ORIGINAL ARTICLE

MAST CELL IN AMYGDALE, THALAMUS, HIPPOCAMPUS OF WISTAR RATS AND ITS CORRELATION WITH CORTICOTROPIN-RELEASING HORMONE (CRH) PLASMA LEVEL AND LENGTH OF ACUTE STRESS EXPOSURE

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Abstract

Objective*: Stress increases CRH level in the blood, which then affects the mast cell's number and function. This study aims to determine whether there is a correlation between the lengths of exposure and changes in the level of CRH and the existence and the activity of the mast cells in the area of amygdale, thalamus and hippocampus in rats exposed to acute stress. *Methods*: Sixteen rats were divided randomly; 4 in the control group and 12 in the exploratory group. The latter were then equally divided into 3 experimental groups, each consisting of 4 subjects. Each of the 12 rats that belonged to the 3 experimental groups was exposed to stress using the psychological stress device for three different lengths of time; 30, 60, and 90 minutes. The CRH plasma was then examined using enzyme-linked immunosorbent assay (ELISA), mast cells in amygdale, thalamus and hippocampus using the light microscope. *Results*: There was no significant difference in the number of mast cells, but the ones in the thalamus tended to increase (p = 0.092), compared to the amygdale (p = 0.269) and hippocampus (p = 0.117), in line with the length of the treatment, amygdale (r=0,291, P=0,274), hippocampus (r=-0.250, *p*=0.350), thalamus (r=0.619, *p*=0.11) in the response to stress level. *Conclusions*: There was a significant correlation between the lengths of exposure, the level of CRH with the number of mast cells in the thalamus, but there was no correlation both in amygdale or hippocampus. *ASEAN Journal of Psychiatry, Vol. 17 (1): January – June 2016: XX XX.

Keywords: Acute Stress, Length Of Exposure, CRH, Mast Cells, Amygdale, Thalamus, Hippocampus

Introduction

More than two decades ago, the brain was believed to be a place with immune privilege, in which blood cells did not exist in the parenchyma. Newer evidence indicates that there are blood cells contained in the brain, and some pass through it. Mast cells functionally include both [1]. Evidence shows that mast cell's transit from medium-sized blood vessels into the brain parenchyma [2]. Mast cells can be triggered by behavior, such as sexual stimulation in pigeons. In such a way, there is an increase in both the number and activity of mast cells in the medial habenula compared to the ones in the control group. Exposure to gonad steroid administered both endogenously and exogenously will increase the number and activity of mast cells in the brain. This shows that hematopoietic

cells can be the target of neuromodulators on specific regions in the brain because they affect the neural-endocrine interactions [1].

The activities of mast cells which are more commonly known as immune modulators are mostly linked to the body's immune system. The presence of allergens would activate mast cells to degranulate and secrete a variety of mediators that functionally play a role in maintaining the body's immune system [3].The The state of stress will increase CRH in the blood [4, 15, 19, 20]. The Increase of CRH in the blood is related to the intensity and the duration of the stress [5] that would bind to the receptor CRH on the surface of mast cells, so that the mast cells hydrate and cause the pores on the surface of the mast cells to enlarge and excrete granules, a situation which is termed as degranulation [6].

This study aims to determine whether the correlation between the lengths of exposure and changes in the level of CRH in the peripheral will be followed by a change of the circumstances, and the existence and the activity of the mast cells in the area of amygdale, thalamus and hippocampus.

Methods and Materials

Subjects

The subjects of the study were 16 male Wistar rats, which were approximately 3 months old with the body weight ranging from 119 to 137 grams prior to the treatment. The rats were acclimatized for 14 days, exposed to acute stress using the psychological stress device (Figure 1).The rats were obtained from the Pharmacology Laboratory of the Faculty of Health Science of Muhammadiyah University Malang, Indonesia. When the rats arrived in the laboratory, each rat was scaled, and a number was tagged on its tail for the purpose of sampling. The rats were kept in wire cages with the dimension of 30 cm x 12 cm x 35 cm. Five rats were placed in each cage, and they were exposed to light and darkness for 12 hours each day, amply fed, and put in a quiet place.

