Spring 2007

The Effect of Advanced Glycation End Product Inhibition on the Development of Diabetic Encephalopathy in Rats

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The Effect of Advanced Glycation End Product Inhibition on the Development of Diabetic Encephalopathy in Rats.

Submitted in Partial Fulfillment of the Requirements for Graduation with Honors to the Department of Natural Sciences at Carroll College, Helena, Montana

Nathanael D. Gay
April 4, 2007
This thesis for honors recognition has been approved for the

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4-5-07

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4/5/2007

Dr. Ron Wilde, Reader
4/5/07
Acknowledgements

I would like to thank Dr. Brehe for her continual support and assistance throughout the duration of this study. Without her expertise and knowledge this study could not have been accomplished. I would also like to thank Dr. Shields who both allowed for flexibility in his correlating course work and consistently gave me constructive criticism on all the drafts of this thesis. I would also like to thank the Carroll College biology program for funding of this project along with the laboratory space and equipment with which to perform the study. Finally, I would like to thank my father, James Gay; without whose help the Morris Water Maze could not have been implemented.
Abstract

Advanced glycation end product (AGE) endogenous proliferation has been identified as a result of long term hyperglycemia associated with diabetes mellitus. These products have been linked to various problems of aging and diabetes. This study used diabetic and non-diabetic rats. Each group received either the placebo, or the AGE inhibitor, aminoguanidine. The animals were then cognitively evaluated after eight weeks. I hypothesized that, diabetic rats receiving aminoguanidine would show reduced cognitive decline as compared with diabetic rats not receiving aminoguanidine. My observations support my hypothesis.
# Table of Contents

Acknowledgments........................................................................................................... ii
Abstract........................................................................................................................... iii
Table of Contents........................................................................................................ iv
List of Tables.................................................................................................................. v
List of Figures................................................................................................................ vi
Introduction................................................................................................................... 1
Literature Review......................................................................................................... 3
Materials and Methods............................................................................................... 14
Results........................................................................................................................... 16
Discussion....................................................................................................................... 26
Literature Cited.............................................................................................................. 29
List of Tables

Table 1: ANOVA results for the time to learn the maze for the four different treatment groups.................................................................19

Table 2: ANOVA results for the average time to complete the maze of the four different treatment groups over the 17-day testing period.................................................................19

Table 3: Multiple comparisons, from Tukey HSD analysis, between normal (non-diabetic) controls (no aminoguanidine), diabetic controls, normal with aminoguanidine treatment, and diabetic with aminoguanidine treatment, for times to complete the maze for the 17 day period.................................................................20

Table 4: Multiple comparisons, from Tukey HSD analysis, between normal (non-diabetic) controls (no aminoguanidine), diabetic controls, normal with aminoguanidine treatment, and diabetic with aminoguanidine treatment, for time to learn maze in days..........................................................................................................................20
List of Figures

Fig. 1: Average number of days taken for rats to complete the maze in 20 seconds or less for three consecutive days.................................................................21

Fig. 2: Average Morris water maze times for all groups........................................22

Fig. 3: Average Morris water maze times for diabetic control and diabetic aminoguanidine groups...........................................................................................................23

Fig. 4: Average Morris water maze times for normal control and diabetic control groups.........................................................................................................................24

Fig. 5: Average Morris water maze times for normal control and diabetic aminoguanidine groups...........................................................................................................25
Introduction

An estimated 180 million humans have some form of diabetes. This condition is defined as a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (Committee, 2003). The hyperglycemia induced by diabetes is known for its resultant complications including, but not limited to: coronary artery disease, arteriosclerosis, nephropathy, neuropathy, peripheral vascular disease, and retinopathy (WHO, 2006). Long-term hyperglycemia has various detrimental effects with root causes tied strongly to endogenous advanced glycation end product (AGE) formation via the Millard reaction (Vlassara, 2003). A reaction of carbohydrates with amino groups of proteins causes the production of AGEs (Wautier, 2001). The oxidative stress that these glycation products put on the body are a primary source of functional change produced in the physiology of hyperglycemic individuals.

