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Resveratrol ameliorates physical and psychological stress-induced depressive-like behavior

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Abstract

Objectives: Depression is a mental disorder that profoundly affects all aspects of life, but currently, antidepressants have some problems with their effectiveness and side effects. Resveratrol is a compound that has the ability to regulate the hypothalamic-pituitary-adrenal axis. This study aimed to determine resveratrol's effect on physical and psychological stress-induced depressive-like behavior.

Methods: Mice were divided into control, physical stress, psychological stress groups. Treatment was conducted with fluvoxamine 20 mg/kg and resveratrol 20, 40, and 80 mg/kg for seven days. The depressive-like state was evaluated using a forced swim test (FST), tail suspension test (TST), and open field test (OFT).

Results: Physical stress and psychological stress induction increase the immobility time on FST and TST. Besides, there is an increase in time in central on OFT, which indicates an anxiety or mental illness-like behavior. However, the OFT examination on sniffing, rearing, grooming, and crossing behavior did not show a significant difference. Resveratrol 80 mg/kg and fluvoxamine 20 mg/kg were significantly reduced immobility time at TST compared to the physical stress group. While in psychological stress, resveratrol 80 mg/kg tended to decrease immobility time but not significant. A significant increase in time in central duration was seen in the resveratrol 40 mg/kg compared to the psychological stress. Stress induction causes increased amygdala corticotrophin-releasing factor (CRF) mRNA expression. However, neither resveratrol nor fluvoxamine affected amygdala CRF mRNA expression.

Conclusions: Resveratrol ameliorates depressive-like behavior induced by physical and psychological stress.

Keywords: depressive-like behavior; fluvoxamine; physical stress; psychological stress; resveratrol; mental illness.

Introduction

Depression is a mental disorder that characterized by low self-confidence, hopelessness, worthlessness, insomnia, fatigue, reduced interest in sex and social interactions, and the onset of suicidal thoughts [1]. There are more than 264 million people affected by depression worldwide [2].

Stress is one of the predisposing factors for depression, anxiety, post-traumatic stress disorder. Both physical and psychological stress is known to cause depressive behavior. Apart from stress, abnormalities of the Hypothalamic-Pituitary-Adrenal (HPA) axis are also associated with depression. This situation begins when the peptide called corticotrophin-releasing factor (CRF) increases due to the stressor response and increases adrenocorticotropic hormone and cortisol in the blood. This peptide is known to affect the limbic system in several brain regions such as the hypothalamus and amygdala [3–5].

Nowadays, selective serotonin reuptake inhibitors are chosen for depression treatment. These antidepressants are known to affect serotonin and the HPA axis. Unfortunately, these antidepressants still have their therapeutic drawbacks. It has been reported that about 60% of patients experienced side effects in the form of insomnia, headaches, nausea, abdominal pain, sexual disturbances, hyponatremia, and serotonin syndrome [6, 7]. Besides, only about 50% of patients experienced an improvement after using antidepressants [8]. Therefore, it is necessary to look for antidepressants from natural herbs which are expected to show fewer side effects such as resveratrol [9].

Resveratrol is a polyphenol compound obtained from grapes, itadori plants, nuts, berries, and chocolate. Resveratrol has many benefits such as anti-inflammatory, neuronal protection effects, antioxidant, cardioprotective effects, preventing obesity and diabetes, and is used for diseases related to aging [10]. Resveratrol is preclinically known to reduce depressive-like behavior induced by lipopolysaccharide,

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corticosterone, and chronic unpredictable mild stress in an animal model [7].

Nowadays, to understand treatment responses and biomolecular markers, an animal model is used. The communication box is an animal model used to study behavioral and physiological changes using physical stress and psychological stress induction. Physical stress induction is performed using footshock stress, while psychological stress induction responds to animals induced by physical stress [11].

In this experimental animal model, depressive behavior can be observed by looking at one of the main symptoms (despair behavior) and an additional symptom in the form of a change in locomotor activity. Besides, there are also conditions associated with depression, such as anxiety-like behavior. Despair behavior can be seen from the increased immobility time using the forced swim (FST) test and tail suspension test (TST). At the beginning of these two tests, the animal tries to get out of the test condition by making an active swimming motion or by trying to reach for its tail so that it is no longer positioned upside down. However, there will be a time when the animal will feel hopeless with the test condition because it has been arranged so that the animal does not can escape. As a result, these animals will show immobility behavior which is used as a reference for symptoms of depressive behavior. Meanwhile, behaviors such as anxiety-like behavior and changes in locomotor activity as symptoms of depressive behavior can be observed using an open field test (OFT). Anxiety-like behavior was observed with a decrease in the duration of the animal being in open space which was described as risk-taking behavior [11, 12].

