THE EFFECTS OF ALCOHOL AND AGE ON ASTROCYTES IN FEMALE RATS FOLLOWING AN INFLAMMATORY STIMULUS

A Senior Scholars Thesis

by

ASHLEY NICOLE SIMPSON

Submitted to the Office of Undergraduate Research
Texas A&M University
In partial fulfillment of the requirements for the designation as

UNDERGRADUATE RESEARCH SCHOLAR

April 2006

Major: Biology

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Approved by:

Research Advisor:	Farida Sohrabji
Associate Dean for Undergraduate Research	Robert C. Webb

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ABSTRACT

The Effects of Alcohol, and Age on Astrocytes in Female Rats Following an Inflammatory Stimulus (May 2006)

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Astrocytes are an important support cell within the central nervous system and are an integral part of the blood brain barrier. They participate in inflammatory processes in the brain and may also be influenced by alcohol and age. Thus, the objective of this research was to determine the relationship between alcohol and age following a lipopolysaccharide-induced (LPS) insult in astrocytes derived from young adult and reproductively senescent female rats.

To understand the effects of age on astrocytes, primary cultures were derived from reproductively-competent young adult female rats or reproductively senescent female rats. The reproductive senescent females are physiologically similar to human menopause. Thus, our aging model is based on ovarian, not chronological, age.

In this study, LPS increased both nitric oxide production and matrix metalloproteinase 9 (MMP-9) activity in young adult and reproductive senescent-derived astrocytes. Matrix metalloproteinases are enzymes that degrade extracellular matrix (ECM) molecules in which cells adhere within the brain. Degradation of the ECM allows for cell migration, but prolonged activation can potentially lead to blood brain barrier degradation. In young adult-derived astrocytes, LPS increased MMP-9 and this was not

affected by ethanol. However, in reproductive senescent female-derived astrocytes, the LPS induced increase in MMP-9 was exacerbated in the presence of ethanol. Interestingly, in astrocytes derived from young adults and reproductive senescent females ethanol had no effect on the LPS-induced increase in nitric oxide (NO).

Inflammation is a necessary process for the brain to fight infection or viruses and to remove cellular debris following injury. Prolonged inflammation, however, can lead to neurodegeneration. This data suggests that, in female adult rat astrocytes, LPS is able to stimulate NO in the presence or absence of ethanol. However, matrix metalloproteinase activity may be differentially regulated in these two physiologically distinct female populations. Thus ethanol may potentially have a more detrimental impact on the mobility of astrocytes and on the blood brain barrier in the aging brain.

To Danielle Lewis, my friend and mentor throughout the entire project

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I would also like to thank the Department of Neuroscience and Experimental Therapeutics at the Texas A&M University Health Science Center and all of the Faculty and Staff. I also want to thank the John William Warren III Brain Tumor Research Memorial Fund and NIH Grant AG19515 for the financial support to complete the project. I also want to thank my friends and family for their encouragement and support.

Finally I would like to extensively thank Danielle Lewis for her help and guidance throughout the entire project.

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NOMENCLATURE

LPS	Lipopolysaccharide
MMP-9	Matrix Metalloproteinase Nine
MMP-2	Matrix Metalloproteinase Two
GFAP	Glial Fibrillary Acidic Protein
iNos	Inducible Nitric Oxide Synthase
ECM	Extracellular Matrix

CHAPTER I

INTRODUCTION[1]

Aging is a natural process that affects the functionality of the brain. In females, advancing age is accompanied by a subsequent decline in hormones such as estrogen, which signals the onset of menopause. This loss of hormone can have a significant impact as estrogen has been shown to be neuroprotective following ischemia [31,12] and excitotoxic brain injury [34]. For instance, in young adult female rats, estrogen attenuated the pro-inflammatory cytokine interleukin-1beta (IL-1b) following an excitotoxic injury but exacerbated IL-1b in reproductive senescent female rats [26]. In this study, we compared the effects of ethanol on two physiologically distinct female populations, young adult, reproductively competent, normal cycling females and reproductive senescent female rats. The reproductive senescent female rats are not chronologically aged but are characterized by their reproductive status (see experimental methods, animals). In essence, the reproductive senescent females mimic aspects of human menopause. Estrogen administered at the time of menopausal onset can be beneficial to the health of the female and may reduce the risk of neurodegenerative disorders such as Alzheimer's and help prevent cognitive decline [32].

Within the central nervous system, glial cells such as astrocytes actively help with the growth and maintenance of neurons. Research has shown that astrocytes play an important role in synaptic repair [29], plasticity [29], and particularly, inflammation. Astrocytes are an important component of the blood brain barrier and are known to

[1] This thesis follows the format of *Neurobiology of Aging.*

increase in activity by increasing expression of GFAP during normal aging [29]. GFAP is an astrocyte specific intermediate filament that increases in expression during aging [6,36] and following neurodegeneration [13]. Since astrocytes play an important role in the process of inflammation within the central nervous system, they are a prime target for research. How astrocytes adapt to the natural aging process is an area that is not well understood.

