ORIGINAL ARTICLE

PLASMA CORTICOTROPINE RELEASING HORMONE (CRH) LEVEL DIFFERENCE BETWEEN WISTAR RATS EXPOSED TO ACUTE STRESS DUE TO PREDATOR AND TO THE PSYCHOLOGICAL STRESS DEVICE

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Abstract

Objective*: Stress triggers and causes psychiatric disorders. This study compared stress generated by different stressors: a cat as the predator of rats and a Psychological Stress Device (PSD) which was developed and modified by the researchers based on the model by Xu and Rocher. *Methods*: Twenty-eight Wistar rats were simple randomly divided into one control group and six treatment groups, each consisting of 4 rats. Each treatment group was individually exposed to stressor for 30, 60, and 90 minutes. The first three treatment groups were treated using the PSD while the other three treatment groups were treated exposed to the cat. Plasma CRH level was measured using the ELISA (Cusabio) method. *Result*: Plasma CRH levels in the rat exposed to stressor using the PSD ranged from 9.89 to 50.22 ng/mL, higher than plasma CRH level in the groups exposed to cat ranged from 0.22 to 23.44 ng/mL with significance level (p<0,05). The average of plasma CRH level in the rats exposed to the PSD for 30, 60, and 90 minutes were 14.83, 28.19, and 36 respectively. 14 ng/mL while in the groups exposed to cats were 11.53, 7.81, and 4.97 ng/mL respectively. The increase of plasma CRH level had positive correlation with the length of exposure to stressor in the group treated with the PSD (r=0.895, p of <0.05) while plasma CRH level in the group exposed to cat did not correlate with the length of exposure (r=-0.043, p>0.05). *Conclusion*: Plasma CRH level of the rats exposed to stressor using the PSD was higher and positively correlate with the length of exposure compared to those exposed to cat. *ASEAN Journal of Psychiatry, Vol. 16 (2): July – December 2015: XX XX.

Keywords: Stressor, Acute Stress, Predator, Psychological Stress Device, Plasma CRH

Introduction

Acute stress is a condition where an individual is exposed to a stressor from a few minutes to several hours [1]. Further, Bhatia et al. (2011) claimed that acute stress on animal under experiment is translated as their being exposed to a stressor once and by the same kind. In the psychiatric study, both acute and chronic stress may trigger or cause psychiatric disorders. One good example is the bipolar disorder which is a mood disorder initiated by real psychological stress. The recurrence of bipolar disorder is mostly triggered by a psychological stress[2], and so are other psychiatric disorders. The stressful condition stimulates paraventricular nucleus (PVN) to release corticotropine releasing hormone (CRH) which will then affect the body functions, immunity system, and psychological functions [1].

In most studies, the treatment of exposing experimental animals to stressors does not differentiate psychological from physical or social stressors. Bhatia, *et al*. (2011), in his study prescribes that inducing psychological stresses to rats can be performed by exposing them to a cat as their predator, noise, neonatal isolation, and light[3].

This study used Psychological Stress Device (PSD) which was developed and modified by the researchers based on the one designed by Xu et al. (1998) and also utilized by Rocher et al. (2004) in his study. Once rats experience acute stress, they will exhibit signs of anxiety, freezing, piloerection, defecating, and urinating while being on the platform [4, 5]. Cats as creatures may tend to be unstable. As a result, it is not a stable source of stressor. The stressor device, on the other hand, is stable and standardized. This study examined the differences in the level of plasma CRH between groups of rats exposed to a cat as their predators and the PSD.

Methods

Subjects

The subjects from the study were 30 Wistar rats obtained from the Pharmacology Laboratory of the Faculty of Health Science of Muhammadiyah University Malang, Indonesia. All the rats used for this study were males, with their initial weight ranging from 93 to 146 grams. The rats were acclimatized for 14 days, kept in six plastic cages with the dimension of 30 cm x 12 cm x 35 cm. Each cage accommodated 5 rats. The cages were woven with strings, and hays were placed inside them. The rats were exposed to light and darkness for each 12 hours a day, amply fed, and put in a quiet place. When the rats arrived at the laboratory, each rat was weighed, and a code was tagged on its tail. The rats were weighed again on the next morning before being given treatment.

Twenty-eight out of the 30 rats with the average weight ranging from 119-188 grams were selected. The other two were not involved in the study due to their insufficient body weight, and it was only 28 rats were needed. The rats were simple randomly divided into 7 groups by drawing the lottery paper. Each group consisted of four rats. The first group was decided to be the control group. The second three groups of rats were exposed to PSD, each group with the length of 30, 60, and 90 minutes consecutively. The third three groups were exposed to their predator, a cat, each group with the length of 30, 60, and 90 minutes consecutively.