In this study, we investigated resveratrol's effect on depressive-like behavior and CRF mRNA in the amygdala using FST, TST, OFT, and RT-PCR.

Materials and methods

Animal

The Ethics Committee of the Faculty of Veterinary Medicine, Airlangga University has approved all experiment protocols. Handling of animals according to the Guidelines for the Care and Use of Laboratory Animals issued by the National Institutes of Health revised in 1985. Male mice weighing 20–30 g, aged 6–8 weeks, procured from Veterinary Farma, Surabaya, Indonesia. Animals are placed under a 12:12 h light/dark cycle in a polypropylene cage, facilitated food and water access, and were maintained at 22–25 °C. The animals were randomly subjected and had acclimatization for one week before the experiment.

Experimental and treatment

Resveratrol (Res) (Tokyo Chemical Industry Co) dose 20, 40, 80 mg/kg and Fluvoxamine maleate (Flu) (Wako Pure Chemical Industries) dose

20 mg/kg were dissolved in 10% of tween 80. Mice were divided into 11 groups (5–6 mice in each group): control, physical stress (FS), psychological stress (PS), FS Flu 20, PS Flu 20, FS Res 20, FS Res 20, FS Res 40, PS Res 40, FS Res 80, and PS Res 80. The experimental method was carried out by modifying the model from Ikeda et al. [13] and Ge et al. [14]. The communication box was divided into 3×3 compartment separated with transparent acrylic (Figure 1). Physical stress exposure with communication box is done with 1 mA electrical footshock for 1 s with 9 s intervals for 5 min. To prevent electric shock, a plastic plate was placed on psychological stress grid floors. Before induction, mice were adapted for 1 h. Induction was carried out for 10 days and the administration of Res and Flu was carried out intraperitoneally from day four to day 10. A day after induction, behavior testing was carried out using OFT, TST, FST, and then sacrifice.

Open field test

In this test, mice were placed in a box with a base measuring 40 \times 40 and 30 cm in height. The box is divided into 16 squares measuring 10 \times 10 cm marked with a line. Mice were placed on the corner face the wall and tested for 5 min. The behavior measured in this tool is the number of crossing, time in central, rearing, grooming, and sniffing. Mice were categorized as experiencing depression and accompanying symptoms in the form of anxiety if there was a decrease in the value of time in central, locomotor activity (crossing, rearing), grooming, and sniffing behavior toward control mice.

Tail suspension test

The mice's tail was hung for 6 min on a hook that was placed 58 cm above the floor. In the last 4 min of the test, the immobility time is measured. The mice were separated from each other during testing. In this test, depressive behavior is described if the time immobility score is significantly higher than that of mice in the control group.

Forced swim test

At FST, animals are placed in tubes filled with water conditioned so that the animals cannot escape. The behavioral cylinder was filled with water maintained at 24-25 °C. Mice get a single exposure session

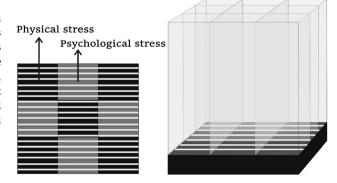


Figure 1: A communication box with electrical gird and transparent acrylic.

for 6 min. The first 2 min is used for the training time and the last 4 min is the measured period of immobility. The mice were placed into dry cages and dried with towels after the test session. In this test, depressive behavior is described if the time immobility score is significantly higher than that of mice in the control group.

RT PCR

The amygdala was dissected, immediately frozen, and kept at −80 °C. Total RNA from amygdala isolated used PureLinkTM RNA Mini Kit (Life TechnologiesTM, USA). Reverse Transcription was performed used GoScript™ Reverse Transcription System (Promega, USA). PCR was conducted using GoTag® DNA Polymerase (Promega, USA). The following primers were used: CRF (Forward: 5′- GAAGAGAAAGG-GGAAAAGG-3′; Reverse: 5′- GCGGTGAGGGGCGTGGAGTT-3′) and β-actin (Forward: 5′-TGTTACCAACTGGGACGACA-3′; Reverse: 5′-AAGGAAGGCTGGAAAAGAGC-3′) PCR was performed on a thermal cycler as follows: initial denaturation for 5 min at 94 °C, followed by 35 cycles of denaturing for 40 s at 94 °C, annealing for 1 min at 55 °C, extension for 2 min at 72 °C and a final extension for 5 min at 72 °C. PCR products were analyzed using electrophoresis (Mupid-ex; Advance, Tokyo, Japan) with 2% agarose LE (Promega, USA) gels. Ethidium bromide (Sigma-Aldrich) was used to stained gel and

photographed with UV transillumination. ImageJ (National Institutes of Health, USA) was used to determine the band intensity.