Ethanol consumption has been known to cause damage to the central nervous system [25], compound the risk for breast cancer [33] and affect osteoporosis [35] in post-menopausal women. The combination of increased ethanol consumption and aging could be potentially damaging to the brain especially following injury. Current research in astroglial cultures has shown that acute ethanol exposure disrupts inflammatory gene expression [10] and induces pro-inflammatory responses [10]. One important effect of ethanol on inflammation in astroglial cells is that it enhances the production of the transcription factor NF-kB via cytokines [10]. NF-kB is a key transcription factor that promotes transcription of several inflammatory markers, particularly inducible nitric oxide synthase (iNOS) [40], which is the enzyme that produces nitric oxide. Nitric oxide is an inflammatory marker produced by astrocytes and microglia in response to injuries in the central nervous system.

The objective of this research was to determine if alcohol interacting with age affects the inflammatory response to LPS in astrocytes derived from reproductive senescent and young adult female rats. Alcohol and age are important factors to study because they can directly affect how the brain responds to inflammatory insults.

CHAPTER II

EXPERIMENTAL DESIGN

Animal Model: The study employed an established animal model routinely used in the laboratory [16]. This animal model consisted of two separate age groups. The young adult age group is designed to mimic normal cycling females that produce estrogen naturally. The female rat estrous cycle consists of four stages: proestrus, estrus, metestrus and diestrus. Normal cycling females complete one menstrual cycle every four to five days. Since these females cycle regularly, they are considered reproductively competent and are approximately three to 4+ months in age [26]. The reproductive senescent group is designed to model post-menopausal females. The reproductive senescent females, are nine to twelve months in age, were previously reproductively competent, and must have subsequently had at least two reproductive failures [16]. Rats normally transition from the normal four day cycle to state of constant estrus and then finally end in a state of constant diestrus [20]. Daily vaginal smears were taken to determine cyclicity/acyclicity in young adult and reproductive senescent animals.

All female rats were purchased from Harlan Laboratories (IN) and were given food and water ad libitum. The animals were maintained in an AALAC-approved facility and all procedures were in accordance with NIH and institutional guidelines governing animal welfare.

Primary Cultures: Primary astrocytes were extracted from the olfactory bulb of the young adult and reproductive senescent female rats. The cells were cultured for ten days in serum containing medium that supports astrocyte growth ([1:1] DMEM:F12 medium, 4.6 g/mL glucose and 10% fetal bovine serum). The cells were fed every 3 to 4

days for the first 10 days. At day 10, astrocytes were re-plated at 50,000 cells per well, in 24-well tissue culture dishes coated with poly-D-lysine (50 μg/mL). The cells were switched to a non-serum containing media (Phenol-free Neurobasal medium, 0.50 mM L-glutamine, 1X N_2 supplement) and cultured for 6 additional days. The cells were treated with ethanol (70 mg/dL) and LPS (10 ug/mL) according to the treatment groups specified in Table 1, 24h prior to harvesting. The low dose of 70 mg/dL represents the blood alcohol content of a light social drinker [1]. After a twenty-four hour treatment period, the astrocyte-conditioned media was collected and stored at -20 ^{o}C. The cells were processed for immunohistochemistry.

Table 1. Treatment Paradigm

Control:	**Media only**
Control + LPS:	**Media + LPS (10 μg/mL)**
Control + EtOH:	**Media + Ethanol (70 mg/dL)**
Control + LPS + EtOH:	**Media + LPS(10 μg/mL) + Ethanol (70 mg/dL)**

Immunohistochemistry: In this study immunohistochemistry was utilized in order to determine the homogeneity of cells within the dish. Cultures were washed in 1X phosphate-buffered saline (dPBS, Invitrogen, CA) and fixed for one hour in 2% paraformaldhyde. The cells were labeled with the glial fibrillary acidic protein (GFAP, 1:80, Chemicon, CA), an astrocytic marker, and the nuclear stain Hoescht (1:500). Alexa Fluor 594 (1:2000, Molecular probes, CA) was used to visualize the GFAP labeling. One set of cells was exposed to the secondary antibody only to ensure that the staining was specific for GFAP. In this study, most of the cells labeled for GFAP indicating that they were astrocytes (Fig. 1).