Certificate of Ethics on Animal Testing and Research License

Before this, study was executed, the researchers applied for the certificate of ethics on Animal testing from the Commission of Ethics of Health Research of Brawijaya University, Malang with the ref. number of 228/EC/KEPK–PPDS/05/2013. The license of research was obtained from the Pharmacology Laboratory and the Physiology of the Faculty of Medicine of the respective University.

Treatment

Exposure to Psychological Stress Device (PSD)

In this study, a platform made of transparent acrylic with the length of 20 cm and width of 21 cm was made. The platform stands on a pole with the height of 100cm from the ground (Figure 1).

Figure 1. The PSD used in this research

In order to make the platform unstable, a ball and socket joint was placed between the pole and the platform whose thickness had been adapted to the platform, thus enabling the platform to move in all directions with the maximum lean angle of 6 degrees, enough to make the rats stressed out. The not-so-steep angle also enabled the rats to keep exploring without falling down from the platform. The device is knock-down in nature. It is made based on Xu et al., 1998 and Rocher et al. 2004.

Each rat was placed on the PSD under a 60 watt lamp. The rats were let to freely explore and move on the platform, and the researchers at the same time observed the signs of stress, which included freezing, piloerecting, defecating, and occasional urinating that the rats exhibited (Figure 2 and 3). The treatments lasted for 30, 60, and 90 minutes.

Figure 2. The rat is on the platform of the PSD

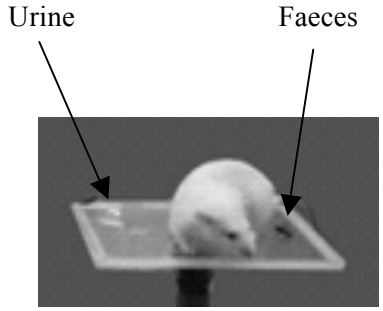


Figure 3. The rat is on the platform of the PSD, some amount of urine and fecal matter was visible because the rat was under a stressful condition

Soon after each treatment was finished within the planned duration of time, the rats were taken from the platform, and they were sacrificed by dislocating their columna vertebra cervicalis. Their abdomens were opened up to the thorax by a pair of scissors. As the heart was spotted, the blood was slowly aspirated from it with a 3 ml syringe for about 2 ml. The blood was then placed inside a bottle containing EDTA, labeled according to the codification, and stored in the refrigerator for the examination of the plasma CRH.

A cat as their predator

The predator involved in this study was a domestic cat kept in a cage with the dimension of 40 cm X 60 cm X 40 cm. The rats were individually placed into a cage with the dimension of 26 cm X 20 cm X 10cm, and then the cage in which the rats were individually placed for 30, 60, and 90 minutes, was positioned inside the bigger cage where the cat was around. (Figure 4).

Figure 4. The rat in its cage was individually

The signs of stress the researchers noticed were freezing, piloerection, defecating, and occasional urinating. Soon after each treatment was finished within the planned duration of time, the rats were taken from the platform, and they were sacrificed by dislocating their columna vertebra cervicalis. Their abdomens were opened up to the thorax by a pair of scissors. As the heart was spotted, the blood was slowly aspirated from it with a 3 ml syringe for about 2 ml. The blood was then placed inside a bottle containing EDTA, labeled according to the codification, and stored in the refrigerator for the examination of the plasma CRH.

Results

The Body weight of the Rats

The body weight of the rats when arriving and before treatment increased compared to when they were just brought to the laboratory. Two rats were excluded from this study for two reasons. First, they were underweight, andsecond;only 28 rats were needed in this study.

The result from the analysis in Table 1 revealed that data of initial weights, weight after 2 weeks, and weight gain had normal distribution in all groups (Shapiro-Wilk $p>0.05$). The variations from the data were homogenous among the groups (Levene's test $p>0.05$). Data analysis using one-way Anova confirmed that there were no significant differences in initial weight, weight after 2 weeks, and weight gains among the groups.

