Combined Effect of Physical and Psychological Stress Exposure during Pregnancy on the Expression of Caspase-3 Cerebrum and Cerebellum of Newborn Mus musculus

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Abstract

Background: Prenatal stress affects fetal development including brain development. When a stressor is felt, the brain as the main target for stress will release a hormone that stimulates the release of pro-apoptotic proteins and activate caspase-3 which acts as an executioner caspase in the cell death process. The aim of the study was to analyze the effect of combined stress during pregnancy on the expression of caspase-3 cerebrum and cerebellum of newborn *Mus musculus*. **Methods:** An experimental study using 24 pregnant mice (*Mus musculus*). Subjects were randomized into four groups, consisting of physical stress exposure group (forced swimming) (G1), psychological stress exposure group (noise) (G2), combination stress exposure group (forced swimming + noise) (G3), and control group (G4). Stress exposure was given on 6^{th}-15^{th} days of pregnancy. From each mother, three Newborn of *Mus musculus* were taken to make preparations from brain tissue. Immunohistochemical examination was performed to assess caspase-3 expression. **Results:** The study shows that the mean and standard deviation of the expression of caspase-3 cerebrum and cerebellum in the physical stress exposure group is 5.70 ± 0.99 and 5.80 ± 1.35, the psychological stress exposure group is 7.23 ± 1.39 and 7.40 ± 1.24, the combined stress exposure group is 8.67 ± 1.09 and 9.30 ± 1.12, and the control group 4.17 ± 1.18 and 3.90 ± 1.06. ANOVAs statistical test results show significant differences among groups with a value of p = 0.000 in the cerebrum and p = 0.000 in the cerebellum. **Conclusion:** Exposure to physical and psychological stress during pregnancy increases the expression of caspase-3 in the cerebrum and cerebellum of newborn mice.

Keywords: *Caspase-3, stress, pregnancy, cerebrum and cerebellum*

Introduction

Pregnancy stress not only has a negative impact on the survival of the pregnancy, it can also affect fetal development and maternal well-being. Prenatal stress affects the fetus resulting in low birth weight, prematurity, and impaired brain development [5, 21]. Studies report that the prevalence of stress during pregnancy in the world ranges from 5.5 to 78%, while in developing countries it ranges from 6% to 52.9% [4, 15].

The brain is the main organ that interprets, responds to,and becomes the target of stress marker hormones [14]. When a stressor is felt, the hormone (CRH) wich acts on the anterior pituitary to promote the secretion of adrenocortico-tropic hormone (ACTH). This hormone then stimulates the adrenal cortex to release glucocorticoids (GCs) into the bloodstream [8]. High glucocorticoids will reduce BDNF expression and stimulate cell apoptosis [18].

Caspase-3 acts as the executioner caspase in the cell apoptosis process [12]. Caspase-3 when activated has the function of controlling cell death [20], causing cleavage of protein kinases, cytoskeletal proteins, DNA repair proteins, endonuclease inhibitory subunits and ultimately to deterioration of cellular function [9].

Increased apoptotic activity of brain cells will reduce the number of cells making up the central nervous system in the fetal brain where there are two main types of cells that make up the central nervous system, namely neurons and glial cells [9]. The cerebellum is a part of the brain that contains more neurons than other parts of the brain [7], the cerebrum and cerebellum are interconnected by means of polysynaptics, forming a system related to cognitive function and neuropsychiatric disorders [1]. There is little literature and researches on the impact of stress and neurobehavioral studies on the impact of stress on the cerebrum or cerebellum.

This study identifies differences in caspase-3 expression in the cerebrum and cerebellum of newborn mice (*Mus musculus*) whose mothers are exposed to physical and psychological stress during pregnancy.

