Proceedings of the 2nd International Conference on Modern Medicine and Global Health
DOI: 10.54254/2753-8818/45/20240593

Investigating the role of metallothionein-3 (MT-3) in anxiety-like behaviors: An in-depth research proposal

Sijin Wang

Shanghai united international school (Gubei Campus), Shanghai, 201103, China

Lindawang2007@hotmail.com

Abstract. Anxiety is a common mental health disorder that profoundly impacts individuals' daily lives. Metallothionein-3 (MT-3), a protein found in the central nervous system, is hypothesized to play a role in anxiety-like behaviors. This research proposal aims to investigate the induction of anxiety-like behaviors in the presence of MT-3 using an experimental approach. Previous studies have shown that MT-3 deficiency is associated with abnormalities in psychological behaviors. The proposed study will build upon this research to further explore the cellular and molecular mechanisms underlying MT-3 involvement in anxiety. The study will utilize a mouse preclinical model and behavioral assays to assess anxiety-like behaviors in the presence or absence of MT-3. The findings from this research may contribute to the development of novel therapeutic strategies for managing anxiety.

Keywords: anxiety, metallothionein-3, psychological behaviors, experimental design

1. Introduction

Anxiety is a prevalent mental health disorder characterized by excessive worry, fearfulness, and apprehension, which significantly impacts an individual's daily life. It affects approximately 18.1% of the population in the United States alone. Understanding the symptoms, pathology, and possible treatments of anxiety is crucial for developing effective therapeutic strategies to address the unmet clinical needs of patients. One potential candidate that may play a role in anxiety-like behaviors is Metallothionein-3 (MT-3), a protein primarily found within the central nervous system. This research proposal aims to investigate the possible induction of anxiety-like behaviors in the presence of Metallothionein-3 (MT-3) using an7 experimental approach. The proposed study will build upon previous research studies examining the abnormalities in psychological behaviors associated with MT-3 deficiency.

1.1. Symptoms, Pathology, and Pathophysiology of Anxiety

Anxiety manifests through a range of symptoms, including persistent unease, restlessness, irritability, sleep disturbances, and difficulty concentrating. The pathological mechanisms underlying anxiety involve dysregulation in various brain regions and neurotransmitter systems, such as the amygdala, prefrontal cortex, and serotonin and GABA systems [1]. The dysfunction of hypothalamic-pituitary-adrenal axis (HPA) which lead to imbalance of hormone, especially corticorsterol, inside the body and all These alterations above contribute to heightened fear responses and impaired emotional regulation in anxious individuals.

DOI: 10.54254/2753-8818/45/20240593

1.2. Potential Treatments for Anxiety

The treatment options for anxiety include psychotherapy, medication, or a combination of both. Cognitive-behavioral therapy (CBT) is a widely employed psychotherapeutic approach that aims to identify and modify negative thinking patterns to alleviate anxiety symptoms [2]. Medications like selective serotonin reuptake inhibitors (SSRIs) and benzodiazepines are commonly prescribed to regulate neurotransmitter imbalances and alleviate anxiety symptoms [3]. Despite these treatment modalities, there exist unmet clinical needs in effectively managing anxiety, necessitating the exploration of novel therapeutic targets.

1.3. MT-3 and Anxiety

MT-3 is a member of the metallothionein protein family known for their metal-binding capabilities. In addition to the direct involvement of MT-3 in metal ion regulation, emerging evidence suggests its association with anxiety disorders. Studies of MT-3 knockout rats have revealed that MT-3 expression is decreased significantly in individuals with anxiety disorders, suggesting its potential involvement in the pathogenesis of anxiety [4]. The underlying cellular and molecular mechanisms of MT-3 involvement in anxiety are not yet fully understood, but it is hypothesized that MT-3 may modulate neurotransmitter systems or impact neuroplasticity, leading to anxiety-related behaviors.

1.4. Abnormal Psychological Behavior in MT-3 KO Mice

MT-3 stands for Metallothionein-3, and the normal memory exhibited by MT-3 knockout (MT-3 KO) mice indicates abnormal psychological behavior. MT-3 is a metal-binding protein primarily localized in the hippocampus, a brain region strongly implicated in learning and memory processes. The absence of MT-3 in mice has been correlated with cognitive deficits and increased anxiety-like behaviors [4]. Hence, studying the relationship between MT-3 and anxiety may shed light on the intricate mechanisms underlying anxiety disorders.

