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Effect of apelin-13 on anxiety like behaviour in male rats

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Abstract

Aim: It is known that apelin-13 is one of the major neuropeptides with a clear and crucial role in the circuits that are involved in mood disorders. In the development and/or maintenance of pathological anxiety, abnormalities of the hippocampus and amygdala play an important role. Here, we assessed the potential anxiolytic effect of apelin-13 on anxiety-like behaviors in male rats.

Materials and Methods: A total of 48 male Wistar rats were divided into 4 groups (n=12). Control (C), Social Isolation (SI), Apelin-13 (A), and Social Isolation + Apelin-13 (SI+A). In the C and A groups, four animals in each cage for 8 weeks. In the SI and SI+A groups, each animal was housed individually for 8 weeks. After that apelin-13 administration was applied by osmotic pomp. Anxiety/depression-related behaviors were evaluated using the Elevated Plus Maze (EPM), Open Field Test (OFT), and Light-Dark boxes (LDB). We also measured the expression of Apelin-13, Apelin receptor (APJ), Brain Derived Neurotrophic Factor (BDNF), Mammalian Atonal Homolog 1 (MASH1), Nestin, Doublecortin (DCX) and Neuritin in the hippocampus. These are important markers indicating the anxiety mechanism in the hippocampus.

Results: The results of our study showed that apelin-13 administration reduced anxiety behaviors. Open arm entires and time spent were higher in the A group. In the open field test, grooming and rearing were lower in the SI group. Moreover, apelin-13 and APJ gene expression was higher in the A group.

Conclusion: The results of the study indicate that apelin-13 infusion may lead to a decrease in anxiety-related behaviors in male rats.

Introduction

Over the last decade, our understanding of mood disorders has expanded rapidly. Stressful experiences in early life can have long-lasting detrimental effects on mood and cognitive function, raising the risk of mental illnesses like anxiety. [1]. Anxiety disorders are among the most prevalent pathologies in clinical psychiatry, accounting for approximately 30% of all psychiatric disorders [2]. The mechanisms underlying the pathogenesis of anxiety are not entirely understood, as multiple factors contribute to the development of anxiety and depression. Notably, stress is a critical inductive factor [3].

Anxiety disorders often manifest in childhood and adolescence, with individuals who experience early life trauma being particularly vulnerable [4]. Recent research has demonstrated that the hippocampus and amygdala are highly susceptible to stress [5]. Chronic stress, including models of SI, leads to neurochemical changes and depression-like behaviors [6]. To investigate these effects, researchers commonly use maternal separation and early deprivation models [7]. For instance, depriving mice of maternal care from 21 to 42 days, post-partum results in anxiety and fear behaviors in adulthood [8]. Similarly, SI during early development in humans contributes to the onset of anxiety and is frequently employed as a measure of mood disorders [9].

The apelinergic system consists of the peptide apelin-13

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and its receptor, APJ. APJ is a G-protein coupled receptor primarily found in various brain regions of rats, notably the hippocampus and hypothalamus. These areas are critical for stress and emotional responses [10,11]. Research indicates that apelin-13 plays a significant role in numerous physiological and pathological processes, including memory modulation, cardiovascular effects, insulin secretion, fluid homeostasis, and the regulation of anxiety and depression [12,13].

Mood disorders like anxiety and depression can be caused by changes in how the brain works because of things in our environment. These changes affect chemicals that can affect pathways. One of these, apelin, is controversial. Some studies say apelin-13 makes people more anxious, while others say it has no effect. This study will look at whether apelin affects anxiety or depression in young male rats that are isolated. It will also look at the role of BDNF and APJ in these effects. Studies have shown that apelin-13 may have anxiogenic [14] or anxiolytic effects [15,16]. However, the precise impact of apelin-13 on mood disorders remains largely unexplored.

Materials and Methods

Test animals Male Wistar rats weighing 230-260 g were purchased from KONÜDAM. The animals were maintained in a 12-hour light/dark cycle and the ambient temperature was set at 21 ± 2 °C. They have open access to food and water and are housed in stainless steel cages. Rats were divided into two groups, SI and C. The isolation group was housed alone and the C group consisted of eleven animals. Tests were conducted during the light cycle (09:00 to 12:00). All experimental procedures were carried out according to the guidelines of KONÜDAM's Ethics Committee. The aim was to minimise animal suffering and distress. The animal experiments were approved by the local ethics committee with decision number 2018-011, dated 23.02.2018, and all procedures in the study were performed according to the ethics committee protocol.