Table 1. Comparison of the body weight of the rats among the groups based on lengths of treatments

Lengths of Measurement	Groups	n	Body Weight (grams)				p
			$\bar{x}$	SD	Min	Max	
Initial weight	Control 0 minute	4	114.75	21.716	97	146	0.815
	PSD 30 minutes	4	104.00	11.518	92	119	
	PSD 60 minutes	4	105.75	7.632	99	116	
	PSD 90 minutes	4	108.75	9.743	101	123	
	Predator(Cat) 30 minutes	4	116.75	16.460	93	130	
	Predator(Cat) 60 minutes	4	99.50	30.447	56	127	
	Cat 90 minutes	4	109.75	12.366	98	127	
Weight after 2 weeks	Control 0 minute	4	144.25	22.882	128	177	0.389
	PSD 30 minutes	4	155.25	22.969	137	188	
	PSD 60 minutes	4	137.50	14.107	123	154	
	PSD 90 minutes	4	139.75	16.820	119	160	
	Predator(Cat) 30 minutes	4	164.00	17.455	141	179	
	Predator(Cat) 60 minutes	4	141.00	19.201	120	163	
	Predator(Cat) 90 minutes	4	149.25	9.811	135	157	
Weight gain	Control 0 minute	4	29.50	12.610	16	46	0.510
	PSD 30 minutes	4	51.25	26.247	18	82	
	PSD 60 minutes	4	31.75	14.385	22	53	
	PSD 90 minutes	4	31.00	18.166	15	54	
	Predator(Cat) 30 minutes	4	47.25	18.661	22	67	
	Predator (Cat) 60 minutes	4	41.50	20.632	23	64	
	Predator (Cat) 90 minutes	4	39.50	8.021	30	49	

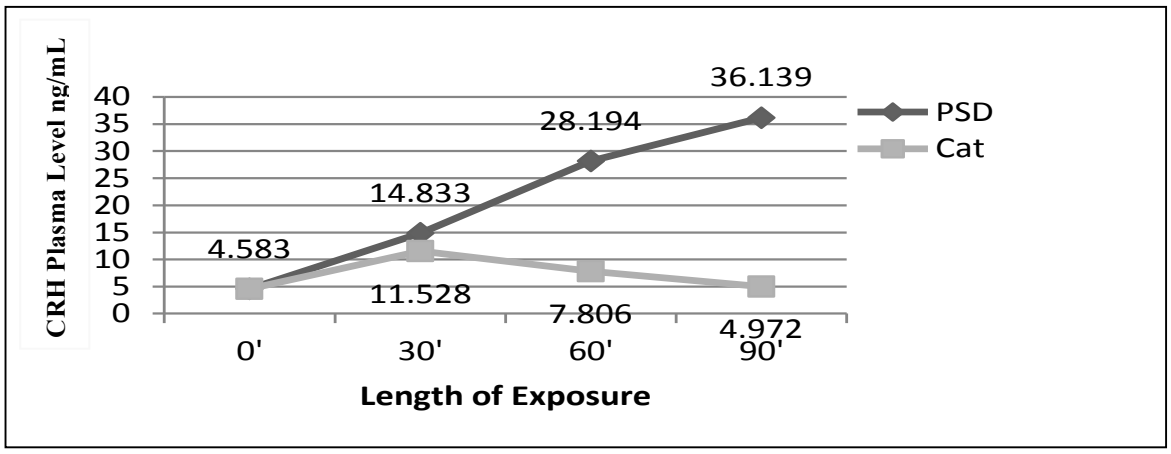
PlasmaCRH Level

The plasma CRH level of the 4 rats in the control group, the group which was not exposed to stressor, ranged from 0.67 to 11.11 ng/mL. There were three groups, which were exposed to the PSD, and each consisted of 4 rats. The plasma CRH level of the first group which was exposed to stressor for 30 minutes varied, with the lowest 0.22 ng/mL and the highest 12.22 ng/mL while the plasma level of the second group which was exposed to stressor for 60 minutes varied, with the lowest 22.56 ng/mL and the highest 34.56 ng/mL. Finally, the plasma CRH level of the last group which was exposed to stressor for 90 minutes also varied, with the lowest 0.35 ng/mL and the highest 50.22 ng/mL. From the

results, it was clear that the longer the treatment was given, the higher the plasma level became ($r=0.895$, $p<0.05$). Furthermore, the last three groups which were exposed to a cat as their predator also consisted of 4 rats each. The plasma level of the first group which was exposed to stressor for 30 minutes varied, with the lowest 0.11 ng/mL and the highest 23.44 ng/mL while the plasma level of the second group which was exposed to stressor for 60 minutes varied, with the lowest 0.56 ng/mL and the highest 20.33 ng/mL. Finally, the plasma level of the last group which was exposed to a stressor for 90 minutes also varied, with the lowest 0.22 ng/mL and the highest 9.44 ng/mL. It can be perceived that the highest plasma CRH level, 23.44 ng/mL, was obtained from the group which was exposed to a cat for 30 minutes, and the lowest plasma CRH level, 0.11 ng/mL, for 90 minutes. It can be summarized that the increase of plasma level is not lineary correlated with the length of treatment ($r=-0.043$, $p>0.05$).