Analytical statistic

All data are represented as mean ± SEM using Graph-Pad Prism version 6.0. One-way analysis of variance and the Bonferroni test were used to compare groups. p<0.05 and p<0.01 were considered significant.

Results

Model of depressive-like behavior

Model of depressive-like behavior in Figure 2 shows no significant difference between the control group compared with crossing (2A), sniffing (2B), rearing (2C), and grooming (2D) behavior, but the induction of psychological stress was able to reduce time in central (2E) significantly. Immobility time (2F) on TST significantly increased in the physical stress group only. Whereas at FST, both stressors increased immobility time (2G) significantly.

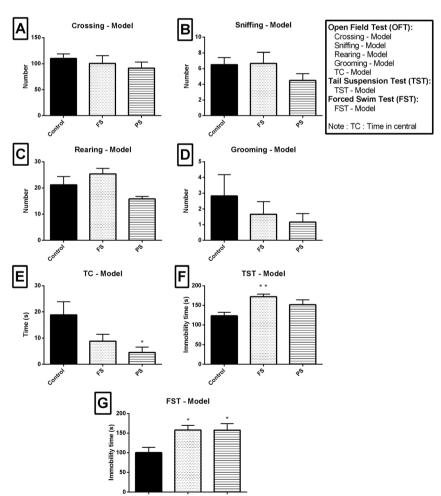


Figure 2: Effect of stress induction on the crossing (A), sniffing (B), rearing (C), grooming (D), time in central (E), tail suspension test (F), forced swim test (G). n=6 mice per group. FS=Physical stress, PS=Psychological stress *p<0.05 vs. control. **p<0.01 vs. control.

Administration of resveratrol alleviated depressive-like behavior

The time in central parameters by administering 20 mg/kg of fluvoxamine or 20 and 80 mg/kg of resveratrol in the physical stress group did not provide a significant change, but this parameter tended to increase when giving resveratrol 40 mg/kg (3A) (Figure 3). Administration of 20 mg/kg of fluvoxamine or 20 and 80 mg/kg of resveratrol in psychological stress group tended of increasing time in central. A significant increase was seen in the resveratrol 40 mg/kg group compared to the other psychological stress group (3B).

In testing using TST, administration of resveratrol 20, 40 mg/kg in animals with physical stress induction gave a trend of decreasing immobility time. A significant decrease occurred in fluvoxamine 20 mg/kg or resveratrol 80 mg/kg group (3C). The parameter of immobility time in the psychological stress group given fluvoxamine or resveratrol tended to decrease but did not occur in the resveratrol 40 mg/kg group (3D).

At FST, there was a trend of decreasing immobility time in the physical stress group given 20 mg/kg of fluvoxamine or 20, 40, and 80 mg/kg of resveratrol. However, based on statistical analysis, this decrease was not significant (3E). The parameter of time of immobility in

animals that were induced by psychological stress and then given fluvoxamine 20 mg/kg or resveratrol 80 mg/kg tended to decrease. However, it did not occur in the resveratrol 20 and 40 mg/kg groups (3F).

Effect of resveratrol on CRF mRNA

The results showed that physical stress induction did not significantly affect CRF mRNA expression, whereas psychological stress induction increased amygdala CRF mRNA expression significantly (4A) (Figure 4). The administration of fluvoxamine or resveratrol did not significantly change the amygdala CRF mRNA expression in mice induced by physical stress (4B) and psychological stress group (4C).

Discussion

Stress induction has been used in many experimental animals resulting in HPA axis dysregulation. In this study, the communication box model's induction shows that depressive-like behavior in mice induced by physical stress and psychological stress. This is indicated by an increase in immobility time on TST and FST. Besides, there is a decrease

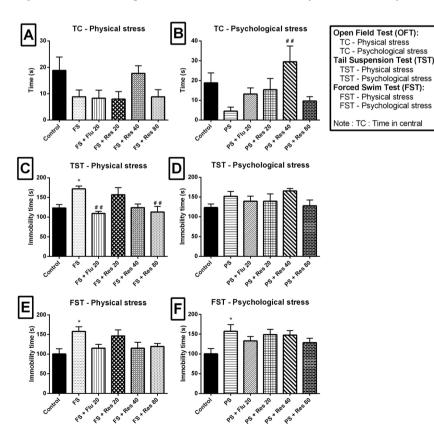


Figure 3: Time in central on physical stress (A) and psychological stress (B); TST immobility time on physical stress (C) and psychological stress (D); FST immobility time on physical stress (E) and psychological stress (F) responses by treatment of fluvoxamine (Flu) and resveratrol (Res). n=5-6 mice per group. *p<0.05 vs. control. **p<0.01 vs. stress group.