Experimental protocol

Rats were taken from their mothers on their 28th day and randomly divided into four groups, as the C (4 animals/cage), A (4 animals/cage) SI stress (1 animal/cage), the SI+A (1 animal/cage) groups and reared for 8 weeks.

C (n = 12): Reared with littermates (4 animals/cage)

A (n = 12): The group without SI and last 2 weeks infused apelin-13 (3.5 g/kg) (4 animals/cage)

SI (n = 12): Animals were administered SI (1 animal/cage)

SI+A (n = 12): After SI applied for 6 weeks, the apelin-13 (3.5 g/kg) was infused subcutaneously for 2 weeks. SI was also applied during the infusion of apelin-13.

Apelin-13 administered by osmotic pump and first dose of apelin-13 infusion started at week 6, same dose repeated up to 14 days. Rats were decapitated and brain tissue stored at -80°C for examination at the end of the experiment. Behavioural tests were used to determine whether apelin-13 had an anxiolytic or anxiogenic effect in both groups after injection, and whether the SI group experienced higher levels of anxiety after SI modelling than the C group. To make these assessments, anxiety-related behaviours were evaluated by OFT, EPM and LDB two different times, after SI and apelin-13 application. Appelin-13, APJ, neuritin, MASH1, nestin, BDNF, and DCX gene expression levels were analyzed to look into alterations in the hippocampus and amygdala during the onset of anxiety.

Drug treatment

Osmotic pumps had an ISV infusion of 200 µg reservoir volume, 5 µl / hour / week. Before apelin-13 application, rats were anesthetized using combination of Xylazine (10mg/kg) and ketamine (mg/kg). Apelin-13 infusion was carried out with osmotic pumps prepared for animals in the A group (C and SI) for 15 days. Osmotic pump was placed subcutaneously and controlled drug release applied.. Rats were decapitated and brain tissues were removed and analyzes were started.

Behavioral tests

EPM, OFT and LDB were used to evaluate the anxiety behaviors and locomotor activities of animals. All tests were done in the same room under the same lighting. All behavior was recorded on video during testing and scored by an observer who was blind to group and testing conditions.

EPM

The EPM mechanism has been introduced to the software program (Ethovision Video Tracking System XT11, Netherlands) and regions are marked as open arms, closed arms, center. The animal was always left in the same direction with the head facing the open area and tested for 5 minutes. The number of times the arms were open and closed, and the time spent with the arms open and closed, were recorded during this period. At the end of each experiment, the apparatus was wiped with 10% ethyl alcohol.

OFT

The animal was introduced to the software program (Ethovision Video Monitoring System XT11, The Netherlands), marked, the whole experiment was recorded by the program. Each animal was left to the apparatus from the same edge at the beginning of the 5-minute process, and during this period, time spent in center, speed, mobility and immobility time were calculated. At the same time, rearing, grooming and defecation scores were calculated by observing during the experiment. The tests were carried out between 09:00 and 12:00 during the day, taking into account the physiological conditions of the animals. At the end of each experiment, the apparatus was cleaned with 10% ethyl alcohol solution.

LDB

The animals were introduced to the software program specially used for LDB (Ethovision Video Monitoring System XT11, The Netherlands), marked with a red dot, the entire experiment was recorded through the program. Each animal was left to the apparatus from the same edge at the beginning of the 5-minute process, and the time spent in the light and dark box were calculated.

