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ORIGINAL ARTICLE

Glucocorticoid levels after exposure to predator odor and chronic psychosocial stress with dexamethasone application in rats

Ana Starcevic [a,*], Sasa Petricevic [b], Zoran Radojicic [c], Vuk Djulejic [a], Andrej Ilankovic [d], Branislav Starcevic [e], Branislav Filipovic [a]

[a] *Institute of Anatomy, Medical Faculty, University of Belgrade, Belgrade, Serbia*
[b] *Institute for Biochemistry, Medical Faculty, University of Belgrade, Belgrade, Serbia*
[c] *Faculty for Organisational Sciences, University of Belgrade, Belgrade, Serbia*
[d] *Psychiatry Clinic, Clinical Center of Serbia, University of Belgrade, Belgrade, Serbia*
[e] *Orthopaedic Clinic, Clinical Center of Serbia, University of Belgrade, Belgrade, Serbia*

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Abstract This study was conducted to explore the effects of specific psychosocial paradigm on predator animal posttraumatic stress model and to test the hypothesis that psychosocially stressed rats would exibit abnormal levels of cortisol and a larger suppression of cortisol levels after the application of dexamethasone. Animals were divided in two groups: experimental and control groups. The experimental group was exposed to two types of stressors: acute immobilization stress, and combined predator stress and daily social stress with application of dexamethasone. Blood sampling was performed at three different times. We found statistically significant results after analyzing the differences between cortisol levels in different times of blood sampling in the group of animals exposed to stress with dexamethasone application. Statistical significance was found when we compared the experimental group with the control group in terms of elevated cortisol levels during blood sampling after stress paradigm exposition. Many significant disruptions in the functioning of the hypothalamic–pituitary–adrenal axis were observed, such as decrease in basal cortisol levels and enhanced dexamethasone-induced inhibition of cortisol levels. These findings are important because their impact can translate to human individuals with posttraumatic stress disorder, which is the most important role of every animal model in research.

Conflicts of interest: All authors declare no conflicts of interest.

* Corresponding author. Bulevar kralja Aleksandra 179, Belgrade, Serbia.
E-mail address: ana.starcevic22@gmail.com (A. Starcevic).

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Introduction

Posttraumatic stress disorder (PTSD) develops in individuals during traumatic periods with prolonged specific chronic stress exposure that follows. PTSD is characterized by an abnormal biological functioning in the endocrine system [1,2]. The endocrine system is one of the most investigated areas in the field of PTSD research, and one of the explanations for this is the low baseline cortisol levels in individuals with PTSD. Decreased levels had been induced by an enhancement of the negative feedback inhibition of the hypothalamic—pituitary—adrenal (HPA) axis. Studies have also used the dexamethasone corticotropin releasing hormone paradigm to investigate abnormal HPA axis functioning in people with PTSD [3,4]. An advantage of this paradigm is that the patients are treated with dexamethasone prior to corticotropin releasing hormone administration, activating negative feedback mechanisms prior to acute HPA axis stimulation. Studies have generally reported reduced adrenocorticotropic hormone (ACTH) levels in dexamethasone-treated PTSD patients who were subsequently treated with corticotropin releasing hormone, which supported the fact that PTSD patients exhibited reduced sensitivity to corticotropin-releasing hormone stimulation. Many studies noted an elevated number of glucocorticoid receptors in PTSD individuals and ACTH following the administration of dexamethasone [5,6]. The literature in this area is not entirely consistent, which likely reflects the heterogeneity in the manifestation of trauma and the measurement of such disorder in different groups of individuals. Most research studies have shown reduced basal cortisol levels and enhanced glucocorticoid negative feedback at the level of the pituitary gland [7]. The development of an appropriate PTSD animal model is crucial for the study of therapeutic and preventive treatments and is more important in defining the neuroanatomical substrates of the disorder [8,9]. The current scientific explanation of the stress impact on the HPA axis in individuals might be enhanced by an animal model that can provide PTSD-like behavioral and physiological abnormalities, and this should be the crucial aim of every PTSD animal model. Some of them have advantages and some of them have disadvantages. Beside the Pavlovian fear conditioning model of PTSD, the second commonly used animal model is the predator exposure PTSD model, which includes exposure of a rodent to a predator, a cat, with various degrees of contact, including direct exposure to a cat, or exposure to a cat odor [10]. The occurrence of PTSD symptoms is often induced by the use of behavioral tests that correspond to a specific symptom in PTSD. We can induce the symptoms of behavioral anxiety and avoidance seen in individuals with PTSD by using elevated plus maze. Hyperarousal observed in PTSD individuals can be modeled by comparing the amplitude and habituation of startle produced in response to a loud noise in rodents. One validated variation of the predator exposure model, which was developed by Zoladz and associates [11], included a combination of predator exposure and social instability, which is possibly the most important item for rodent stability and organization. During a cat exposure, rodents are immobilized in order to maximize the expression of an intense fear response and helplessness, which are the main characteristics of stress events that trigger PTSD in humans. The addition of social instability provides the possibility that the stress event will indeed produce PTSD symptoms in rodents. These characteristics make predator animal models much more effective in investigating neurobiological cues in trauma [11]. Another variation of the predator exposure model of PTSD was developed by Cohen and Zohar [12]. It is based on the fact that the vast individual differences in susceptibility to PTSD in response to stress event are the main reason for triggering the disorder. The core of the investigation refers to the cutoff criteria based on behavior on startle response [13].