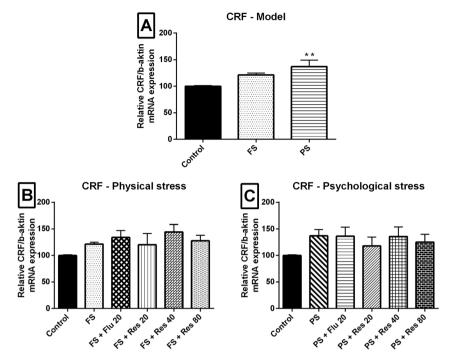


Figure 4: Physical (FS) and psychological stress (PS) effect on CRF mRNA expression (A). Effect of fluvoxamine (Flu) and resveratrol (Res) on CRF mRNA expression induced by physical stress (B) and psychological stress (C). n=3 per group. **p<0.01 vs. control.

in time in central, which indicates anxiety-like behavior. However, stress induction using the communication box model did not cause changes in the locomotor activity. The presence of depressive-like behavior is consistent with the previous study that uses social stress induction for psychological stress and physical stress induction with chronic unpredictable mild stress [15, 16].

Resveratrol 40 mg/kg decreased the time in central duration. It is indicated that resveratrol has the potential to treat anxiety-like behavior. This finding is consistent with the study in a post-traumatic stress disorder model [17]. The results of immobility time using FST and TST show that resveratrol has a similar antidepressant-like effect to fluvoxamine as behavioral therapy for depression in mice induced by physical and psychological stress. These results also show that the resveratrol antidepressant effect is more effective in physical stress than psychological stress. This can occur because the induction of physical stress and psychological stress can provide different molecular changes and metabolites associated with nerve development functions and cellular proliferation [18]. The differences were also observed in behavior patterns, corticosterone levels, and gene expression associated with plasticity [19].

Stress induction increased the amygdala CRF mRNA expression, consistent with the previous study [20]. Resveratrol 20, 40, 80 mg/kg and fluvoxamine 20 mg/kg did not show any changes in the amygdala CRF mRNA expression. This suggests that resveratrol and fluvoxamine do not act as antidepressants by affecting CRF projected from the amygdala. CRF mRNA from the amygdala is

projected predominantly to the bed nucleus of stria terminalis, locus coeruleus, and minor projections to the ventral tegmental area and lateral hypothalamus [21].

There is evidence that 15 mg/kg of resveratrol for 16 days can eliminate depressive behavior by reducing despair behavior and reduce CRF mRNA expression in the hypothalamus [22]. Furthermore, resveratrol 40 mg/kg for 18 days can reduce CRF protein levels in the hippocampus, hypothalamus, and amygdala and reduce anxiety-like behavior [17]. However, a different effect was reported in another study, which showed that 20 mg/kg of resveratrol for seven days reduced depressive-like behavior and the amount of corticosterone but did not decrease hypothalamus CRF mRNA expression. The difference in duration of therapy using resveratrol may affect the results of the CRF biomarker test. This study improves the hypothesis that resveratrol reduces depressive behavior in experimental animals by more influencing the function of the HPA axis through the peripheral route than through the central route [14].

Besides, it is also possible that resveratrol influenced other depressive pathways such as antioxidant activities and increased amygdala and hippocampus brain-derived neurotrophic factor (BDNF) protein level to lead to anti-depressant effect [14, 16].

Conclusions

In conclusion, both physical and psychological stress induction increased depression-like behavior in mice.

Fluvoxamine as well as resveratrol reduced depression-like behavior but did not affect amygdala CRF mRNA expression.

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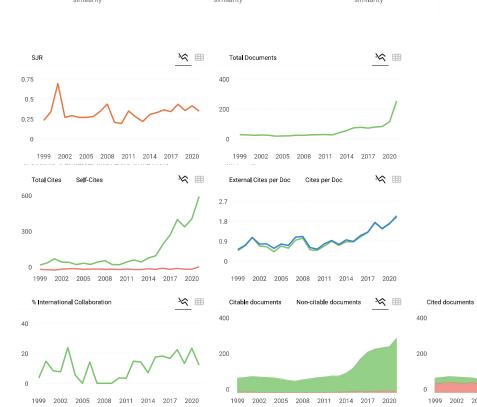
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