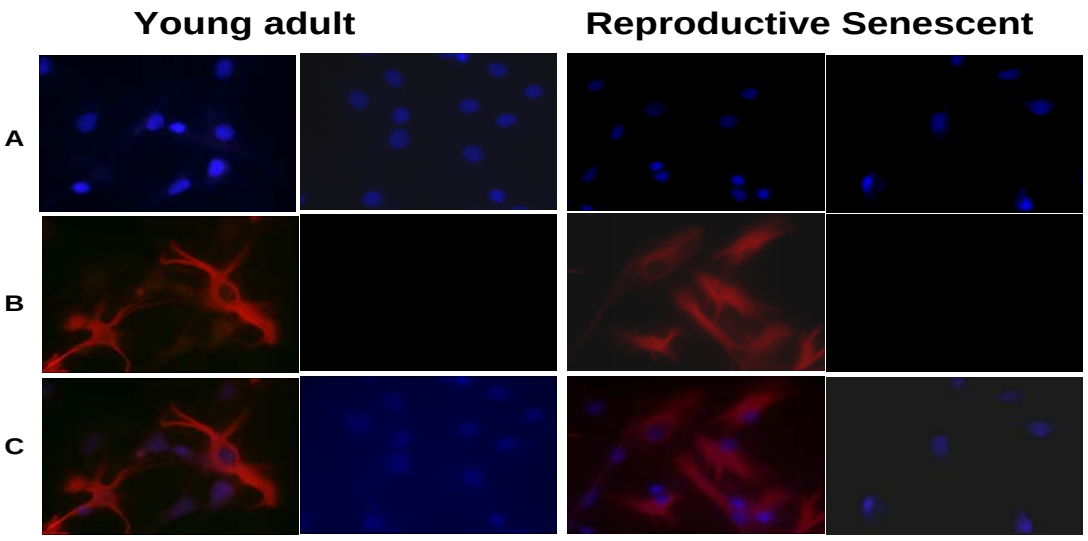

Figure 1: Primary astrocyte cultures from the olfactory bulb of young adult females stained for GFAP. Primary astrocyte cultures derived from young adults and reproductive senescent females were stained with the antibody to GFAP (b, red labeling), an astrocytic marker and Hoescht nuclear stain (a, blue labeling). Image (c) was taken with a filter that recognizes both GFAP and Hoescht. Images were captured with a 40X objective. The second column is the negative stain.

Astrocyte-Conditioned Media Analysis: Three inflammatory markers analyzed in this study were nitric oxide, MMP-9, and MMP-2. These inflammatory markers were measured in astrocyte-conditioned media. Nitric oxide is an important inflammatory mediator and the production of nitric oxide indicates that the LPS treatment was effective in stimulating an inflammatory response. This was analyzed by an ELISA assay (Cayman Chemical, MI). Matrix metalloproteinase lytic activity was measured in media using gelatin zymography.

Statistical Analysis: All statistical analysis was performed using a two-way ANOVA (dependent variables=LPS treatment and ethanol), using the software package SPSS® 11.0. Group differences were considered significant at $p \leq 0.05$.

CHAPTER III

NITRIC OXIDE

Nitric oxide is an important inflammatory mediator that is present within cultures after stimulation with lipopolysaccharide. Nitric oxide may also play a key role in biological aging as its concentration within the central nervous system increases with age and excessive levels can lead to neurodegeneration [18]. Since nitric oxide participates in inflammatory events as the brain ages, this study focused on nitric oxide and its role as an inflammatory marker.

A potential mechanism by which ethanol could affect the inflammatory cascade in the central nervous system is suggested by a recent study. In astroglial cells, ethanol was shown to increase NF-kB activity [10]. NF-kB activates many genes, particularly inducible nitric oxide synthase (iNOS), which is responsible for synthesizing nitric oxide present within the central nervous system and in response to injuries [7].

To measure the concentration of nitric oxide in the astrocyte-conditioned media, a colorimetric ELISA assay was used (Cayman Chemical, MI). Nitric oxide by nature is very reactive and is difficult to measure directly. Thus, this assay measures the concentration of nitrates and nitrites as they are the stable by-products of nitric oxide metabolism. Established laboratory procedures [17] and manufacturer's recommendations were followed.

Analysis of the astrocyte-conditioned media revealed that within the young adult treatment group, lipopolysaccharide (LPS) increased nitric oxide levels (Figure 2). This was also true for the reproductive senescent females (Figure 3). Furthermore, LPS significantly increased nitric oxide in the presence and absence of ethanol (Figure 2,3).

The LPS-induced increase in nitric oxide suggests that both young adult and reproductive senescent females show a normal inflammatory response [19].