The existence of cognitive decline in hyperglycemic individuals, known as diabetic encephalopathy, has been established (Biessels, 1996). Observations have been made that link physiologic hyperglycemic conditions with changes in cell morphology in the hippocampus region of the brain (Magarinos, 2000). Diabetic rats have deficits in learning and memory (a function for which the hippocampus is required) as indicated by decreased maze performance (Biessels, 1996).

Aminoguanidine is a well known inhibitor of AGE formation (Vlassara, 2003). The terminal amino group of this compound prevents detrimental cross-linking of proteins
within the body (Vlassara, 2003). Vascular problems caused by diabetes are prevented by treatment with aminoguanidine (Vlassara, 2003).

Hyperglycemia has thus been linked to cognitive decline and structural changes in the central nervous system. The mechanism by which this diabetic encephalopathy occurs remains elusive. AGE’s have a close affiliation with vascular alterations and nephropathic conditions as mentioned. The question remains whether these products are responsible for the processes that contribute to cognitive impairment in diabetics.

I hypothesize that streptozotocin-induced diabetic rats receiving the drug aminoguanidine will show reduced cognitive decline as compared with streptozotocin induced diabetic rats receiving a placebo.
Literature Review

Diabetes

Diabetes mellitus is defined as a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (Committee, 2003). There are three main classes of diabetes. One of these classes consists of type 1 diabetes (Committee, 2003). This class of diabetes results from pancreatic β-cell destruction. People with type 1 diabetes are prone to ketoacidosis (Committee, 2003). The most prevalent class of diabetes is type 2, which results from insulin resistance coupled with a lack of insulin secretion (Committee, 2003). Gestational diabetes mellitus is another class which is characterized by glucose intolerance during pregnancy which can either disappear or continue after pregnancy (Committee, 2003). Individuals may fit into more than one of the previously described classes at a given time, and determining the type of diabetes that an individual is experiencing depends on the circumstances present at the time of diagnosis (Committee, 2003).

Type 1 diabetes, the class of diabetes which the experimental conditions in this study replicated, consists of variable β-cell destruction between individuals (Committee, 2003). Type 1, also identified by the names insulin-dependant or juvenile-onset diabetes, is the product of a cellular mediated auto-immune destruction of insulin secreting β-cells (Committee, 2003). Although β-cell destruction is initially variable and insulin
requirement for survival may be delayed, most type 1 diabetics will require insulin for survival and be at risk for ketoacidosis at some point (Committee, 2003).

Type 2 diabetes (also known as adult on-set or non-insulin dependant diabetes) appears to have many different causes not including autoimmune destruction of β-cells. The majority of people with type 2 diabetes are obese (Committee, 2003). Obesity alone causes insulin resistance. Concentration of fat in the abdominal regions in non-obese people can lead to insulin resistance in individuals lacking a standard obese classification (Committee, 2003). Due to the gradual development of symptoms, this form of diabetes may go undiagnosed in individuals for long periods of time (Committee, 2003). There is a stronger genetic component for this class of diabetes than for type 1, however the genetics appear complex and an explanation has not been elucidated (Committee, 2003). It should be emphasized both that the risk of developing this form of diabetes increases with age, obesity and deficiency of physical activity and that the physical complications associated with type 2 diabetes are similar with the other classes of diabetes discussed (Committee, 2003). Over 90% of type 2 diabetes cases occur in older adults (Pompei, 2006). Aging has been associated with glucose intolerance by way of reduction in pancreatic function as well as a decline in the insulin-signaling mechanisms. These mechanisms limit the mobilization of glucose transporters needed for insulin-mediated glucose uptake and metabolism in muscle and fat (American Diabetes Association, 2006).
**Streptozotocin**

