The Role of Estrogen in the Neurodegenerative Disorder Alzheimer's in the Female Rat.



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Abstract

Alzheimer's disease (AD) is a major neurodegenerative disorder that affects many people worldwide. There are many reported causes of this disease such as, genetic or environmental. Women are disproportionately affected by AD than men, indicating that the absence of estradiol might play a role in this disease. Recent studies have found that the primary sex steroid hormones, especially estradiol, play a larger role than initially believed in the etiology and progression of AD. This study examines the possibility of estradiol as a potential treatment for AD in female rats. A total of 7 female *Sprague Dawley* rats age ranging from 12-18 months were used. Subjects were food deprived for 10 hours; latency and errors to completion of the maze were recorded. Five trials per day per subject were performed to collect data for both baseline and treatment doses of estradiol. Two doses of estradiol were used, a low dose 28.0 and a high dose 56.0 (mg/kg).The major finding of this study is that following 4 days of estradiol administration performance of the subjects in the Triple T-Maze most closely resembled those before the Alzheimer's like state was induced. Kainic acid 25.0 (ng/ml) was injected into the dorsal hippocampus to induce a condition similar to Alzheimer's.

Keywords: Alzheimer's, Sex steroid hormones, Estradiol

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Alzheimer's is a neurodegenerative disease that causes impaired memory and learning ability. Alzheimer's is named after Dr. Alois Alzheimer a German physician who first discovered this disease in 1906 by accident while performing an autopsy on a patient who had severe confusion and memory impairments for years before they had died. During this autopsy he discovered dense deposits surrounding nerve cells in the brain, which are now known as plaque, and inside these nerve cells he discovered twisted bands of fibers (Murray, 2013). In the initial stages of Alzheimer's these fibers begin tangling and forming plaques in the cortex and hippocampus, which is associated with memory and learning (Alzheimer's Association). These plaques begin to increase in this part of the brain affecting the individual's ability to speak, understand speech, and organize thoughts. As the plaque spreads to other parts of the brain it causes wide spread cell death (Necrosis), causing the brain to shrink. As Alzheimer's progresses, individuals may experience changes in personality, behavior and have trouble recognizing friends and family members. The cause of this disease is still unknown, many speculate the causes as being genetic, environmental, or due to gender differences. The risk for Alzheimer's increases every year over sixty-five (Murray, 1994).

This disease disproportionately affects more women than men. The likely hood for women to develop Alzheimer's disease (AD) is 1.5 times as likely than it is in males (Alzheimer's Association), even after controlling for the fact that women have an increased lifespan women are still affected more often with AD (Moser, 2015).

There is evidence that sex-steroid hormones are protective against AD in both sexes. Low brain and circulating estrogen in women and testosterone in men have been associated with increased AD risk (Moser, 2015).

Researchers believe this could be correlated with the loss of the sex hormone estrogen in women as a result of menopause. Studies have found that the estrogen β receptor plays an important role in a broad spectrum of brain activities from development to aging (Zhao, 2015). The apoliproprotein (apoE4) is the strongest genetic risk factor for AD, which has a greater effect in women, as compared to men. So much so that a single copy of apoE4 increases the risk factor four fold in women, without significantly affecting risk factor of AD in men (Moser, 2015).

Some studies show that estrogen is a good neuroprotective agent against central nervous system damage, as well as traumatic brain injuries (Chakrabarti, 2015). A study done by Manly et al found that patients with AD had lower estradiol levels than their normal controls. Another study using males found that patients with AD had lower serum testosterone levels than their control, but there was no difference in testosterone levels of women with AD (Hogervorst, 2001). This suggests that these primary sex steroid hormones play a role in the development and cause of AD in both sexes. Bodor also believed that estrogen might help delay the process of Alzheimer's type dementia in post-menopausal women (Bodor, 2012). An MRI based study done by The University of California Davis found that the hippocampus had shrunk in female AD patients, which was larger in post-menopausal women who were taking estrogen replacement therapy compared to post-menopausal women not taking this therapy.

In the present study estradiol is examined as a potential treatment for AD, based off of previous research that describes loss of primary sex steroid hormones as the possible reason for AD.

In order to induce an Alzheimer's like state Kainic acid was injected into the dorsal hippocampus. This acid was used based off of the findings of Park et al, which found that intrahippocampal injections of this acid caused severe neuronal loss and profound learning and memory deficits (Park, 2012).

Method

Animals

Seven female *Sprague Dawley* rats age ranging from 12-18 months were used in this experiment. These subjects were initially housed in three different cages; post surgery they were individually housed for the remainder of the experiment.

Surgery

Subjects were anesthetized with a mixture of 70mg ketamine and 6mg xylazine (.1mg/kg) total per subject. The subjects were then placed in a sterotaxic frame. The cranium was exposed and four small holes were made using a dental drill. Kainic acid (25ng/ml) was used to lesion the dorsal hippocampus. Dorsal hippocampal lesions were made in two locations bilaterally: (1) AP= -4, ML= ± 3.0, DV= 3.0 from bregma; (2) AP= -5,

ML= ±3.0, DV= 3.0. Four injections of Kainic acid (2μl) per injection were administered into the dorsal hippocampus. After surgery the wound was sutured and the subject was placed on a heating pad for 10 min before being placed back into their cage. The subjects were then given a week to recover from surgery before further testing.