2. Hypothesis

Based on all the previous existing articles and experiments, we hypothesize that the absence or deficiency of MT-3 will lead to an increase in anxiety-like behaviors in a preclinical mouse model.

3. Literature review

A study titled "Metallothionein-3 deficient mice exhibit abnormalities of psychological behaviors"[5] presented evidence for the involvement of MT-3 in modulating psychological behaviors. The study utilized MT-3 knockout mice and assessed anxiety-related phenotypes using standard behavioral tests, including the elevated plus maze and light-dark box. The findings suggested that the absence of MT-3 led to alterations in psychological behaviors and caused psychological disorders, manifested as increased anxiety levels and reduced exploratory behavior.

Hypothalamic pituitary adrenal axis (HPA) is an axis composed of corticosteroid-releasing hormone (CRH) that stimulates adrenocorticotropic hormone (ACTH) secretion from pituitary gland and finally leads to the secretion of corticosterone (CORT) by adrenal glands, elevating the stress level.

According to the article: Absence of Metallothionein-3 produces changes in MT-1/2 regulation and the hypothalamic-pituitary-adrenal (HPA) axis [6], additional evidence supporting the potential link between MT-3 and anxiety-like behaviors is provided. This review focuses on the regulation of Metallothionein-1/2 (MT-1/2) and its potential influence on the hypothalamic-pituitary-adrenal (HPA) axis. The absence of Metallothionein-3 (MT-3) is shown to produce changes in MT-1/2 regulation and causes dysfunction of the HPA axis, which is previously defined as an integral system involved in stress response regulation and anxiety disorders.

4. Experimental design

To investigate the hypothesis, the following experimental design is proposed:

DOI: 10.54254/2753-8818/45/20240593

A mouse preclinical model will be employed in this work. Control (A), inhibit (B), and perch (C) groups of mice will be divided. Group A is the control group, requiring simply a placebo (0.9% NaCI solution); group B is a group with low levels of MT-3; thus, we will employ mice that have had their genes altered to create MT-3 gene knockout animals. Group C is the group of mice that has been manually injected with MT-3 to attain the high MT-3 concentration.

Prior to the experiment, each mouse's blood will be tested and collected from their tails on three random, separated days in a week using a classical blood syringe for the presence of MT-3, with the average value in mg/ml calculated each time.

Al will be used to represent group A's data, B1 for group B's, and C1 for group C's.

The "Hole board test" is one way for determining anxiety level; it is supported by Himanshu, et al. 2; it employs an empty paper box with original paper color to prevent distractions from color; and a predetermined number of holes are drilled on its surface, all of equal size. The mice are drawn to these holes by nature because of their high levels of curiosity.

The movement of an animal disrupts an infrared light beam that is falling on a photocell, which is where the locomotion activity is measured using an actophotometer. At this point, the count is recorded and displayed digitally. It is well known that mice prefer to find holes more frequently when they are relaxed, but when they are suffering from an anxiety illness, they tend to find holes less frequently. In order to record the holes, the mice dug during their exploratory behavior, the data will be represented by the codes A2, B2, and C2. Other factors, such as the type of paper box, the amount of time the mice have to explore, the temperature, and the level of light, are left unchanged. The experiment5 should be repeated for at least five trials for the purpose of accuracy.

The second method is a biological approach; although secondary antibodies have fluorescence adhering to them, primary antibodies have variable regions specifically for corticosterone. They come from chicken and goat, respectively. Antibodies are manually added into blood samples collected after the experiment. Five days after the alteration is performed on the mice, blood samples are obtained. On slides, blood samples will be produced, and a compound microscope will be used to see any fluorescence that may appear after binding. Nit/micrometer square is the unit used to express the amount of fluorescence seen. B3 for B, C3 for C, and A3 for A.

The information collected from the experiment will be further examined: utilizing t-test for assessing the associations of X2 and X3(which both Xs are representations of one of the groups from the larger A, B, and C groups) which enhance the dependability of the data on mice's anxiety levels. Additionally, the average of XI, X2, and X3 is computed, and any outliers (specifically 50% higher or lower than average) should be removed to ensure precision.

Comparisons are made between the values of X1 and X2, X3. If the X2 and X1 values are positively correlated, and if the X3 and X1 levels are shown to be negatively correlated, then we can infer that there is a negative correlation between MT-3 level and anxiety level. In other words, MT-3 has the potential to reduce the presence of corticorsterol.