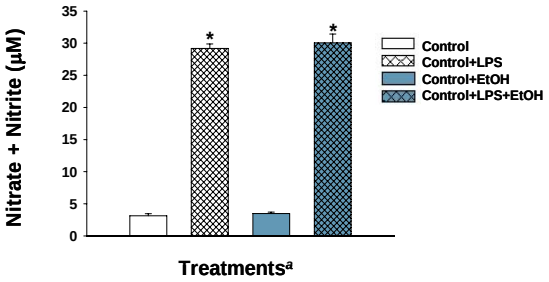

Figure 2: Nitric oxide expression following LPS treatment in astrocytes derived from young adult females. It is evident that LPS is increasing nitric oxide levels in the control and ethanol treated cultures. *a*=main effect of LPS, * indicates significant differences between treatments at $p \leq 0.05$. LPS=lipopolysaccharide (10mg/mL), EtOH = ethanol (70mg/dL)

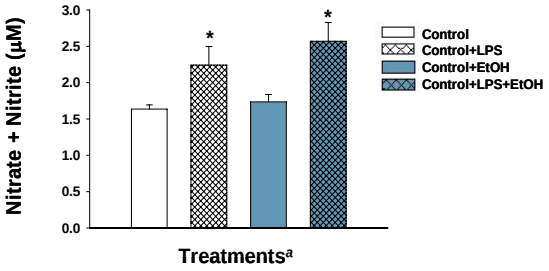

Figure 3: Nitric oxide expression following LPS treatment in astrocytes derived from reproductive senescent females. It is evident that LPS is increasing nitric oxide levels in the control and ethanol treated cultures. *a*=main effect of LPS, * indicates significant differences between treatments at $p \leq 0.05$. LPS=lipopolysaccharide (10mg/mL), EtOH = ethanol (70mg/dL)

However, contrary to what was observed in the study by Blanco et.al. (2004, 2005) and Davis & Syapin (2004), ethanol alone had no affect on nitric oxide production. The origin of the astrocytes, the timing of treatment and the ethanol dose may explain the

differences. In human A172 astroglial cells ethanol treatment resulted in increased protein expression of NF-κB following acute ethanol exposure [10]. As stated previously, NF-kB is the transcription factor required to transcribe the gene responsible for NO production [7]. In prenatal astrocyte cultures, a similar treatment regime resulted in increased expression of the inflammatory cytokine receptor, interleukin-1 beta receptor, NF-κB and a subsequent increase in iNOS [5] and cyclooxygenase 2 (COX-2) [4]. In both aforementioned studies, a 50mM dose of ethanol was used to generate the inflammatory response needed to increase iNOS activity. Further, Blanco et al. (2005) reported that an ethanol concentration of 10-50 mM was sufficient to stimulate the signaling response. The concentration of ethanol in our study was comparable to the lower concentration of ethanol (approximately 10-15 mM) used in the Blanco et al. (2005) study and our time points were also comparable. Thus, it is possible that adult rat astrocytes respond differently than human astroglial cells or prenatal astrocytes cultures.

It is also possible that in female rat astrocytes, ethanol affects other physiological events unrelated to nitric oxide. Research has shown that acute ethanol treatment inhibits the function of N-methyl-D-aspartate (NMDA) receptors causing an increase in glutamate-induced cytotoxic responses [11]. This in turn, can cause neuronal cells to become sensitive to excitotoxic insults and lead to ethanol-induced brain damage [14]. In addition to inhibiting NMDA receptors, ethanol has also been shown to reduce availability and alter the receptor function of brain-derived neurotrophic factors [8], and reduce the number of receptors for nerve growth factors [23], which could lead to impairment of intracellular signaling pathways that are responsible for controlling cell survival and death [14].

CHAPTER IV

MATRIX METALLOPROTEINASES

Matrix metalloproteinases are enzymes that are important for the normal functioning of the brain and are also involved in the inflammatory response following injury or in neurological diseases [21]. Research has shown that MMP's play a key role in angiogenesis, a process by which new blood vessels are synthesized [21]. It is known that matrix metalloproteinases degrade the extra cellular matrix therefore increasing blood brain barrier permeability [21]. The blood brain barrier is an important structure responsible for protecting the brain from toxins that may be floating freely within the blood stream along with other blood borne cells and proteins [38]. This structure is important because it is selectively permeable allowing molecules such as glucose to diffuse through, but blocks toxins from entering the central nervous system [24]. Matrix metalloproteinases, in particular, MMP-9 are responsible for cleaving the extra cellular matrix of astrocytes. Astrocytes and endothelial are essential components of the blood brain barrier [2], so elevated levels of MMP-9 could compromise the integrity of the blood brain barrier and cause astrocytes to become more mobile. MMP-9 is produced by astrocytes following an inflammatory stimulus [18]. As astrocytes are important for the integrity of the blood brain barrier, increases in MMP-9 could potentially be detrimental to the cell. MMP-2 is also important to the integrity of the central nervous system because it also cleaves extracellular matrix molecules aiding in synaptic remodeling [27].