In the present study, rats were induced with diabetes through the injection of a compound called streptozotocin. Streptozotocin along with alloxan are two of the most widely recognized and experimentally potent diabetogenic agents known (Takasu, 1991). Streptozotocin acts in several characteristic ways which ultimately lead to β-cell destruction and/or lack of insulin secretion. The cytotoxic characteristics of Streptozotocin are due to DNA alkylation as well as DNA damage brought about through increased intracellular concentrations of nitric oxide and reactive oxygen species. Streptozotocin is absorbed by pancreatic β-cells by way of the glucose transporter GLUT2 (Szkudelski, 2001). Once inside the cell streptozotocin induces changes in the DNA of pancreatic β-cells which leads to its fragmentation (Szkudelski, 2001). The main reason for β cell death after streptozotocin exposure has now been experimentally attributed to alkylation of DNA (Szkudelski, 2001). Streptozotocin has been shown to alkylate the DNA via its nitrosourea moiety at the sixth oxygen position of guanine (Szkudelski, 2001). Pancreatic β-cells that were exposed experimentally to streptozotocin also displayed a reaction that was similar to that of nitric oxide action (Szkudelski, 2001). Nitric oxide is liberated when streptozotocin is metabolized inside cells and thereby brings about the death of pancreatic islet cells (Szkudelski, 2001). Further, streptozotocin has been found to also produce reactive oxygen species that play a role in DNA fragmentation along with other negative cell effects (Szkudelski, 2001). Streptozotocin also acts by inhibiting the Krebs cycle. Oxygen consumption by the mitochondria is
therefore reduced which in turn causes an increase in the formation of superoxide anions (Szkudelski, 2001). The DNA damage induced by both nitric oxide and reactive oxygen species activates poly ADP-ribosylation (Szkudelski, 2001). This leads to the exhaustion of cellular NAD+, further drop of the ATP content, and succeeding inhibition of insulin production and secretion from β cells (Szkudelski, 2001).

**Diabetic Complications**

Diabetes causes chronic hyperglycemia which is associated with a myriad of ailments (David, 2004). Diabetic retinopathy is a condition that affects the blood vessels in the retina (David, 2004). Causes of retinopathy remain unknown however two stages are characteristic of retinopathic development (David, 2004). The first consists of leaking of small vessels and creation of deposits of protein and fat which can impair vision, and the second is characterized by severely diminished blood flow that causes the retina to try to repair itself, leading to further complications and often vision loss (David, 2004). Other eye conditions that have been observed at higher rates in people with diabetes include cataracts and glaucoma (David, 2004).

Neuropathy, which is damage to the nervous tissue, is also a result of hyperglycemic conditions associated with diabetes. Again, the actual mechanism by which this damage occurs remains unknown, however some suggest that it may occur when glucose attaches to or affects proteins in nerve cells (David, 2004). Peripheral neuropathy is especially
prevalent and affects feeling and sensitivity in the extremities of diabetics due to nerve damage (David, 2004).

Kidney disease occurs in about 10% to 35% of people with diabetes and can lead to total loss of kidney function (David, 2004). Capillary membranes thicken, and the glomeruli can become damaged and distorted in response to extended exposure to hyperglycemic conditions (David, 2004). The hyperglycemic state appears to affect blood pressure control and causes hypertension in a long term diabetic state (Brands, 2001). Although a direct mechanism by which glucose can increase blood pressure has not been elucidated, it appears that hyperglycemia may stimulate a hypertensive agent such as Angiotensin II and then as progressive glomerular injury occurs, sustained hypertension results (Brands, 2001).

Seventy five percent of people with diabetes die from heart disease or stroke (David, 2004). Type 2 diabetes, specifically, is often associated with diverse heart disease risk factors such as high blood pressure, high cholesterol, high triglycerides, and obesity (David, 2004). Additional contributory factors to cardiovascular disease in diabetics include kidney disease, increased tendency to form clots, increase oxidation tendency, and amplified inflammatory damage (David, 2004).

Essential to this study is the existence of yet another important, yet more subtle and less understood complication of diabetes. Insulin regulation problems have been associated with an increased risk for cognitive impairment and neurodegenerative disorders.
including Alzheimer’s disease (Friedrich, 2006). Type 2 diabetics have been shown to display lower cognitive scores when compared with non-diabetics (Strachan, 2003). Links have also been made between type 2 diabetes and Alzheimer’s disease. Plasma concentrations of both insulin and amyloid β42 (Aβ42), which are the major pathogenic events associated with diabetes and Alzheimer’s respectively, have been shown to have a significant positive linear correlation with one another (Odetti, 2005). Epidemiological studies further show that type 2 diabetes and hyperinsulinemia are risk factors for sporadic Alzheimer’s disease (Odetti, 2005). The link is revealed through evidence that both insulin and Aβ42 are degraded by the same insulin degrading enzyme (IDE), and that a polymorphism of the IDE region on chromosome 10 has been genetically associated with type 2 diabetes as well as late-onset Alzheimer’s (Odetti, 2005).