The aim of our investigation was to explore if rats exposed to a psychosocial stress paradigm would display abnormalities in glucocorticoid levels under baseline conditions, and abnormalities in cortisol levels and in response to trauma, stress, and dexamethasone administration.

Methods

We used adult male Wistar albino rats weighing about 225 g for the experiment. The animals were raised in the vivarium of Galenika a.d., Belgrade, under the conditions of alternating 12-hour light and dark intervals, in macrolon cages with steel wire covers that were properly marked. Temperature and relative humidity in the animal room were 18°C and 22°C and 55—65%, respectively. All animals had free access to food, which contained a full feed mixture for rats with 20% raw protein content, and water. The experimental group consisted of eight animals that were exposed to a psychosocial stress paradigm. The control group consisted of four animals that were kept away from stress. The stress experimental group was exposed to two types of stressors comprising the experimental paradigm: double exposure to acute immobilization stress, and combined predator-threat stress and daily social stress. Our experimental paradigm is based on the procedures described by Zoladz and Zohar and associates [13,14]. The time prior to stress exposure is considered the acclimatization time, which was 7 days prior to the experiment. The 1st day of our experimental psychosocial paradigm procedure included immobilization of the experimental animal group in plastic rat-immobilization tubes for 20 minutes, after which the animals were released and taken back to their cages. The same treatment was repeated 10 days later. Starting from the 2nd day of the experiment, the experimental group was exposed to social stress on a daily basis. After the second acute stress exposure, we divided the experimental stress group into two subgroups and applied dexamethasone (50 μg/kg, b.m.) via a subcutaneous injection. Blood samples from all animals (experimental and control groups) were taken after the animals had been exposed to the second acute stress according to the following procedure: blood samples were first taken from the animals (baseline cortisol, prior to stress exposition, cortisol 0) followed by immediate exposure to stress by placing the animals into plastic immobilization tubes for 20 minutes. The second blood sampling was performed after they had been released from the immobilization tubes and placed in cages to rest (stress cortisol, in the time of stress

exposition, cortisol 1). Blood was sampled for the third time 2 hours after animal release from the immobilization tubes (return to baseline cortisol, after stress exposition, cortisol 2). The blood sampling method we used is a modified version of the procedure described by Zeller et al. [15]. In this procedure, blood samples were taken from the sublingual vein, punctured by a 0.6-mm gauge needle in a sampled amount of 0.5 mL of blood. The sample was allowed to stay at room temperature to clot for about 20 minutes and centrifuged at 5000 rev/min for 5 minutes to separate the serum. All serum samples for cortisol levels were tested by a contractual laboratory. The animals were sacrificed after the third blood sampling by decapitation. The experiment was conducted in compliance with the current national (Animal Welfare Law) and European (Directive 2010/63/EU; European convention for the protection of vertebrate animals used for experimental and other scientific purposes) regulations.

Statistical analysis

When we tested for the normality of the data, the statistical analysis showed that there was no normal distribution ($p < 0.05$). We used exploratory analysis for describing the group. The nonparametric Mann–Whitney *U* test was used for comparing the results between the groups. Nonparametric Friedman test was used for analysis within the groups. The statistical package SPSS version 23 (SPSS Inc., Chicago, IL, USA) was used for data analysis and testing.

Results

As for group description, we used exploratory analysis and we did not find statistically significant results after analyzing the differences in body mass between the experimental animals and controls. We used the value of 20 as the referent value for determining other cortisol level values, which was also the time in the acclimatization period before the psychosocial paradigm started or the values prior to stress exposition and cortisol levels in different times of blood sampling when we compared all groups of animals. Oscillations are noticeable between female and male rats, and we took the upper limit of the normal value and the maximum concentration that occurs during the day, and that value is 20 nmol/L; we also obtained this value from our laboratory as the upper limit (Table 1) [16,17].