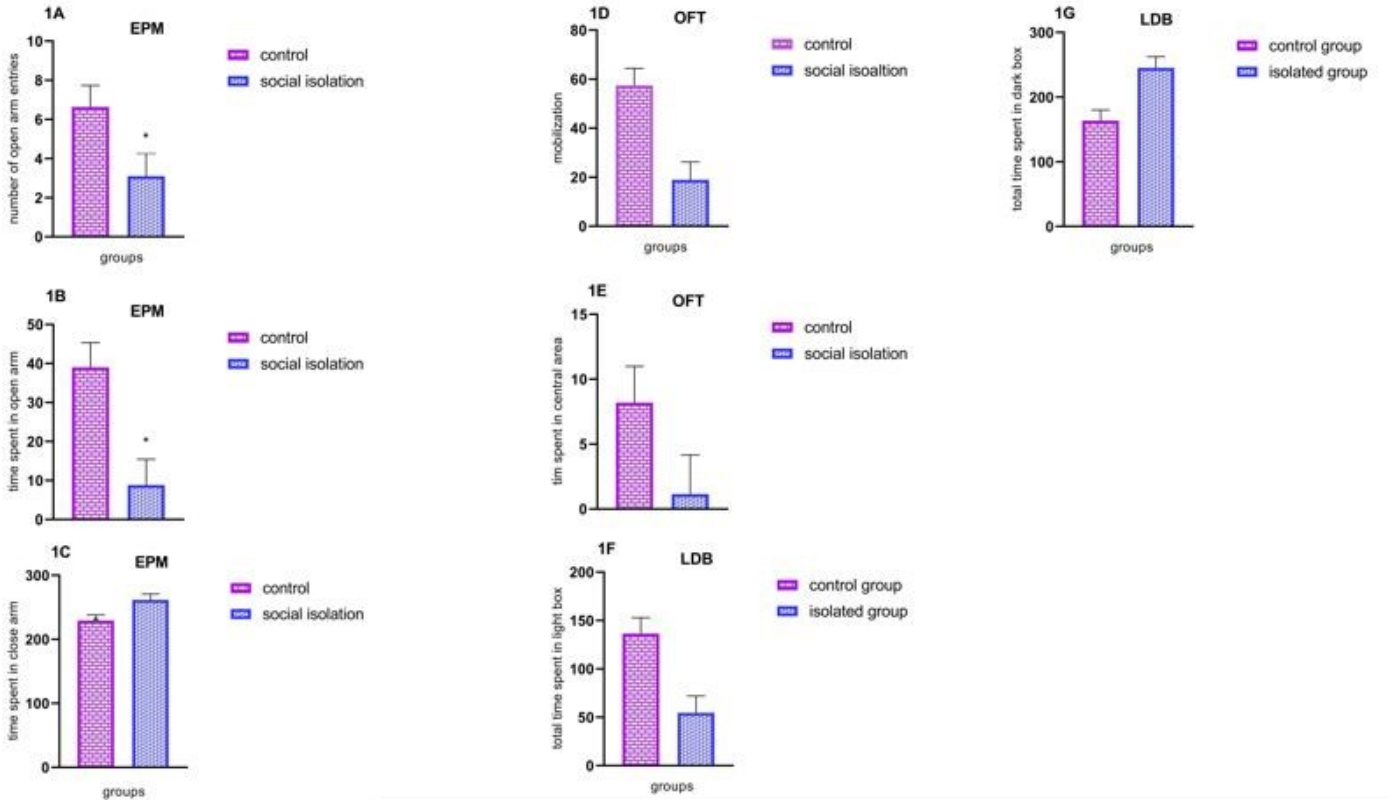

Figure 1. Effect of SI on behavioural tests: EPM, OFT and LDB. A: Open arm entires, B: Time spent in open arm, C: Time spent in close arm, D: Mobilization, E: Time spent in central area, F: Time spent in light box, G: Time spent in dark box. The statistics are presented with mean±SEM (n=12 per group). One-way ANOVA with Tukey HSD for multiple comparisons. Abbreviations: EPM, Elevated Plus Maze; OFT, Open Field Test; LDB, Light-Dark Box. All data were expressed as mean ± SE values and analysed by one-way factorial ANOVA with Tukey post-hoc test. *p<0.05 is considered significant.