Apparatus

A Triple T-maze was used. This T-maze was equipped with a guillotine door and holding chamber.

Procedure

The animals were subjected to ten hours food deprivation, and were tested at the same time every day. The first five days of the experiment the subjects were trained to complete the Triple T-maze. A small drop of peanut butter was used as the reinforcer, and was placed in a small dish at the end of the maze. After they were trained each subject was run five times individually, and latency and errors was recorded for baseline measurements.

After these baseline values were collected, in order to induce an Alzheimer's like state Kainic acid was used to chemically lesion the dorsal hippocampus. After they recovered the subjects were then run in the Triple T-maze again and values were recorded for baseline post-surgery. The animals were then given two different doses of estradiol, 28.0 low dose and 56.0 mg/kg high dose. The subjects were first given the low dose of estradiol, after the 15 min pre-treatment time the subjects were then placed into the Triple T-maze, time and number of errors were recorded. The following day they were tested again in the Triple T-maze, but were given no drug to test chronic affects of estradiol on performance. After the four days of the low dose was tested the high dose testing began. Time and error were both recorded for each subject. A total of four days per dose were done, two days of drug and two days without drug. After completion of behavioral studies, animals were euthanized using CO_2.

Results

Two different doses of estradiol were used, 28.0 and 56mg/kg. It was found that there was no significant difference (p=.155) in time before and after surgery (see figure 1). There was a significant difference (p=.024) with errors made before and after surgery (see figure 2). Day four of both the low and high doses show a significant decrease in errors in comparison to baseline before surgery.

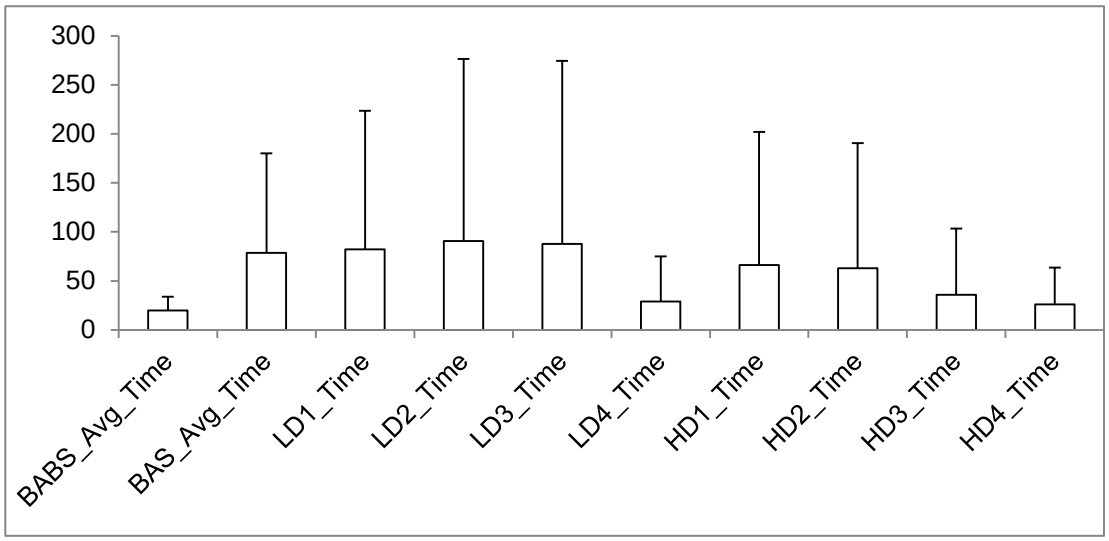


Figure 1. Average time in seconds to completion of Triple T-maze (± 1 SEM). BABS represents baseline before surgery, BAS is baseline after surgery. LD represents the low (28mg/kg) estradiol dose, and HD represents the high (56mg/kg) estradiol dose. Before testing began there was a 15-minute pretreatment time for each dose. A total of four days per dose was tested and recorded. Days two and four there was no drug administered to test chronic affects the drug may have had.