The data of plasma CRH level had normal distribution in all groups ($p>0.05$) and had homogenous variations among all groups ($p>0.05$). The comparison with the CRH level between groups explained that there were significant differences at least between 2 groups ($p<0.05$).

Briefly, the result of the analysis is depicted in the following diagram.



Length of Exposure

Figure 5. The fluctuation of the plasma CRH level stimulated by PSD (dark grey) and a cat as predator (light grey)

In figure 5, clearly the increase with the plasma level stimulated by PSD was lineary correlated to the length of the exposure, while the increase of plasma CRH level in the groups exposed to a cat as their predator only took place until the 30th minute, making the changes much less significant. The CRH level of the groups exposed to the PSD differed significantly along with the durations of the treatment ($p<0.05$). Table 3 reveals that the longer exposure to the PSD, the higher the CRH level.

The plasma CRH level of the groups exposed to a cat as their predator did not increase significantly over length of treatments ($p>0.05$). Table 4 exhibits the comparison of the plasmaCRH levelof the rats stimulated by a cat as their predator among the observation periods. The plasmaCRH level was only increasing during the first 30 minutes, and it was gradually decreasing afterwards. The gradual decrease over the periods of the treatments was suspected due to the rats' being able to adapt themselves with the existence of their predator over the time.

Table 5 reveals the comparison with the plasma level of the rats stimulated by the PSD and a cat as their predator within the

observation period of 30 minutes. The result from the analysis revealed that there were no significant differences in the plasma level ($p>0.05$) both in the groups that were exposed to the PSD and to the ones that were exposed to a cat as their predator.

Table 6 reveals that the comparison with the plasma CRH level of the rats stimulated by and a cat as their predator within the observation period of 60 minutes was significantly different ($p<0.05$). Further; the plasma level was higher in the groups that were exposed to the PSD compared with the ones that were exposed to a cat as their predator. Table 7 reveals the comparison with the plasma level of the rats stimulated by PSD and a cat as their predator within the observation period of 90 minutes. The result from the analysis revealed that the plasma level was higher for the groups that were exposed to the PSD compared with the ones that were exposed to a cat as their predator.

Tabel 2. The Comparison of plasma CRH Level among Treatments

Groups	n	CRH level (ng/mL)				p
		$\bar{x}$	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	
Predator(cat) 30 minutes	4	11.528	8.730	2.5556	23.4444	
Predator(cat) 60 minutes	4	7.806	9.091	0.5556	20.3333	
Predator(cat) 90 minutes	4	4.972	3.807	0.2222	9.4444	

Table 3. The comparison of the plasma CRH level of the rats stimulated by psychologicalstress device among groups and observation periods

Groups	n	CRH Plasma Level (ng/mL)				P
Groups	n	Plasma CRH Level (ng/mL)				p
		x	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	

Table 4. The comparison of the plasma CRH level of the rats stimulated by a cat as their predator among groups and observation periods

Groups	n	Plasma CRH Level (ng/mL)				p
		x	SD	Min	Max	
Control 0 minute	4	4.583	4.604	0.6667	11.1111	0.494
Predator(cat) 30 minutes	4	11.528	8.730	2.5556	23.4444	
Predator(cat) 60 minutes	4	7.806	9.091	0.5556	20.3333	
Predator(cat) 90 minutes	4	4.972	3.807	0.2222	9.4444	

Table 5. The comparison of the plasma CRH level of the rats stimulated by PSD and a cat as their predator within the observation period of 30 minutes

Groups	n	Plasma CRH Level (ng/mL)				P
		x	SD	Min	Max	
PSD 30 minutes	4	14.833	5.253	9.8889	22.0000	0.540
Predator(cat) 30 minutes	4	11.528	8.730	2.5556	23.4444	

Table 6. The comparison of the plasma CRH level of the ratsstimulated by PSD and a cat as their predatorwithin the observation period of 60 minutes

Groups	n	Plasma CRH Level (ng/mL)				p
		x	SD	Min	Max	
PSD 60 minutes	4	28.194	5.733	22.5556	34.5556	0.009
Predator(cat) 60 minutes	4	7.806	9.091	0.5556	20.3333	

Table 7. The comparison of the plasma CRH level of the rats stimulated by PSD and a cat as their predator within the observation period of 90 minutes

Groups	n	Plasma CRH Level (ng/mL)				p
		x	SD	Min	Max	
PSD 90 minutes	4	36.139	10.283	25.5556	50.2222	0.001
Predator(Cat) 90 minutes	4	4.972	3.807	0.2222	9.4444	

Discussion

This experiment aims at comparing the changing of the level of CRH in the plasma of the rats exposed by the psychological stress device (hence: PSD) and the ones by predator, in this study a cat. Cat is a natural predator of rats. Therefore, the behavior of a cat as a living being, especially its aggressiveness is tremendously influenced by the surrounding as well as by the inner emotion of the cat itself. As a result, its behavior is unsteady. On the other hand, the stressor produced by the PSD is steady, measurable, adjustable, and standardized.