Materials and Methods

This research is an experimental study on mice (*Mus musculus*) which was conducted from January to March 2021 at the Laboratory of the Faculty of Veterinary Medicine, UNAIR, Surabaya. This study used 24 adult female mice (*Mus musculus*) aged 2-2.5 months of pregnancy which were exposed to stress during pregnancy on the 6th until 15th days of pregnancy. The research subjects were divided into 4 groups which were randomly selected (G1, G2, G3, and G4) with 6 mice in each group.

First group: Exposure to physical stress in the form of forced swimming for 5 minutes once a day in a special box measuring 50x30x25 cm filled with water with a height of 18 cm with a water temperature of 24 ^{o}-28^{o} C and put in a dark box cage, exposure time is 09.00 am.

Second group: The group of exposure to psychological stress by giving noise with an intensity of 90 dB once a day for 1 hour successively in a dark and soundproof enclosure measuring 1x1x2 m, TrueRTA software (real time audio analyzer) was used to produce noise. Noise intensity was measured with a real time sound analyzer (TES 1358) each day prior to exposure to experimental animals, by placing the analyzer in animal cages at several locations, and taking the average of the different readings.

The third group: The stress exposure group, a combination of physical and psychological, was given a noise of 90 dB for 1 hour, then given a break of 5 minutes and then be swam for 5 minutes per day, exposure time starts at 09.00 am.

Fourth group: Control group with standard treatment without stress exposure.

Sampling Inspection

Mus musculus mothers were anesthetized then the pups were born by sectio caesarea (SC) on the 16th day of pregnancy. The pups of *Mus musculus* which were to be sacrificed were anesthetized first, and then the cranium was cut in the sagittal direction from caudal (occipital) to rostral (frontal), right between the two hamisters of their brain. Furthermore, the brain was released. The separated brain was weighed, and then put in a 10% formalin solution for organ preservation; the cerebrum and cerebellum were taken. Furthermore, immunohistochemical preparations and Hematoxylin-Eosin (HE) staining were made.

Data Analysis

To see the normality of the data, the Shapiro-Wilk test was used. If the data obtained are normally distributed, then the ANOVA test is used followed by LSD (Least Significant Difference) to see the differences in all groups. If the data obtained are not normally distributed, the Kruskall Wallis test and the Mann Whitney test are used. This study uses a significance level of $P<0.05$. To simplify statistical calculations, researchers used the SPPS tool version 21.

Results and Discussion

Results

The results show the highest expression of caspase-3 in the cerebrum and cerebellum in the combination of physical and psychological stress exposure group compared to the physical stress exposure group, psychological stress exposure group, and the control group (Table 1). The results of the normality test using

the Shapiro-Wilk test on the treatment group obtained a significance value (p-value)> 0.05, which means that the data distribution is normally distributed, so the Analysis of Variance (ANOVA) test was used to test whether there were differences in the treatment groups on the expression of caspase-3 in the cerebrum and cerebellum.

Based on the ANOVAs test results in table (2), it is known that there are significant differences among the groups in the expression of caspase-3 in cerebrum and cerebellum of newborn *Mus musculus*.

Table (1) Mean and standard deviation of caspase-3 expression in cerebrum and cerebellum of newborn *Mus musculus*.

Group of Treatment	Mean ± Standard Deviation	
	Cerebrum	Cerebellum
G.1	5.70 ± 0.99	5.80 ± 1.35
G.2	7.23 ± 1.39	7.40 ± 1.24
G.3	8.67 ± 1.09	9.30 ± 1.12
G.4	4.17 ± 1.18	3.90 ± 1.06

Table (2) Anova test results on Caspase-3 Expression in Cerebrum and Cerebellum of newborn *Mus musculus*.

Variable	p-value
Expression of Caspase-3 in Cerebrum	0.000*
Expression of Caspase-3 in Cerebellum	0.000*

* Significantly different <0.05

Figure (1) The differences in the description of caspase-3 expression in the cerebrum tissue of newborn mice. The red arrow indicates the presence of Caspase-3 expression which is indicated by the presence of brown chromogen. The combination of physical and psychological stress exposure group (G3) is the strongest among the physical stress group (G1), psychological stress group (G2), and the control group (G4). The expression of caspase-3 in the control group is the weakest. IHC is with a magnification of 400 times.