In addition, type 2 diabetic obese rat performances on learning and memory tests as compared with performance of normal rats show an impaired performance among the obese rats in functions requiring the hippocampus (Winocur, 2005). Fasting blood glucose reduction in human diabetic patients via insulin and oral treatments has also shown a correlation with increased performance on cognitive tests (Araki, 2004).
**Advanced Glycation End Products**

Compounds termed advanced glycation end products (AGEs) have been demonstrated to be causative agents in many of the aforementioned diabetic complications (Sharp, 2003). These products are formed in both diabetics and aging non-diabetics because of long term exposure of proteins, lipids, and nucleotides to glucose (Sharp, 2003). AGE’s are formed via a non-enzymatic Maillard reaction that occurs between the amino groups on the specific macromolecule and the reducing sugar (Thomas, 2005.) Depending on the type of macromolecular substrate, different stable products, known as Amadori products, will initially result from the Maillard reaction (Thomas, 2005). The formation of AGEs from these Amadori products results from subsequent dehydration, oxidation, rearrangement, and fragmentation reactions which occur very slowly within the human body (Thomas, 2005). In both type 1 and type 2 diabetes, the formation of AGEs is accelerated resulting in modification of both short-lived and long-lived proteins (Forbes, 2004).

AGEs have been implicated in a variety of pathogenic pathways associated with diabetes. The process of AGE formation has deleterious effects on platelet function, coagulation, and fibrinolytic systems which cause cardiovascular disease (Takenaka, 2006). AGE accumulation also occurs within pericytes (which reside in the retina) and have a detrimental effect on their survival and function (Yamagishi, 2006). The AGE induced mechanism of diabetic retinopathy includes platelet aggregation and fibrin stabilization which contribute to the retinopathy via thrombogenesis (Yamagishi, 2006). It has been shown that AGEs act on the central nervous system. These actions include:
neurotoxicity, increased oxidative stress, and acceleration of aggregation and cross-linking of soluble B-amyloid peptides.

AGEs accumulate in senile amyloid plaques and neurofibrillary tangles in Alzheimer’s disease, suggesting a possible role of AGEs in cognitive impairment (Araki, 2004). As aging occurs in normal humans, AGEs accumulate in central nervous tissue to such a degree that immunohistochemical analysis of AGE concentration within pyramidal neurons of the CA4 regions of the brain yields information about the age of unknown cadavers (Sato, 2001). More specifically, AGEs such as imidazolone and pentosidine are observed to accumulate in these regions of the brain (Jono, 2002). The presence of AGEs in the neurofibrillary tangles and senile plaques of post-mortem diabetic brains contributes to the idea that increased toxic engagement of AGEs with various molecules within the central nervous system is contributing to the development of diabetic encephalopathy (Stewart, 1999).

Aminoguanidine

Aminoguanidine prevents AGE formation in diabetic mammals. The terminal amino group of this small nucleophilic hydrazine compound reacts as a cross-link inhibitor (Vlassara, 2003). Severe diabetic nephropathies in rats are alleviated through oral aminoguanidine administration (Wilkinson-Berka et al, 2002). In addition aminoguanidine significantly inhibits retinopathy when given to diabetic rats (Renu, 2000). In treated rats, glomerulosclerosis and medullary pathology were also eliminated
Aminoguanidine also can act to prevent the age-related aortic stiffening and cardiac hypertrophy (Chang, 2004.) Detected via radioimmunoassay, AGE concentrations are shown to increase three weeks after diabetes onset in rats and this increase is attenuated by treatment with aminoguanidine (Youssef, 1999).