MMP-2 and MMP-9 degrade gelatin so their lytic activity can be measured using gelatin zymography. The astrocyte-conditioned media was fractioned on a 10% SDS polyacrylamide gel containing 0.1% gelatin. The polyacrylamide gel was then incubated

overnight at 25°C in developing buffer containing 50mM Tris, 0.2M NaCl, 5mM $CaCl_2$, 0.02% Brij 35. Following the overnight incubation, the lytic activity was visualized by staining the gels with a 0.25% coomassie blue solution (50% methanol, 20% acetic acid) for 30 minutes and destaining in 20% methanol: 10% acetic acid until the bands were distinct. The gels were dried, scanned and quantified using the Molecular Analyst Program (BioRad, CA).

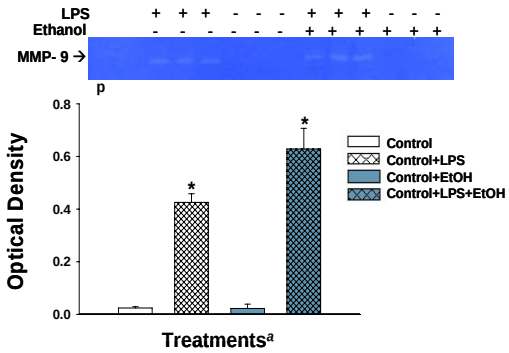

Figure 4: MMP-9 lytic activity increases with LPS treatment in astrocytes derived from young adult female rats. *a*=main effect of LPS; * indicates significant differences between treatments at p≤0.05. Ethanol and LPS treatments are indicated by a (+) or (-). p=positive; LPS=lipopolysaccharide (10mg/mL); EtOH = ethanol (70mg/dL)

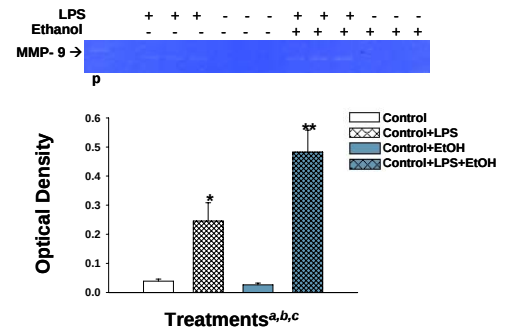

Fig 5. MMP-9 lytic activity increases in astrocytes derived from reproductive senescent female rats. *a*=main effect of LPS, *b*=main effect of EtOH, *c*=interaction LPS x EtOH; * indicates significant differences between treatments at p≤0.05. Ethanol and LPS treatments are indicated by a (+) or (-). p=positive; LPS=lipopolysaccharide (10mg/mL); EtOH = ethanol (70mg/dL)

Data from this study revealed that LPS increased MMP-9 levels in the young adults (Figure 4) and reproductive senescent (Figure 5) astrocytes. However, in reproductive senescent females, ethanol enhanced the LPS-induced increase in MMP9 in control treated cultures. While the LPS increase in MMP-9 was expected, the ethanol enhanced increase in LPS induced MMP-9 was novel. This suggests that ethanol might activate specific signaling pathways in the aging astrocyte. This may be a consequence perhaps, of age-related changes in ethanol metabolism. Further research will be needed to investigate these effects.

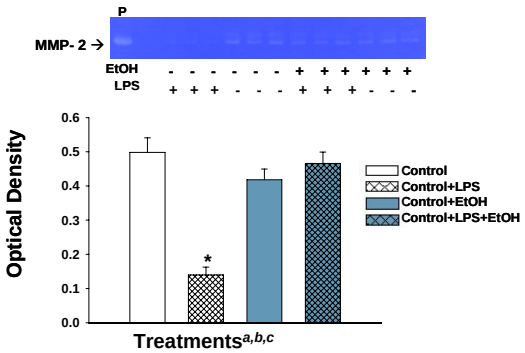

Fig 6. MMP-2 lytic activity decreases in astrocytes derived from reproductive senescent female rats. LPS decreased MMP-2 lytic activity in the absence of ethanol, but had no effect on MMP-2 in the presence of ethanol. *c*=interaction of LPS vs EtOH; * indicates significant differences between treatments at $p \leq 0.05$. Ethanol and LPS treatments are indicated by a (+) or (-). p=positive; LPS=lipopolysaccharide (10mg/mL); EtOH = ethanol (70mg/dL)

MMP-2 activity was also determined by gelatin zymography using one modification of the above described procedure. Since MMP-2 lytic activity is more abundant in astrocyte media, the volume of astrocyte-conditioned media was reduced to 10µl for these assays. The results from this analysis revealed that in young adult and

reproductive senescent females, LPS decreased MMP-2 production in the absence of ethanol.