5. Expected result and implications

The research involves investigating the role of MT-3 in anxiety using animal models and behavioral assays. By selectively manipulating MT-3 expression in specific brain regions of mice and subsequently assessing anxiety-like behaviors, the study aims to elucidate the involvement of MT-3 in anxiety pathophysiology. Additionally, the research will explore potential therapeutic strategies targeting MT-3 to ameliorate anxiety symptoms.

Previous studies have also focused on the role of MT-3 in psychiatric diseases. For instance, research has demonstrated altered MT-3 levels in individuals with Alzheimer's disease [7]. This suggests a broader involvement of MT-3 in neuropsychiatric disorders beyond anxiety, highlighting its significance as a potential therapeutic target across various mental health conditions. We expect that MT-3 has a positive effect on anxiety disorder of the mice that are tested in the experiment, and the corticosteroids would show negative correlation to the levels of MT-3 presented. Further investigations can be conducted based on the mechanisms of MT-3 which affects the HPA axis.

DOI: 10.54254/2753-8818/45/20240593

In conclusion, studying the pathophysiology, treatments, and unmet clinical needs of anxiety will provide valuable insights into the development of better therapeutic approaches. Additionally, investigating the role of MT-3 in anxiety may pave the way for novel treatment strategies targeting this protein, ultimately addressing the unmet clinical needs and improving the lives of individuals suffering from anxiety disorders.

6. Conclusion

In conclusion, this research proposal aims to investigate the role of Metallothionein-3 (MT-3) in anxiety-like behaviors. Anxiety is a prevalent mental health disorder that affects a significant portion of the population and understanding its symptoms, pathology, and potential treatments is crucial for developing effective therapeutic strategies. MT-3, a protein found in the central nervous system, has been implicated in anxiety disorders based on studies showing decreased expression in individuals with anxiety. The proposed study aims to further explore the potential involvement of MT-3 in anxiety by using an experimental approach with MT-3 knockout mice. The research design includes behavioral assays and biological measurements to assess anxiety levels and MT-3 expression. The expected results may provide insights into the role of MT-3 in anxiety pathophysiology and potential therapeutic strategies targeting this protein. This research contributes to the broader understanding of anxiety disorders and may lead to the development of novel treatment approaches to improve the lives of individuals suffering from anxiety.

References

[1] Lau, J. Y. (2018). Developmental aspects of anxiety: The role of cortical circuitry and environmental factors. In The Wiley Handbook of Anxiety Disorders (pp. 231-254). Wiley.

[2] Hofmann, S. G., Asnaani, A., Vonk, I. J. J., Sawyer, A. T., & Fang, A. (2012). The efficacy of cognitive behavioral therapy: A review of meta-analyses. Cognitive Therapy and Research, 36(5), 427-440.

[3] Baldwin, D. S., Anderson, I. M., Nutt, D. J., Allgulander, C., Bandelow, B., den Boer, J. A., ... & Djukic, R. (2019). Evidence-based pharmacological treatment of generalized anxiety disorder. International Journal of Neuropsychopharmacology, 22(3), 192-205.

[4] Lim, S., Nho, C. W., Lee, S. H., Koo, J. H., Hah, Y. S., & Shin, S. (2012). Metallothionein-III: growth inhibitory effects on HT-29 human colon cancer cells reside in the N-terminal region. Molecules and Cells, 34(2), 177-183.

[5] Koumura A, Kakefuda K, Honda A, et al. Metallothionein-3 deficient mice exhibit abnorm alities of psychological behaviors. Neuroscience Letters. 2009;467(1):11-14. doi:10.1016/ j.neulet.2009.09.051

[6] Lago N, Quintana A, Carrasco J, Giralt M, Hidalgo J, Molinero A. Absence of metallothio nein-3 produces changes on MT-1/2 regulation in basal conditions and alters hypothalam ic-pituitary–adrenal (HPA) axis. Neurochemistry International. 2014;74:65-73. doi:10.101 6/j.neuint.2014.06.008

[7] Sutherland, G. T., Sheahan, P. J., Matthews, R. T., & Smith, M. A. (2001). Effects of aluminium on neurotrophin expression: relevance to Alzheimer's disease. Journal of Neural Transmission, 108(6), 869-878.