Figure (2) The differences in the description of caspase-3 expression in the cerebellum tissue of newborn mice. The red arrow indicates the presence of Caspase-3 expression which is indicated by the presence of brown chromogen. The combination of physical and psychological stress exposure group (G3) is the strongest among the physical stress group (G1), psychological stress group (G2), and the control group (G4). The expression of caspase-3 in the control group is the weakest. IHC is with a magnification of 400 times.

Discussion

The developing brain is the most sensitive organ to the effects of stress during the prenatal period, due to

substantial changes in structural growth and connectivity during fetal life [2]. During the gestational period, there are process of proliferation, differentiation, migration, and aggregation of fetal neurons which are strongly influenced by environmental factors [2]. Prenatal stress exposure shows a long-term effect that includes both behavioral and molecular changes [6, 13].

When a stressor is felt, the hypothalamus will release the hormone cortisol. During pregnancy, fetal exposure to maternal cortisol is limited by the placental enzyme 11β-HSD-2 which functions to convert cortisol to cortisone, an inactive glucocorticoid [17]. High glucocorticoids under stress reduce placental expression of 11βHSD2 which is associated with intraute-rine growth restriction [19].

In acute stress, the binding of glucocorti-coids and glucocorticoid receptors increases the tissue-plasminogen activator (tPA) which helps the process of converting proBDNF to mature BDNF (mBDNF) and increases proteolytic processing of proBDNF in mBDNF which can increase BDNF levels during stress, but in chronic stress due to decreased tpA hence the process of proBDNF to mBDNF is inhibited and BDNF expression is reduced. If proBDNF is not processed into mBDNF then proBDNF will bind more highly to the p75NTR receptor which induces a pro-apoptotic signaling pathway [3]. The proBDNF binding to the p75NTR receptor will activate the apoptotic pathway through the co-receptor bond, namely sortilin. Sortilin will activate jun-N terminal kinase (JNK) which will then phosphorylate C-Jun which will activate pro apoptotic proteins such as p53, Bad, BIM, BAX so that it will stimulate mitochondria to release cytochrome-C which then activates caspase [12].

It is known that there are two types of caspases that have been identified, namely the initiator caspase and the effector / executioner caspase. Caspase 8 and 9 are the initiator caspases, while caspase-3 is the effector / executioner caspase [22]. Caspase executioner mediates cell death during apoptosis, caspase-3 has the ability to partially cleave caspase substrates and its activity is required to induce cell death [10].

The results of our study indicate that there are significant differences in the expression of caspase-3 in cerebrum and cerebellum of newborn mice among treatment groups. Com-bined stress exposure show the strongest expression of caspase-3 compared to the physical, psychological, and control stress exposure groups. This finding is supported by a study by Qiao Y et al in 2020, where the combination of stress exposure caused significantly more hyper-activity of the HPA system as indicated by increased serum cortisol, CRH and ACTH levels [16].

High caspase-3 expression can cause an increase in apoptotic activity of brain cells and will decrease the number of cells making up the central nervous system in the fetal brain [9]. A study conducted by Kinsella et al in 2009 shows that chronic stress during the prenatal period interferes with fetal neurodevelopment. Sandman et al found that prenatal stress impaired cognitive performance during infancy and decreased brain volume [11].

Conclusion

Stress exposure during pregnancy increases the expression of caspase-3 in cerebrum and cerebellum of newborn *Mus musculus*.

Sources of Funding: This research was self-funded by the author.

Conflict of Interest: There is no conflict of interest in this study.

Ethical Approval : This study has obtained ethical eligibility permit based on the Research Ethics Committee of the Faculty of Veterinary Medicine, Airlangga University No: 2.KE.001.01.2021.

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