Learning and Memory

Escape from a water maze has been established in the scientific community as a uniform motivator by which rats’ spatial navigation and cognitive abilities may be tested (Morris, 1981). Tasks such as escaping from water, which use elemental associations, and involve the response of an animal to a given cue, will always produce the same outcome (Morris, 1981). The rats’ association of escaping the water with the platform located at a certain location in reference to external cues, allows for a consistent and accurate assessment of learning. These cognitive associations correspond to specific biological mechanism occurring with the brains of the rats. James, (1950) states that no matter how complex a thought process or memory pathway may be, there is indeed a perfect biological complement to that pathway, and these physical pathways are responsible for every aspect, no matter how subtle, of human thinking. Learning and memory is defined and described in relation to what is known about the biological causes of both phenomena in order for objective and comparative analysis to be attained.
There are at least three memory systems existent in the brain. These systems hold information over time and include sensory, short-term, and long term memory (Byrne, 2001). Briefly, the sensory memory system seems to be involved in the immediate neuronal activity response to a stimulus (Byrne, 2001). Short-term memory systems include mechanisms of neuronal integration with small storage capacities (Byrne, 2001). Long term memory systems include the integration processes involved in the creation of relatively permanent memories (Byrne, 2001). The physiological process of the development of long term memory is described as long term potentiation (Byrne, 2001). This process involves a persistent enhancement in the strength of the synaptic connection produced as a result of delivering a brief high frequency burst of neural activity (Byrne, 2001). Long term potentiation processes have been found to occur in specific regions of the brain, such as the hippocampus, that are classically associated with memory (Byrne, 2001).

Spatial learning, which is the main type of learning analyzed in this experiment, is defined here as an organism acquiring the ability to have a representation of its location in the environment and thus navigate effectively. Without the hippocampus, learning of this type cannot occur (Byrne, 2001).

There are multiple known proteins that appear to be involved in the processes of memory formation. One specific protein that is specifically involved in long-term potentiation is the NMDA receptor (Byrne, 2001). This receptor, which leads to an increase in calcium concentrations in the postsynaptic neuron, is crucial in long term potentiation and long
term memory formation (Byrne, 2001). One of the differentiating factors between long-term and short memory formation is the requirement of protein formation in the former (Byrne, 2001). Cascades of protein interactions, beginning for example with PKA, form complex biochemical pathways which are the basis for learning (Byrne, 2001). Indeed disruption or toxic modification (via AGE formation for example) of any one of these proteins could potentially have an effect on learning and memory.
Materials and Methods

The protocol for induction of diabetes was modified from Biessels et al. (1996). Male Wister albino rats, weighing 250g, were given subcutaneous injections of streptozotocin at 50 mg/kg. Based on blood glucose concentrations this dosage yielded two diabetic animals. Intraperitoneal injections were then administered at a dose of 60mg/kg, which yielded 72 hour blood glucose concentrations greater than 300 mg/dl. Clinical criteria (among others) for diabetes diagnosis is generally accepted as a causal blood glucose concentration above 200 mg/dl (Committee, 2003). These rats were deemed diabetic and were given aminoguanidine via drinking water at a concentration of 1 gram per liter for 8 weeks. Diabetic controls were given normal drinking water. Non-diabetic control rats were given normal water for 8 weeks, and a second non diabetic group was given 2 grams per liter of aminoguanidine via drinking water. Attempted adjustment for the differential fluid intakes between diabetic and non-diabetic groups was made through adjustment of aminoguanidine concentrations in the water.

To test cognitive performance, the protocol was modified from Morris (1981). A pool of water with a diameter of 6 feet and a depth of 2 feet was made opaque with the use of a non-toxic water coloring, and a 4 inch by 4 inch platform was placed 2 cm beneath the water’s surface. Animals were placed into the pool at the same location upon each iteration and were given 2 minutes to find the platform. If unsuccessful, they were placed on the platform for 25 seconds to observe surroundings and then placed back into their cages. Times correlated to initial contact with the water and front paw contact with the
platform. Tests were repeated daily at the same time of day. Once the animals found the platform they were allowed to sit for 25 seconds and then returned to their cage. A rat was deemed to have learned maze when he could find the platform in less than 20 seconds for three consecutive days.