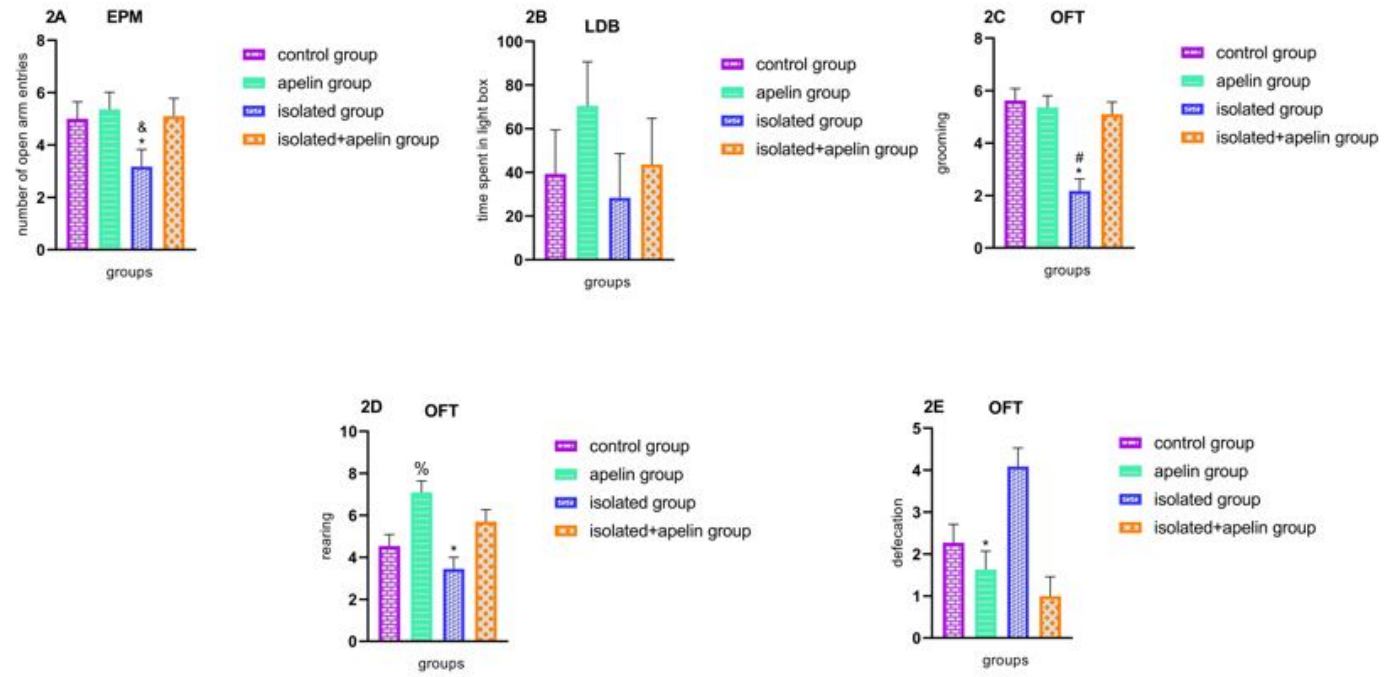

Figure 2. Effect of apelin administration on behavioural tests: EPM, OFT and LDB. A: Open arm entires, B: Time spent in light box, C: Grooming, D: Rearing, E: Defecation. The statistics are presented with mean±SEM (n=12 per group). One-way ANOVA with Tukey HSD for multiple comparisons. Abbreviations: EPM, Elevated Plus Maze; OFT, Open Field Test; LDB, Light-Dark Box. All data were expressed as mean ± SE values and analysed by one-way factorial ANOVA with Tukey post-hoc test. *p<0.05 is considered significant.

Gene expression analysis

For gene expression assessment, RNA was isolated from hippocampal and amygdala tissue using the TRIzol method. The concentration and quality of the total RNA samples were checked by spectrophotometric and agarose

Figure 3. Effect of apelin administration on gene expression levels. A: Gene expression levels of Apelin, APJ, BDNF, Neuritin, Nestin, DCX and MASH1 in the hippocampus. After the SI, B: Gene expression levels of Apelin, APJ, BDNF, Neuritin, Nestin, DCX and MASH1 in the hippocampus. After the apelin administration. All data were expressed as mean ± SE values and analyzed by one-way factorial ANOVA with Tukey post-hoc test. There is no significant difference between the groups (p>0.05).

gel electrophoresis methods. mRNA-level expression of the candidate and reference genes was detected by qRZR. PZR products of all genes were observed in the agarose gel (2%) electrophoresis.

Statistical analysis

In accordance with earlier research, a minimum sample size of n=12 per group was determined using a mean difference of 0.4 and a standard deviation of 0.1, which yielded 90% power at a 95% confidence level.ith reference to.

To analyze the gene expression data, all genes' Ct values were compared to the reference genes' Ct values (PGK1, RPL13A, and GAPDH), from which ΔCt values were obtained. Differences in gene expression among the groups were reported as mean ± SE values and analyzed using one-way factorial ANOVA followed by the Tukey post-hoc test. A significance level of *p<0.05 was applied.

Results

SI causes anxiety-like behavior in rats

The EPM, OFT and LDB tests were used to assess the effects of SI on anxiety-like behaviour in ratsIn the EPM test, the SI group spent significantly less time in the open arms than the C group, and they also entered the open arms significantly less frequently than the C group.n the EPM test, entries into the open arms (p<0.05, Figure 1A-B). However, time spent in closed arms did not change significantly (p>0.05, Figure 1C). No significant differences were found between the groups in the OFT and LDB tests (p>0.05, Figure 1D-F).