Hans Selye in his concept General Adaptation Syndrome defines stress as a condition where there is a homeostatic disruption [6]. Furthermore, stress is defined as a chain of occurrences, including stimulus (stressor) which precipitates the reaction from the brain (the perception and processing of stress). This sequence activates the physical system of

"fight-or-flight" in the body, which is a normal response to stress [1]. Basically, both definitions refer to the struggle of living beings to adapt themselves to their environment in order to survive. Previous researches that induced stress to tested animals generally make use of the term of stress, but they do not further differentiate whether it is psychological, physical, or social stress [7, 8]. In reality, the first onset, relapse and recurrent of psychiatric disorder are likely to be preceded by a psychological stressor [2] although physical stress can also trigger psychiatric disorder.

The stress which was induced by non-traumatic physical restrain on the rats being tested increased the CRH level (8). However, it is quite far from being a real psychological stressor in the real sense, although there was a factor of hopelessness. The result from the analysis reveals that the rats in all groups had a normal distribution of body weight both in the beginning and within two weeks (Shapiro-Wilk $p>0.05$). The gaining of the body weight was homogenous among the groups (Levene's test $p>0.05$). The one-way Anova analysis also confirmed the result that there was no significant change in the body weight among the groups both in the beginning and within two weeks. A drop in body weight and appetite was identified in the tested animals which had been suffering from depression [7].

The rats, having been acclimatized in the laboratory for two weeks, gained more weight, signifying that none of the rats used for this experiment experienced stress prior to the treatment. The weight increase between the two groups, however, was not significantly different, and it. Consequently, did not influence the rise of the CRH level in the plasma. Previous researchers have presented the evidence that a stressful condition increases the CRH level [9], and the one by Eposito et al (2001) confirmed a similar result. On the other words, stress is closely related with the increase of CRH level [8].

All the rats exposed to PSD visually showed more severe signs of stress; being anxious, freezing, defecating, or urinating [5, 4] (Figure 3) compared to the other groups treated using a cat as their predator. Those signs of stress were in accordance with the significant progressive rise to the CRH level in the plasma of the groups exposed to a stressor generated by the PSD compared with the one exposed to a stressor by introducing a cat as their predator.

The CRH level in the plasma on the groups which were induced to stress by a cat as their natural predator only kept rising within 30 minutes, and a continuous decline was found afterwards. There were two reasons underlying this phenomenon. First, the rats must have adapted themselves with the presence of the cat as their predator, and second the aggressiveness of the cat as a creature tended to be unstable.
Meanwhile, the CRH level in the plasma on the groups which were induced to stress by PSD kept rising starting from 30, 60, to 90 minutes in a consistent and linear manner since PSD as a mechanic device was stable, adjustable, and measurable (Figure 1).

The condition of acute stress activates the HPA axis which then releases CRH [8]. In this research, it can be concluded the level of CRH rises significantly in a linear manner with the treatment duration of 30, 60, and 90 minutes. Nakamura in his international workshop about International Animal Research Regulation: Impacton Neuroscience Research: NCBI Bookshelf proposes about animal welfare, and minimizing pain and distress [10].

The clinical implication from this study is to know the relationship of psychological stress and pathopsychobiology of psychiatric disorders. With the understanding of this relationship, prevention and treatment would be better developed. The study limitation is we cannot use various species of experimental animals (Rats and Cats), because it will be more complicated and require lots of studies, although there is a possibility that the result will be different if using various species. For the PSD, rats placed upon the PSD platform in this study sometimes fall from the platform because it cannot avoid the rats from jumping.

The researcher recommends the discrimination of physical, psychological, and social stressors as well as fair animal treatment. All researchers, especially those involving animals

in a direct manner, should pay a close attention to their welfare as well as minimizing their pain and distress. In conclusion, conclusion, the body weight of the rats under study was not significant to the rising level of CRH in the plasma of the rats. The rise of CRH plasma in the groups induced to stress by PSD turned out to be not only higher but also more stable compared to both the control groups, and the ones induced to stress by a cat as their predator. The rise of CRH plasma in the groups induced to stress by PSD was linear along with the length of the exposure.

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