**Statistical Analysis**

ANOVA was used to analyze for effects of time, treatment, and treatments over time on the average water maze times. Post hoc multiple comparisons were also done via Tukey HSD analysis to examine treatment effects on the times of the four different groups.
Results

Differences exceeding errors bars for the average number of days to learn the maze occurred between the normal control group and the diabetic control group (Fig. 1). Such differences also occurred between normal aminoguanidine and diabetic aminoguanidine groups as well as between the diabetic control and diabetic aminoguanidine groups (Fig. 1). It should be noted that normal control groups learned the maze quicker than did diabetic controls, the diabetic aminoguanidine learned the maze faster that diabetic controls, and that the diabetic aminoguanidine group learned the maze faster than normal aminoguanidine (Fig. 1).

ANOVA analysis of the data showed a statistically significant difference in time to learn the Morris water maze (3 days under 20 seconds in a row) across all four of the different treatment groups (Table 3).

Tukey HSD analysis of time to learn water maze between specific treatment groups yielded significant differences between the normal control group and the diabetic control group (p= 0.0197). A difference was also statistically observed between the diabetic aminoguanidine group and the diabetic control group (p=0.00076). Additionally a significant difference between the diabetic aminoguanidine and the normal aminoguanidine group was observed (p=0.025) (Table. 4).
ANOVA analysis of the data revealed a statistically significant difference in Morris water maze completion times across the different treatment groups (Table 1). There was also a significant difference in Morris water maze times across the 17 day time period. Significant time differences between the treatment groups over time were not observed (Table 1).

Analysis of water maze time between specific treatment groups yielded a significant difference between the normal control group and the diabetic aminoguanidine group. Also, a statistically significant difference was found between the diabetic rats receiving aminoguanidine and those diabetic rats not receiving aminoguanidine. This latter difference was more significant ($p= 0.0036$) than the former ($p=0.048$). Statistical significance was not observed between any other of the treatment groups (Table 2). Figure 1 shows the daily times runs for all four groups.

For every trial period except for day 1 the diabetic aminoguanidine group outperformed the diabetic control group (Fig. 2). Differences exceeding error bars occurred on days 2, 4, 7, 8, 9, 10, 11, and 12. No significant difference was observed between diabetic controls and normal controls but differences were observed between daily trials (Fig. 3). Eleven of the sixteen trials revealed a faster time by the normal control than by the diabetic control. Time differentials exceeded errors bars on days 3, 10, 11, and 12 with normal controls being faster on three of these days. Significance was also observed between the diabetic aminoguanidine and normal control groups. Graphical analysis shows large differences on trials 2 and 3 (Fig. 4). Differences exceeding errors bars also
occurred on trials 8 and 9, and the diabetic aminoguanidine group out performed the normal control in 15 of the 16 trials.
Table 1: ANOVA results for the four different treatment groups over the 17-day testing period.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>3</td>
<td>9.10</td>
<td>5.16</td>
<td>0.0045</td>
</tr>
<tr>
<td>Time</td>
<td>15</td>
<td>20.38</td>
<td>30.36</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment ( \times ) Time</td>
<td>45</td>
<td>0.72</td>
<td>1.07</td>
<td>0.858</td>
</tr>
</tbody>
</table>

Table 2: Multiple comparisons, from Tukey HSD analysis, between normal (non-diabetic) controls (no aminoguanidine), diabetic controls, normal with aminoguanidine treatment, and diabetic with aminoguanidine treatment, for time to complete maze over the 17 day period.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Normal Control</th>
<th>Diabetic Control</th>
<th>Normal Amino.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>1.0</td>
<td>0.64</td>
<td>0.63</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>0.64</td>
<td>1.0</td>
<td>0.12</td>
</tr>
<tr>
<td>Normal Aminoguanidine</td>
<td>0.63</td>
<td>0.12</td>
<td>1.0</td>
</tr>
<tr>
<td>Diabetic Aminoguanidine</td>
<td>0.048</td>
<td>0.0036</td>
<td>0.46</td>
</tr>
<tr>
<td>Source</td>
<td>df</td>
<td>MS</td>
<td>F</td>
</tr>
<tr>
<td>--------------</td>
<td>----</td>
<td>-----</td>
<td>------</td>
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<tr>
<td>Treatment</td>
<td>3</td>
<td>33.86</td>
<td>7.19</td>
</tr>
</tbody>
</table>