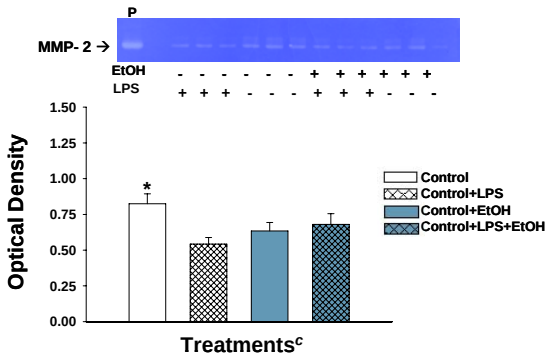

Fig 7. MMP-2 lytic activity decreases in astrocytes derived from young adult female rats LPS decreased MMP-2 lytic activity in the absence of ethanol, but had no effect on MMP-2 in the presence of ethanol. *c*=interaction of LPS vs EtOH; * indicates significant differences between treatments at $p \leq 0.05$. Ethanol and LPS treatments are indicated by a (+) or (-). p=positive; LPS=lipopolysaccharide (10mg/mL); EtOH = ethanol (70mg/dL)

Figure 6 and 7 illustrates these effects. While MMP-2 has been implicated in synaptic remodeling [27] very little research has been conducted on the lytic activity of MMP-2 following and inflammatory event. When taken into context, the LPS induced decrease in MMP-2 may shed some light on cell function when challenged with a stressor such as LPS. At the time of injury, it may not be beneficial for the cell to divert energy to synthesize MMP-2 for synaptic remodeling. The cell may instead need to invest it energy into synthesizing MMP-9 in and effort to mobilize astrocytes to the site of injury. This data suggests the possibility of a molecular switch within the central nervous system in response to injuries. The present data also suggests ethanol prevents this switch and may therefore cause unnecessary expenditure of cell resources.

CHAPTER V

CONCLUSIONS

Inflammation is a necessary process for the brain to fight infection or viruses and to remove cellular debris following injury [37]. The inflammatory markers analyzed in this study are necessary players in the inflammation signaling process of the central nervous system. Prolonged inflammation caused by excessive production of nitric oxide or MMP-9, however, can lead to neurodegeneration [15]. It is important to note that in both treatment groups, young adult and reproductive senescent females, inflammation was successfully achieved with LPS treatment. This was confirmed by the increase in nitric oxide and MMP-9 levels in control and ethanol-treated astrocyte cultures. Among the two treatment groups, the reproductive senescent females were the most responsive to ethanol treatment suggesting that alcohol effects aged astrocytes differently than it affects young adults.

In our study, ethanol did not have a strong effect on the primary astrocyte cultures derived from young adult animals. It is possible ethanol may be effecting these astrocytes in another way. Astrocytes are important support cells that are responsible for maintaining the environment for surrounding neurons [3]. Astrocytes are responsible for maintaining synaptic repair [29], synaptic plasticity [29], regulation of the neurotransmitters L-glutamate and γ-aminobutyric acid (GABA) [3], and regulation of extracellular potassium and calcium concentrations [3]. Thus future studies will focus on the "repair" function of astrocytes.

One of the most important functions of astrocytes is their participation in the permeability of the blood brain barrier [3]. The blood brain barrier consists of endothelial cells, astrocytic end feet and pericytes [2]. In this study, the LPS-induced increase in MMP-9 in senescent females was exacerbated by ethanol treatment. The data suggests that ethanol treatment in turn could compromise the integrity of the blood brain barrier following an LPS insult by allowing the astrocytes to become more mobile. Increasing permeability of the blood brain barrier could leave the central nervous system vulnerable to infection and damage.

The reported increase in MMP-9 with LPS treatment is consistent with other reports in the current literature (e.g. [18]). However, in the aforementioned study, no change in MMP-2 expression was observed when the cultures were treated with LPS [18]. This contrasts with the findings of the present study. However, these data are consistent with Wright et.al. (2003) where ethanol failed to regulate MMP-2 in the hippocampus and pre-frontal cortex [39].

The results from this study provide an important new avenue for research in the context of astrocyte function following and inflammatory stimulus. All the present analysis was conducted on media from the astrocyte cultures harvested 24h following the initial insult with LPS. Thus, this represents only a small window for astrocyte response to LPS and treatment. The levels of NO and MMP's reported here may be rising and falling during this time period. It is possible that the peak ethanol effects of treatment on the cultures occur at another time point not examined in this study. Future research will therefore need to focus on a range of time points of analysis.

