulation of the canonical Wnt-3A and Sonic hedgehog signaling underlies melanocortin-induced neurogenesis after cerebral ischemia. Eur. J. Pharmacol. 707, 78–86.
Sternberger, L.A., Sternberger, N.H., 1983. Monoclonal antibodies distinguish phosphorylated and nonphosphorylated forms of neurofilaments in situ. Proc. Natl. Acad. Sci. U. S. A. 80, 6126–6130.
Stone, T.W., Darlington, L.G., 2013. The kynurenine pathway as a therapeutic target in cognitive and neurodegenerative disorders. Br. J. Pharmacol. 169, 1211–1227.
Strand, F.L., Lee, S.J., Lee, T.S., Zuccarelli, L.A., Antonawich, F.J., Kume, J., Williams, K.A., 1993. Non-corticotropic ACTH peptides modulate nerve development and regeneration. Rev. Neurosci. 4, 321–363.
Taylor, A.W., Kitaichi, N., 2008. The diminishment of experimental autoimmune encephalomyelitis (EAE) by neuropeptide alpha-melanocyte stimulating hormone (alpha-MSH) therapy. Brain Behav. Immun. 22, 639–646.
Ter Laak, M.P., Brakkee, J.H., Adan, R.A., Hamers, F.P., Gispen, W.H., 2003. The potent melanocortin receptor agonist melanotan-II promotes peripheral nerve regeneration and has neuroprotective properties in the rat. Eur. J. Pharmacol. 462, 179–183.
Thompson, A.J., Kennard, C., Swash, M., Summers, B., Yuill, G.M., Shepherd, D.I., Roche, S., Perkin, G.D., Loizou, L.A., Ferner, R., et al., 1989. Relative efficacy of intravenous methylprednisolone and ACTH in the treatment of acute relapse in MS. Neurology 39, 969–971.
Trapp, B.D., Peterson, J., Ransohoff, R.M., Rudick, R., Mork, S., Bo, L., 1998. Axonal transection in the lesions of multiple sclerosis [see comments]. N. Engl. J. Med. 338, 278–285.
Turski, W.A., Malaczewska, J., Marciniak, S., Bednarski, J., Turski, M.P., Jablonski, M., Siwicki, A.K., 2014. On the toxicity of kynurenic acid in vivo and in vitro. Pharmacol. Rep. 66, 1127–1133.
Vamos, E., Pardutz, A., Klivenyi, P., Toldi, J., Vecsei, L., 2009. The role of kynurenines in disorders of the central nervous system: possibilities for neuroprotection. J. Neurol. Sci. 283, 21–27.
Werner, P., Pitt, D., Raine, C.S., 2001. Multiple sclerosis: altered glutamate homeostasis in lesions correlates with oligodendrocyte and axonal damage. Ann. Neurol. 50, 169–180.
Zemek, F., Drtinova, L., Nepovimova, E., Sepsova, V., Korabecny, J., Klimes, J., Kuca, K., 2014. Outcomes of Alzheimer's disease therapy with acetylcholinesterase inhibitors and memantine. Expert Opin. Drug Saf. 13, 759–774.