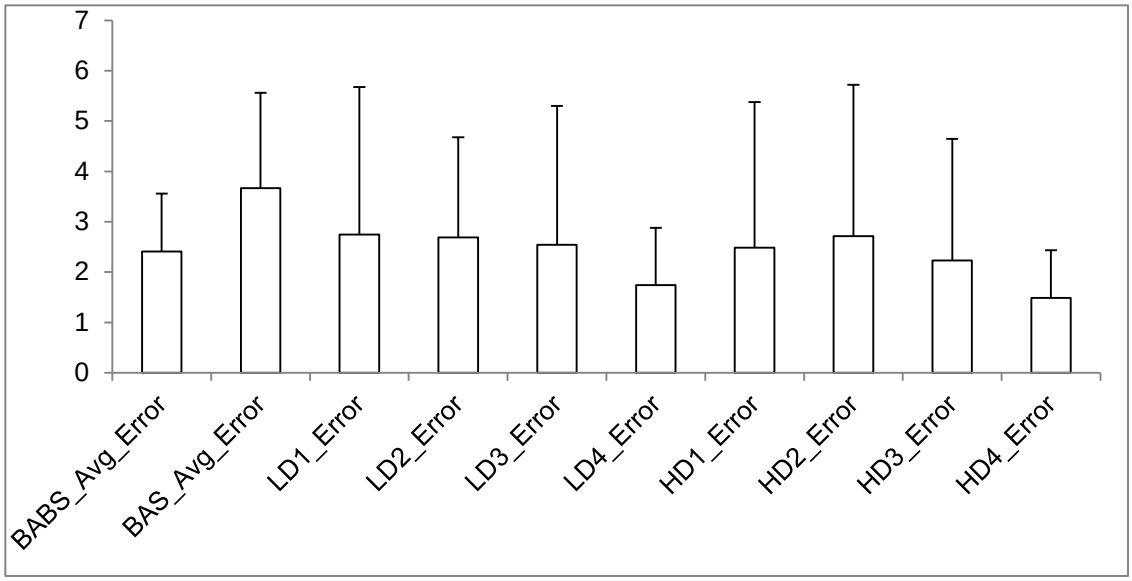


Figure 2. Average number of errors made by animals before completion of Triple T-maze (± 1SEM). BABS represents baseline before surgery, BAS is baseline after surgery. LD represents the low (28mg/kg) estradiol dose, and HD represents the high (56mg/kg) estradiol dose. Before testing began there was a 15-minute pretreatment time for each dose. A total of four days per dose was tested and recorded. Days two and four there was no drug administered to test chronic affects the drug may have had.

Discussion

The purpose of this study was to test estradiol as a treatment for Alzheimer's disease. Due to Alzheimer's affecting women more than men it was hypothesized that estradiol played a larger role in the development of AD than previously thought. In order to test this older rats were used with the assumption that their low levels of estrogen would mimic the hormone cycle of a human female.

It was found on day four for both 28.0 and 56.0 mg/kg that the errors emitted were significantly less than that of baseline after surgery, and more closely resembled the baseline before surgery. This indicates that this experiment needs to be over a prolonged period of time, instead of four days per dose. Since both doses showed a significant decrease in errors on the

fourth day, one can conclude that brain chemistry and circulating levels of estradiol might take longer to take effect.

During the experiment one of the subjects produced data that could be considered an outlier. This subject emitted on average 235 seconds for latency and 6.5 errors. Data like this was not thrown out due to the possibility that the surgery was successful in this case and not in the other animals.

The results found in this study somewhat coincides with those found by Moser et al, in the way that the sex hormone estradiol is a factor in the Alzheimer's mystery. This study is different from most studies because estradiol is used as a treatment, rather than a neuroprotective mechanism that was used by Chakrabarti et al.

One of the possibilities for the next experiment conducted in continuation should be at minimum a month long with a similar drug procedure. That way the effects of estradiol on this disease could be better understood. Based off of the results produced from this experiment it shows that more days per dose are required to see clearer results.

In order to improve the already existing experiment perfusions would be necessary to see if the intended part of the brain was affected or not. Also, to see if surgery was totally successful in generating a proper Alzheimer's like state. Through perfusions it is also possible to see if estradiol had any positive affects in reversing or slowing down AD.

The surgery performed in this study could have also caused a mild to moderate level of AD instead of the beginning stages. A study performed by Mulard et al found that hormone replacement therapy, on participants that had mild to moderate AD, did not slow disease progression or improve cognitive or functional outcomes in both humans and other animals.

Our study might not have given high enough doses of estradiol, or giving it orally could have affected concentrations internally in a way not intended. Testing for levels of estradiol in the blood during the experiment would have helped to give insight into whether or not the doses given were appropriate for desired effects.

Also, since estrogen is a lipophilic endogenous steroid it is poorly retained in the brain, and in order to maintain therapeutically significant concentrations frequent or sustained doses have to be administered (Bodor, 2002). Another study performed could give constant injections for a week and test the effects at the end of the week. Then the following week do something similar, but either increase or decrease the dose based off of the results from the previous dose. This study further hints at the true importance of estrogen in the fight against Alzheimer's.

Although some of the data seems inclusive it brings us closer to understanding how these mechanisms work, instead of how we think they should work. The ultimate goal of this research was to find another puzzle piece in this complex system in order to help other researchers continue the fight. These findings are relevant because they show overall that estradiol does have some reversal affect in the cycle of AD, but we still are not sure exactly what that is. With this disease being the second leading cause of death in the United States alone, research like this is important in order to prevent further suffering and bring us closer to truly understanding and combating this disease.

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