Future studies should also take into consideration different doses of ethanol that correspond to clinically-relevant drinking patterns in the human population. As pointed out earlier, the 70 mg/dL dose used in this study was used to model the blood alcohol content of a moderate social drinker [1]. Previously published reports by Blanco et.al. (2004 & 2005) and Davis & Syapin (2004) reported higher acute ethanol doses in order to generate an ethanol-related response via inflammatory markers [4,5,9]. It is possible that the ethanol dose used in this study was not significant enough to invoke the increases in inflammatory markers that were expected.

REFERENCES

[1] Adachi J, Mizoi Y, Fukunaga T, Ogawa Y, Ueno Y, Imamichi H. Degrees of alcohol intoxication in 117 hospitalized cases. J Stud Alcohol 1991; 52(5): 448-53.

[2] Ballabh P, Braun A, Nedergaard M. The blood-brain barrier: an overview structure, regulation, and clinical implications. Neurobiol Dis 2004; 16(1):1-13.

[3] Benarroch EE. Neuron-astrocyte interactions: partnership for normal function and disease in the central nervous system. Mayo Clin Proc 2005; 80(10): 1326-1338.

[4] Blanco AM, Pascual M, Valles SL, Guerri C. Ethano-inducted iNOS and COX-2 expression in cultured astrocytes via NF-kB. Neuroreport 2004; 15(4): 681-5.

[5] Blanco AM, Valles SL, Pascual M, Guerri C. Involvement of TLR4/type I IL-1 receptor signaling in the induction of inflammatory mediators and cell death induced by ethanol in cultured astrocytes. J Immunol 2005; 175(10): 6893-9.

[6] Bronson RT, Lipman RD, Harrison DE. Age-related gliosis in the white matter of mice. Brain Res 1993; 609(1-2): 124-8.

[7] Chao CC, Lokensgard JR, Sheng WS, Hu S, Peterson PK. IL-1-induced iNOS expression in human astrocytes via NF-kB. Neuroreport 1997; 8: 3163-3166.

[8] Climent E, Pascual M, Renau-Piqueras J, Guerri C. Ethanol exposure enhances cell death in the developing cerebral cortex: role of brain-derived neurotrophic factor and its signaling pathways. J Neurosci Res 2002; 68: 213-225.

[9] Davis RL, Syapin PJ. Ethanol increases nuclear factor-kB activity in human astroglial cells. Neurosci Lett 2004; 371(2-3): 128-32.

[10] Davis RL, Syapin PJ. Interactions of alcohol and nitric-oxide synthase in the brain. J Brainresrev 2005; 49: 494-504.

[11] Dodd PR, Beckmann AM, Davidson MS, Wilce PA. Glutamate-mediated transmission, alcohol, and alcoholism. Neurochem Int 2000; 37: 509-533.

[12] Dubal DB, Kashon ML, Pettigrew LC, Ren JM, Finklestein SP, Rau SW, Wise PM. Estradiol protects against ischemic injury. J Cereb. Blood Flow Metab 1998; 18:1253-1258.

[13] Fujita K, Yamauchi M, Matsui T, Titani K, Takahashi H, Kato T, Isomura G, Ando M, Nagata Y. Increase of glial fibrillary acidic protein fragments in the spinal cord of motor neuron degeneration mutant mouse. Brain Res 1998; 785(1): 31-40.

[14] Harper C, Matsumoto I. Ethanol and brain damage. Curr Opin Pharmacol 2005; 5(1): 73-78.

[15] Ilzeka J, Stelmasiak Z, Dobosz B. Matrix metalloproteinase-9 (MMP-9) activity in cerebrospinal fluid of amyotrophic lateral sclerosis patients. Neurol Neurochir Pol 2005; 35: 1035-1043.

[16] Jezierski MK, Sohrabji F. Neurotrophin expression in the reproductively senescent forebrain is refractory to estrogen stimulation. Neurobiol Aging 2001; 22(2): 309-319.

[17] Johnson AB, Sohrabji F. Estrogen's effects on central and circulating immune cells vary with reproductive age. Neurobiol Aging 2005; 26(10): 1365-74.

[18] Lee WJ, Shin CY, Yoo BK, Ryu JR, Choi EY, Cheong JH, Ryu JH, Ko KH. Induction of matrix metalloproteinase-9 (MMP-9) in lipopolysaccharide-stimulated primary astrocytes is mediated by extracellular signal-regulated protein kinase 1/2 (Erk1/2). Glia 2003; 41(1): 15-24.

[19] Lee SC, Dickson DW, Liu W, Brosnan CF. Induction of nitric oxide synthase activity in human astrocytes by interleukin-1 beta and interferon-gamma. J Neuroimmunol 1993; 46(1-2): 19-24.