The Certificate of Research Ethics

The certificate of research ethics was obtained from the Commission of Ethics of Health Research of Brawijaya University, Malang while the research license from the Pharmacology Laboratory, the Anatomy Histology Laboratory, and the Physiology of the Faculty of Medicine of the respective University.

Treatment with the Psychological Stress Device

After the rats had been acclimatized, they were randomly sampled, and divided into four groups. Each group consisted of four rats; one control group was given no treatment, and the three experimental ones received 30, 60, and 90 minutes of treatment each. The rats in the experimental groups were exposed to acute stress using the Psychological Stress Device (PSD) by putting them on a platform made of transparent acrylic with the length of 20 cm and width of 21 cm. The platform was able to move in all directions with the maximum lean angle of 6 degrees. The platform stands on a pole at a height of 100 cm from the ground, above which a 60 watt bulb was hanging. Soon after each treatment was finished within the planned lengths of time, the rats were exterminated by dislocating their columna vertebra cervicalis in a rapid manner by a trained laboratory assistant. The abdomen of the rats was then dissected using a pair of scissors until the hearts that were still beating were visible. The blood of the rats was aspirated from the ventricle for as much as 2 ml by using a 3 ml syringe. Afterwards, the blood was placed in tubes, which contained EDTA, kept in the refrigerator with the temperature of more or less 4 degrees Celsius before the CRH plasma level in the blood was examined using ELISA (Cusabio).

Figure 1. The knock-down Psychological Stress Device used in this study. This device is a modification of the ones of Rocher [7]; and Xu [8] As quoted in Daeng et al., 2015

Specimen preparation

Immediately after the blood sampling was completed, the heads of the rats were removed. The cranium was opened, and the brain was extracted and fixed with 10% formalin solution. The same procedure was conducted on the control group. After 18-24 hours, the brain was processed and made into paraffin blocks, sliced in sagittal direction to reclaim the area of amygdale, thalamus and hippocampus. The preparatory was then stained with Toluidin Blue [5]. After that, the mast cell inspection was performed by the Olympus light microscope.

Results

CRH Plasma

In the groups exposed using the PSD, the increase of the CRH plasma was linear with the length of the exposure.

Table 1. The comparison of the Rat's CRH plasma exposed to PSD between Observation Groups

Groups	n	CRH Level (ng/mL)				*p*
		Mean	SD	Min	Max	
Control 0 minute	4	4.583	4.604	0.6667	11.1111	$p< 0.001$
PSD 30 minutes	4	14.833	5.253	9.8889	22.0000	
PSD 60 minutes	4	28.194	5.733	22.5556	34.5556	
PSD 90 minutes	4	36.139	10.283	25.5556	50.2222	

(n = number, SD = standard deviation, Min = minimum, Max = maximum)
As quoted in Daeng et al., 2015

The groups that received stimulation by the device showed their CRH levels varied along with the lengths of the exposure ($p<0.05$). Table 1 reveals that the longer the treatment was, the higher the CRH level became. The correlation between the lengths of exposure to the CRH level is represented by the following figure:

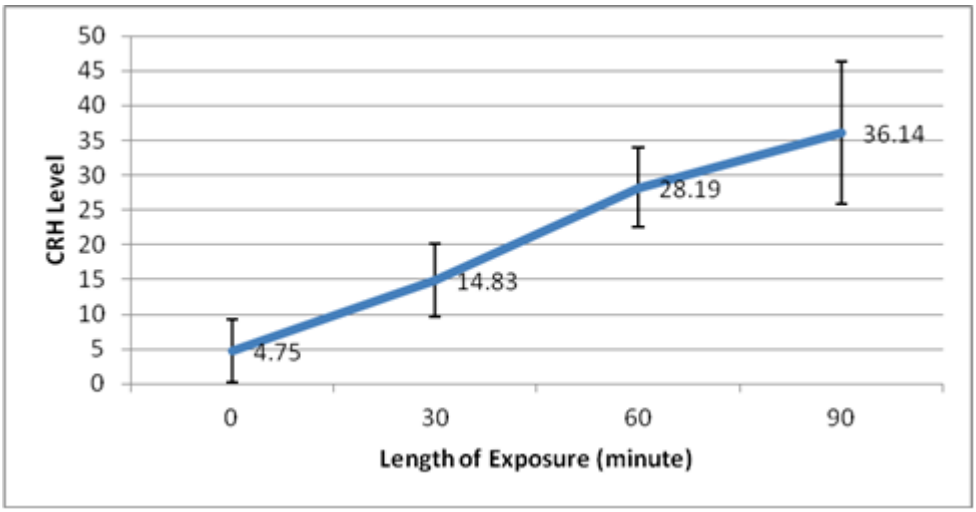

Figure 2. The correlation among the lengths of exposure (minute) using PSD with the CRH level (ng/mL) (r=0.894, p<0.05)