Table 3: ANOVA results of time to learn maze for the different treatment groups.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Normal Control</th>
<th>Diabetic Control</th>
<th>Normal Amino.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>1.0</td>
<td>0.0197</td>
<td>0.37</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>0.0197</td>
<td>1.0</td>
<td>0.47</td>
</tr>
<tr>
<td>Normal Aminoguanidine</td>
<td>0.37</td>
<td>0.47</td>
<td>1.0</td>
</tr>
<tr>
<td>Diabetic Aminoguanidine</td>
<td>0.46</td>
<td>0.00076</td>
<td>0.025</td>
</tr>
</tbody>
</table>

Table 4: Multiple comparisons, from Tukey HSD analysis, between normal (non-diabetic) controls (no aminoguanidine), diabetic controls, normal with aminoguanidine treatment, and diabetic with aminoguanidine treatment, for time to learn maze in days.
Fig. 1: Average number of days taken for rats to complete the maze in 20 seconds or less for three consecutive days.
Fig. 2: Average Morris water maze times
Fig. 3: Average Morris water maze times
Fig. 4: Average Morris water maze times
Fig. 5: Average Morris water maze times
Discussion:

Normal control groups learned the maze quicker than did diabetic controls. The diabetic aminoguanidine group learned the maze faster than diabetic controls, and the diabetic aminoguanidine group learned the maze faster than normal aminoguanidine group (Fig. 1). These differences were upheld through Tukey HSD analysis (Table 4). The fact that normal control groups significantly learned the maze quicker than diabetic control groups demonstrates the reproduction of diabetic encephalopathy within the present study. Faster learning times by the diabetic aminoguanidine group as compared with the diabetic control group supports the hypothesis that aminoguanidine will inhibit cognitive decline in diabetic rats.

There was a difference in Morris water maze completion times across the 17 day time period. As the trial iteration increased, the rats learned where the platform was located with reference to external cues and were able to find the platform more quickly. A difference exists between all four treatment groups. This means that at least two of the treatment groups behaved in different ways. Further analysis showed that these differences occurred between the normal control group and aminoguanidine treated diabetics as well as between the diabetic controls and aminoguanidine treated diabetic rats. The statistical significance of the differences between the diabetic rats treated with aminoguanidine and normal control rats were less than the differences between the diabetic rats treated with aminoguanidine and diabetic controls.
The difference between the aminoguanidine diabetics and diabetic controls was significant \((p = 0.0036)\). The aminoguanidine treated diabetics displayed better memory and psychomotor skills than did the non-treated diabetics. These results point strongly to prevention of diabetic encephalopathy in rats via treatment with aminoguanidine.

A statistical difference in completion time between normal and diabetic control groups was not observed, via Tukey HSD analysis. As stated previously however, a statistical difference in the time to learn the water maze was observed between these two groups. Diabetic encephalopathy was therefore significantly reproduced within this experiment. It should be noted that there were several rats in the normal control group which displayed unusually slow learning times on several of the trial days. Due to the relatively small number of animals used in this study, any unusually slow individual might have a large effect on the overall data. In order to link AGE inhibition via aminoguanidine treatment to the prevention morphological damage in the hippocampus, further neurophysiological studies should be done.

The cognitive decline in humans due to extended periods of hyperglycemia has been shown to be moderate and often short-lived. Because of these rather mild effects, little research has been undertaken to explain these cognitive impairments. From this study it appears that AGE formation in rats is in fact involved in cognitive decline. The acute manner in which diabetic encephalopathy in humans occurs leads to questions of whether AGE’s are being formed in hippocampal regions and then being broken down by the body after the glucose levels are lowered. Currently, there is clinical interest in
molecules that can safely break such cross linking between proteins (Ulrich, 2001). An endogenous natural pathway could be involved if, in fact, the acute nature of the cognitive decline is a function of natural breaking of cross-links over time.
Literature Cited