[20] LeFevre J, McClintock MK. Reproductive senescence in female rats: a longitudinal study of individual differences in estrous cycles and behavior. Biol Reprod 1988; 38(4): 780-9.

[21] Mandal M, Mandal A, Das S, Chakraborti T, Chakraborti S. Clinical implication of matrix metalloproteinases. Mol Cell Biochem 2003; 252: 305-329.

[22] Mendelowitsch A, Ritz M-F, Ros J, Langemann H, Gratzl O. 17β-estradiol reduces cortical lesion size in the glutamate excitotoxicity model by enhancing extracellular lactate: a new neuroprotective pathway. Brain Res 2001; 901:230-236.

[23] Miller R, King MA, Heaton MB, Walker DW. The effects of chronic ethanol consumption on neurotrophins and their receptors in the rat hippocampus and basal forebrain. Brain Res 2002; 950: 137-147.

[24] Mun-Bryce S, Rosenberg GA. Gelatinase B modulates selective opening of the blood brain barrier during inflammation. Am J Physiol 1998; 274(5pt2): R1203-11.

[25] Neiman J. Alcohol as a risk factor for brain damage: neurologic aspects. Alcohol Clin Exp Res 1998; 22(7suppl): 346S-351S.

[26] Nordell, VL, Scarborough MM, Buchanan AK, Sohrabji F. Differential effects of estrogen in the injured forebrain of young adult and reproductive senescent animals. Neurobiol. Aging 2003; 5798:1-11.

[27] Paggi P, Destefano ME, Petrucci TC. Synaptic remodeling induced by axotomy of superior cervical ganglion neurons: involvement of metalloproteinase-2. J Physiol Paris 2006; 99(2-3): 119-24.

[28] Pawlak, J, Brito V, Kuppers E, and Beyer C. Regulation of glutamate transporter GLAST and GLT-1 expression in astrocytes by estrogen. Brain Res. Mol. Brain Res 2004; 1381: 1-7.

[29] Rozovsky, I, Wei M, Morgan T, and Caleb E. Finch. 2005. Reversible age impairments in neurite outgrowth by manipulations of astrycytic GFAP. Neurobiol. Aging 2005; 5: 705-15.

[30] Shumaker SA, Legault C, Rapp SR, Thal L, Wallace RB, Ockene JK, Hendrix SL, Jones III, BN, Assaf AR, Jackson RD, Kotchen JM, Wassertheil-Smoller S, Wactawski-Wende J. Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women. The women's health initiative memory study: a randomized controlled trial. JAMA 2003; 289: 2651-2662.

[31] Simpkins JW, Rajakumar G, Zhang Y-Q, Simpkins CE, Greenwald D, Yu CJ, Bodor N, Day AL. Estrogens may reduce mortality and ischemic damage caused by middle cerebral artery occlusion in the female rat. J. Neurosurg 1997; 87:724-730.

[32] Simpkins JW, Yang SH, Wen Y, Singh M. Estrogens, progestins, menopause and neurodegeneration: basic and clinical studies. Cell Mol Life Sci 2005; 62(3): 271-80.

[33] Singletary KW, Gapstur SM. Alcohol and breast cancer: review of epidemiologic and experimental evidence and potential mechanisms. JAMA 2001; 286(17): 2143-2151.

[34] Sohrabji F, Peeples KW, Marroquin OA. Local and cortical effects of olfactory bulb lesions on trophic support and cholinergic function and their modulation by estrogen. J. Neurobiol 2000; 45:61-74.

[35] Turner RT, Sibonga JD. Effects of alcohol use and estrogen on bone. Alcohol Res Health 2001; 25(4): 276-81.

[36] Wagner AP, Reck G, Platt D. Evidence that V+ fibronectin, GFAP and S100 beta mRNAs are increased in the hippocampus of aged rats. Exp Gerontol 1993; 28(2): 135-43.

[37] Wang T, Rumbaugh JA, Nath A. Viruses and the brain: from inflammation to dementia. Clin Sci (Lond) 2006; 110(4): 393-407.

[38] Wilson CJ, Finch CE, Cohen HJ. Cytokines and cognition-the case for a head-to-toe inflammatory paradigm. J Am Geriatr Sci 2002; 50(12): 2041-56.

[39] Wright JW, Masino AJ, Reichert JR, Turner GD, Meighan SE, Meighan PC, Harding JW. Ethanol-induced impairment of spatial memory and brain matrix metalloproteinases. Brain Res 2003; 963(1-2) 252-61

[40] Xie QW, Kashiwabara Y, Nathan C. Role of transcription factor NF-kappa B/Rel in induction of nitric oxide synthase. J Biol Chem 1994; 269(7): 4705-8.

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