Table 2. The number of mast cells in amygdale, hippocampus, and thalamus

Location	Length of Exposure (minutes)	N	Number of the Mast Cells				(p)
			Mean	SD	Min	Max	
Amygdale	0	4	1.25	0.96	0	2	0.269*
	30	4	1.50	2.38	0	5	
	60	4	0.75	1.50	0	3	
	90	4	3.00	1.83	1	5	
Hippocampus	0	4	4.50	1.73	2	6	0.117**
	30	4	6.75	4.03	2	11	
	60	4	0.75	0.96	0	2	
	90	4	4.00	3.92	0	9	
Thalamus	0	4	0.25	0.50	0	1	0.092*
	30	4	1.00	0.82	0	2	
	60	4	1.50	1.29	0	3	
	90	4	2.50	1.73	1	5	

(* Kruskal-Wallis test. ** Brown-Forsythe test)

The analysis showed no significant difference in the number of mast cells in the amygdale, hippocampus, and thalamus among the lengths of exposure; 0, 30, 60, and 90 minutes (p> 0.05). However, of these three areas, there was a visible trend that the number of mast cells increased along with the duration of the exposure in the thalamus which was proven with the significant correlation between the length of the exposure and the number of mast cells in the thalamus (p <0.05), as represented by the following Table:

Table 3. Correlation of the lengths of exposure with the number of mast cells in amygdale, hippocampus, and thalamus

Location	Correlation of CRH levels with the number of mast cells	
	r	*p*
Amygdale	0.291	0.274
Hippocampus	-0.250	0.350
Thalamus	0.619	0.011*

Note: *Significant at α=0.05

Table 4. Correlation of CRH levels with the number of mast cells and the location of mast cells in amygdale, hippocampus, and thalamus

Mast Cell Location	Correlation of CRH levels with the number of mast cells	
	r	***p***
Amygdale	0.213	0.427
Hippocampus	-0.327	0.217
Thalamus	0.530	0.035*

*Level of significant at $\alpha = 0.05$

Table 4. The analysis showed a significant correlation between the CRH levels with the number of mast cells in the thalamus ($p < 0.05$), but without the correlation in the amygdale and hippocampus.

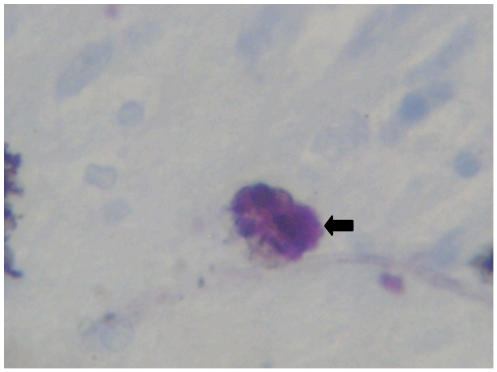

Figure 3. One non-degranulated mast cell (black arrow) seen in thalamus region stained with Toluidine Blue depicting visible granules in the cytoplasm and round nucleus of cell in the middle with darker color, 400X magnification with an Olympus light microscope.

Discussion

This study was experimental in nature, and the subjects of the study were 16 male Wistar rats exposed to psychological stress, which were approximately 3 months old with the body weight ranging from 119 to137 grams prior to the treatment.

This study only used male rats in order to make the samples homogenous since sex differences might influence the mood of the rats [16], which is analogous to human beings. Mood disturbances in humans are likely found twice as much in females [16, 17]. Nevertheless, this study did not, in particular, measure or investigate the correlation between mood and the age of the rats. Additionally, vascular depression, an age-related mood disorder, is found in human beings [18]. Further, body weight did not correlate with a CRH plasma level in the rats [5].