Apelin-13 administration may reduce anxiety-like behaviors in rats

In order to assess the effects of apelin-13 on behaviour, the EPM test was carried out. Open arm entries were significantly lower in the SI group compared to both the C and A groups (p<0.05, Figure 2A). However, in terms of time spent in open arms, there were no significant differences between the groups (p>0.05, Figure 2B).

In the OFT, the SI group showed fewer grooming and defecation behaviors compared to the C and A groups. Conversely, rearing in group A was significantly higher than the others (p<0.05, Figure 2C-E).

No significant differences between groups were observed for any parameter in the LDB test (p>0.05).

SI stress may have varying effects on different regions of the brain

According to the results of real-time PCR analysis, there was a significant decrease in the expression of the MASH1 gene in the hippocampal tissue of the SI group compared to the C group (p<0.05, Figure 3A). In rats that received apelin-13 treatment following SI, there were no significant differences in gene expression in the hippocampus and amygdala tissues (p>0.05, Figure 3B).

Discussion

The mechanisms underlying the psychopathological effect of stress remain largely unknown. Several stress methods used in animal modeling are important in terms of exposing this mechanism and have shown that there is a relationship among behavior and brain areas such as hypothalamus, hippocampus, and amygdala. It has been shown that there is apelin-13 and apj gene expression in these brain regions [17] which are considered to be central points for stress and emotional responses [18]. The existence of apelin-13 and APJ in these regions suggests that apelin-13 may have a potential role in the behavioural process. Moreover, different special biomarkers are expressed at the stages that take place in the neurogenesis process. Through biomarkers, the neurogenesis process and the path of newborn cells can be observed in detail and clearly [19,20]. The present investigation demonstrates activation of the BDNF, Nestin, Neuritin, MASH1 and DCX expression levels in hippocampus and amygdala following apelin-13 infusion in the rat. After creating anxiety with the SI model in rats, apelin-13 was injected as subcutaneous for 14 days. To find out how apelin-13 affected anxiety, behavioral tests and the expression levels of multiple genes in the hippocampus and amygdala were analyzed.

In the OFT, the scores for the time spent in the central area were significantly higher in the A group than in the B group. The decreasing of time spent in the central part of the square is considered as an indicator of anxiety, anxiolytic agents increase research behavior and prolong this time [21,22]. In the current study, the SI group had a significant decrease in time in the centre after application, while the A group had an increase in time in the centre after application.

Another parameter is the exploratory behavior of the rats to investigate and obtain information. The frequency of this behavior decreases anxiety [23,24]. We also determined that the scores of rearing and grooming were significantly lower in the SI group in the OFT. It is seen that the increase in the rearing after application of the A group supports the anxiolytic effect of the apelin-13.

The distance parameter measured at OFT gives information about the locomotor activity of rats. The study's findings indicate that the rats in the SI group were less distant than those in the C group, but that this difference was reversed following the administration of apelin-13.ccording to the results of this study, rats in the SI group decreased. Defecations increases in anxiety due to activation of the autonomic system [25]. Considering the C group, there has been significant increase defecations in the SI group. Apelin-13 application significantly reduced defecations. However, both views are available in grooming behavior [26]. Decrease in grooming activity can be attributed to an increase in anxiety behaviour however, anxiolytic drugs can reverse this by increasing grooming activity [27]. Grooming decreased in the SI group, while apelin-13 increased in the group applied. According to the current findings, considering that anxiety decreases after apelin-13 application, the increase in grooming behavior after apelin-13 application can be considered as a decrease in anxiety.

Open arm entries and time spent in open arms are the main parameters considered in the EPM [28]. In a study conducted by [29] they investigated the effect of fluoxetine, an effective antidepressant treatment, on anxiety and they reported that the duration of the animals receiving fluoxetine increased in the open arm. We determined that the open arm entires and time spent in open arm. We observed an increase in both groups of apelin-13 may have occurred due to anxiety reduction.

LDB scored the time spent in the light box and the time spent in the dark box. An indicator of anxiety may be the prolonged time spent in the dark box in the SI group. Anxiogenic agents increase the number of passes to the bright box and the residence time, while anxiolytic substances increase the number of passes to the dark box and the length of stay in this region [30]. A potential correlation between hippocampal neurogenesis and anxiety-related behaviors has been proposed. The inhibition of neurogenesis has been linked to an increase in certain types of behaviour associated with anxiety. According to our findings, it was determined that the number of passes from dark box to bright box decreased in the SI group, and this transition behavior increased with the application of the apelin-13.