The examination of mast cells stained with Toluidine Blue observed by the Olympus light microscope with the magnification of 400X (Figure 3). Results of the analysis of the Kruskal-Wallis test and Brown-Forsythe test showed no significant difference in the number of mast cells in the amygdale, hippocampus, and thalamus among the lengths of 0, 30, 60, and 90 minutes of exposure ($p > 0.05$).However, However, among the three regions, it was observable that there was an increase in the number of mast cells along with the length of the exposure (amygdale $p = 0.269$, hippocampus $p = 0.117$, thalamus $p = 0.092$). It is proven with the significant correlation between the length of the exposure with the number of mast cells in the thalamus ($p < 0.05$). In other words, there was a relationship between length of the exposure with the number of mast cells and CRH levels in the thalamus.

The question that arose was why it took place in the thalamus, but not in the amygdale and hippocampus. The role of the thalamus in acute psychological stress might have been more dominant. Most of the mast cells in the

Central Nervous System (CNS) of healthy mammals are found in the thalamus [9, 10] whereas in rats B 10 PL, most of the mast cells are found in the hippocampal formation, and not in the thalamus [10]. A Study in the subordination chronic stress conducted by Cirulli, et al (1998) discovered an increase of the mast cells in the CNS (thalamus and hypothalamus) of the experimental rats [11]. In this study, the researchers also discovered the existence of the mast cells in the thalamus which is in line with the result of the previous study by Silverman et al (2000) who found a lot of mast cells in the thalamus from newborn rats [2] So perhaps naturally mast cells are likely to be found in the thalamus region in certain experimental species, and this is similar to the results of this research. However, there has been no research yet that answers the functions of the mast cells in the thalamus and the amount of it. It may seem that the increase in the amount, the distribution, and the activities of the mast cells in the brain are very much influenced by individuals, species, kinds, and the magnitude of the stress [11, 12]. From the analysis of the CRH level and the mast cells, it is obvious that the significant correlation observable was between the CRH levels and the mast cells, not the CRH levels with the degranulation of the mast cells. One important question to be further addressed and investigated is under which CRH level and/or after how long the granulation of the mast cells in the mood region of the rat's brain, in this case thalamus, takes place following the increase of the CRH level.

According to Stahl (2008), thalamus is hypothetically one of 11 centers of mood function whose function regulates signs and symptoms of mood [13]. Mast cells which degranulate due to the influence of CRH will release a mediator in the form of serotonin. Serotonin, dopamine, and norepinephrine regulate one another through the appropriate receptors. Norepinephrine functions as a brake on the release of norepinephrine by presynaptic α2 auto receptors and regulates the release of norepinephrine through receptors α2 somatodendritic. Norepinephrine also acts as the accelerator of the release of serotonin.

Norepinephrine controls serotonin in 2 directions; receptor 5HT2A regulates norepinephrine and dopamine, and receptor 5HT2C regulates norepinephrine and dopamine [13, 14]. In other words, serotonin, norepinephrine and dopamine regulate each other to achieve a functional equilibrium. The clinical implication of this study was to determine the patophysiopsychobiology of psychiatric disorders, especially mood disorders, associated with acute stress situation, and the role of mast cells, so that remedies for attitude management can be better developed. The limitation of this study is that the results cannot be generalized to human beings since the presence of mast cells in the brain depends on the individual, species, location, types, kind, and magnitude of the stress.

Conclusion

The number of mast cells in the thalamus was higher, and correlated with the lengths of exposure, and the CRH levels in the experimental rats that were exposed to acute stress, compared with the amygdale and hippocampus.

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References

1. Silver, R., Silverman, A-J., Vitković, L., and Lederhendler II, Mast cells in the brain: Evidence and functional significance. 1996;*Trends Neurosci,* 19 (1): 25-31.

2. Silverman, A-J, Sutherland, AK, Wilhelm, M, Silver, R, Mast cell migrate from blood to brain, The Journal of Neuroscience, 1 January 2000, 20(1): 401-408

3. Denburg, JA, Allergy and Allergic Diseases: the new mechanism and therapeutics. 1998; Totowa, Humana Press, In Befus AD, Denburg JA,

Basophilic Leucocytes : Mast Cells and Basophils in Lee, GR, Foerster J, Lukens J, Paraskevas F, Greer JP, Rodgers GM,Wintrobe's Clinical Hematology tenth edition. 1999; USA, Lippincott Williams & Wilkins.

4. Esposito P, Gheorghe D, Kandere K, Pang, X., Connolly R, Jacobson S, Theoharides, TC, Acute Stress Increases Permeability of the Blood-Brain Barrier through activation of Brain Mast Cells, USA, Brain Research. 2001; 888: 117-127.

5. Daeng BH, Wardhana AW,Widodo A,Sujuti H, Mintaroem K,Widjajanto, E, Plasma Corticotropine Releasing Hormone (CRH) Level Difference Between Wistar Rats Exposed to Acute Stress Due to Predator and to The Psychological Stress Device, ASEAN Journal of Psychiatry, Vol. 16 (2), July - December 2015: 193-202.

6. Wilhelm M, Silver R, Silverman AJ,Central Nervous System Neurons Acquire Mast Cell Products via Transgranulation, European Journal of Neuroscience.2005; (22): 2238-2248.

7. Rocher C, Spedding M, Munoz C, Jay TM, Acute Stress-induced Changes in Hippocampal /Prefrontal Circuits in Rats: Effects of Antidepressants, Cerebral Cortex. 2004; 14:224-229.

8. Xu L, Holscher C, Anwyl R, Rowan MJ, Glucocorticoid receptor and protein/RNA synthesis-dependent mechanism underlie the control of synaptic plasticity by stress. ProcNatlAcadSci USA. 1998; 95:3204-3208.

9. Brenner T, Soffer D, Shalit M, Levi-Schaffer F, Mast cell in experimental allergic encephalomyelitis: characterization, distribution in the CNS and in vitro activation by myelin basic protein and neuropeptides, J. Neurol. Sci.1994; 122:210-213.

10. Hendrix S, Warnke K, Siebenhaar F, Peters EMJ, Nitsch R, Maurer M., The Majority of brain mast cells in B 10.PL mice is present in the hippocampal formation, Neurocience Letter.2005;392:174-177.

11. Cirulli F, Pistilo L, AcetisLd, Alella E, and Aloe L, 1998, Increased Number of Mast Cell in the Central Nervous System of Adult Male Mice Following Chronic Subordination Stress, Academic Press, Neurocience Letters, 392 (2006) 174-177.

12. Silver R and Curley JP, Mast Cell on the mind: new insights and opportunities. 2013; Elsevier Ltd.

13. Stahl SM, Depression and Bipolar Disorder, Stahl's Essential Psychopharmacology, 3rd Edition. 2008; Cambridge University Press, New York.

14. PaeCh-U, Serretti A, Patkar AA, and Masand PS, Aripriprazole in the Treatment of Depressive and Anxiety Disorders, CNS Drugs. 2008; 22(5):367-388.

15. Theoharides TC, Stewart JM, Panagiotidou S, Melamed I., Mast cells, brain inflammation and autism. Eur J Pharmacol. 2015 May.

16. Seney, ML and Sibille, E, Sex difference in mood disorders: perspectives from humans and rodent models.Biol Sex Differ. 2014; 5: 17.

17. Kelly, MM, Tyrka, AR, Anderson, GM, Price, LH, andCarpenter, LL., Sex differences in emotional and physiological responses to the Trier Social Stress Test. J BehavTherExp Psychiatry. 2008 Mar; 39(1): 87-98.

18. Samaras, N, Rossi, G, Giannakopoulos, P, Gold, G, Vascular depression. An age-related mood disorder.European Geriatric Medicine. September 2010; 1(4): 220-225.

19. Esposito, P, Gheorghe, D, Kandere, K, Pang, X, Connolly, R, Jacobson, S, Theoharides, TC. Acute Stress Increases Permeability of the Blood-Brain Barrier through activation of Brain Mast Cells, USA, Brain Research. 2001; 888: 117-27.
20. Theoharides, TC, Roznieckі, JJ, Sahagian, G, Jacobson, S, Kempuraj, D, Conti, P, and Kalogeromitros, D. Impact of Stress and Mast Cells on Brain Metastases, ClicalKey, Elsevier. 2008; 12-15Z: 205(1-2): 1-7.

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