Apelin-13 gene expression has been shown to decrease in brain regions as a result of neurodegenerative diseases [31]. Injection of intrahippocampal apelin-13 has been reported to have a modulatory effect on mood disorders such as anxiety and depression by modulating various cellular signalling pathways such as ERK and PI3K [32]. In our study, apelin-13 gene expression levels of rats investigated in the hippocampus and amygdala tissues, but no significant changes were found.

APJ is a receptor found in many tissues such as hippocampus, hypothalamus, amygdala in addition to peripheral tissues in rats [33]. The increase of APJ in the hippocampus, where the stress response occurs, can be effective in maintaining moods by increasing the apelin-13 binding capacity. Recently, it was reported that apelin-13 regulates stress response by increasing the expression of the APJ found in the hippocampus [34]. We did not detect a significant change on the APJ expression level in SI group.

Neuritin is a protein that regulates neuronal plasticity [35] and increases the level of BDNF in the hippocampus in a similar way to antidepressants [36]. Researchers have demonstrated that long-term stress lowers the expression of neuritin in the rat hippocampal CA1 and CA3 regions [37]. In agreement with the recent experimental data indicating increased expression of neuritin by chronic antidepressant treatment in the rat brain [38], we also observed that the expression level of neuritin in the A group increased compared to the C group but not statistically significant. This may be due to the removal of the total hippocampus tissue, not specifically the CA1 and CA3 sections.

DCX is used to measure the degree of neurogenesis and is only expressed in neonatal neurons [39]. It was reported that the level of DCX expression decreased in anxiety group. They applied Tamoxifen injection to suppress neurogenesis [40]. We also determined that the level of DCX expression was lower in the SI group. Decreased in DCX expression level may be considered as postpartum depression [41]. It has been reported that maternal separation reduces hippocampal cell proliferation but does not have a mitigating effect on DCX expression [42]. According to our findings, Decrease in DCX expression in the SI group, but increase in DCX expression after apelin-13 injection. These results may give clues that neurogenesis is suppressed because of anxiety and the injection of apelin-13 has a positive effect on neurogenesis.

Many areas of the brain, including the amygdala and hippocampal regions, express BDNF and as hypocampal neurogenesis increases, there is also an increase in BDNF gene expression [43]. It has been noted that apelin-13 plays a healing role in memory loss by increasing the level of hippocampal BDNF [44]. According to our findings, anxiety has reduced the level of BDNF gene expression in hippocampus tissue. Gray et al. demonstrated that stress is a factor that causes a decrease in BDNF levels in the CA1 and CA3 regions of the hippocampus, which is consistent with our findings [45].

Nerve stem cells divide and differentiate in the neurogenesis phase, forming the precursor cells. Nestin is a precursor biomarker [46]. Recently a study noted that the level of the positive nesting cell increased with anxiety treatment [47]. In our experiment, apelin-13 infusion decreased the level of nestin gene expression in hippocampus tissue. This may

be due to the extraction of the entire hippocampus tissue or insufficient infusion time. In amygdala tissue, it has been observed that apelin-13 increased the level of nestin gene expression. It may support the idea that apelin-13 has a positive effect on neurogenesis by increasing neural precursor cells in different parts of the brain.

MASH1 is an important transcription factor that regulates differentiation processes during neurogenesis and differentiates neural progenitor cells [48]. Research has demonstrated that adult DC ischaemia is associated with elevated MASH1 cell production and MASH1 mRNA expression. This implies that neurogenesis is significantly influenced by the growth of these cells [49]. Therefore, we focused on MASH1 protein expression in the hippocampus and investigated its physiological effects. According to the findings of our study, MASH1 gene expression was significantly decreased in hippocampus tissue in both SI and A groups. This may be due to the activation of neural precursor cells as a result of negatively affected neurogenesis.

Conclusion

In conclusion, when all these findings are evaluated, it is likely that hippocampal neurogenesis may play a role as an intermediary mechanism under the anxiolytic effect of apelin-13. Apelin-13 may have increased hippocampal neurogenesis, so it causes a decline in anxiety behaviours. The present findings indicate that SI has increased anxiety-like behaviours and have a suppressive role in hippocampal neurogenesis. Behavioural experiments have demonstrated that apelin-13 has inhibition of anxiety.

Ethical approval

Ethical approval was obtained for this study from Necmettin Erbakan University KONÜDAM Experimental Medicine Application and Research Center Animal Experiments Local Ethics Committee (Decision number: 2018-011).

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