The nonparametric Mann–Whitney *U* test showed statistical significance when we compared the stress experimental group and the control group. When we compared the stress experimental group (exposed to psychosocial stress paradigm, with dexamethasone treatment) with the control group (animals not exposed to any kind of stress or dexamethasone), we found statistical significance in increased cortisol levels of the experimental group at the time of stress paradigm exposition ($p = 0.021$; Table 2).

We found statistical significance at different times of cortisol measurement or blood sampling. When we used the nonparametric Friedman test to compare cortisol values at

Table 1 Descriptive analysis of the groups.

Statistics						
Group			Body mass	Cortisol 0—prior to exposition	Cortisol 1—in time of exposition	Cortisol 2—after exposition
Group 1—Stress	*N*	Valid	4	4	4	4
		Missing	0	0	0	0
	Mean		348.5000	20.00	42.975	20.000
	Median		347.0000	20.00	42.500	20.000
	SD		22.76694	0.000	2.1884	0.0000
	Percentiles	25	328.5000	20.00	41.175	20.000
		75	370.0000	20.00	45.250	20.000
Group 2—Stress and dexamethasone	*N*	Valid	4	4	4	4
		Missing	0	0	0	0
	Mean		340.7500	20.00	34.425	25.900
	Median		332.5000	20.00	33.200	20.000
	SD		18.31893	0.000	16.7762	11.8000
	Percentiles	25	330.0000	20.00	20.000	20.000
		75	359.7500	20.00	50.075	37.700
Group 3—Control	*N*	Valid	4	4	4	4
		Missing	0	0	0	0
	Mean		357.0000	20.00	32.300	26.150
	Median		362.5000	20.00	31.050	23.800
	SD		19.64688	0.000	4.0025	8.0720
	Percentiles	25	336.2500	20.00	29.400	20.000
		75	372.2500	20.00	36.450	34.650

SD = standard deviation.

Table 2 Mann–Whitney *U* test results between the groups.

	Body mass	Cortisol 0—prior to exposition	Cortisol 1—in time of exposition	Cortisol 2—after exposition
Group 1 (Stress) vs. Group 2 (Stress & dexamethasone)	1.000	1.000	1.000	0.317
Group 1 (Stress) vs. Group 3 (Control)	0.468	1.000	0.021	0.131
Group 2 (Stress & dexamethasone) vs. Group 3 (Control)	0.237	1.000	1.000	0.741

different times of measurement (within the stress experimental group), the results indicated a statistical significance between the group of experimental rats that were given dexamethasone and those that were not. A greater suppression of cortisol levels was shown in the group of stress experimental rats that were administered dexamethasone ($p = 0.018$; Table 3).

Discussion

Chronic stress generally induces major changes in the body and greatly affects the basic endocrine axis. As the primary stress hormone, cortisol modifies other control mechanisms in the body, disrupting homeostasis and thereby induces specific changes that are reflected in changes in behavior and in the manifestation of various psychiatric disorders, primarily PTSD. All this leads to fatigue and pathological modifications in neuroanatomical substrate that, in synergy with other modified structures, manifest in aberrant behavior and manifestation of the disorder [18–21]. Many researchers used several types of stressors to provide different aspects of PTSD in rodents such as electric shock, underwater trauma, stress–restress and single prolonged paradigms, and predator exposure model. All of them used different types of stressors as a main cue to provide a common manifestation of PTSD in humans, which is defined as anxiety, enhanced fear conditioning, and reduced social interaction. We considered exposure to a predator as the most suitable model for our investigation [11]. We also found cat odor to be more efficient, as it provides the maximization of intense fear response and helplessness as the main characteristic of the stress event that triggers PTSD in humans. The addition of social instability increases the likelihood that the stress event will indeed produce PTSD symptoms in rodents. Based on this premise, in our investigation we found increased cortisol levels in the group of experimental rats compared with the control group at the time of the second blood sampling. This was after the exposure to the psychosocial stress paradigm, before the rats were taken back to their cages to rest. The findings are consistent with data in the current literature [14]. In our investigation, we found that cortisol levels in experimental animals that were treated with dexamethasone prior to exposure to stress was much lower than those found in animals that were not treated with dexamethasone, which means that cortisol levels were higher in the experimental group of animals that were given dexamethasone treatment and were exposed to the psychosocial stressors paradigm experiment. Previous studies have shown significantly larger test result values for dexamethasone suppression, as well as greater values for cortisol level decrease [9]. The investigations of PTSD concerning the animal model showed significantly reduced baseline glucocorticoid levels resulting from the use of either a single prolonged stress paradigm or a stress–restress paradigm consisting of situational reminders of the original stress experience [21–23]. Experimental animals that were psychosocially stressed did not exhibit significant cortisol level increase compared with the control animals, probably because blood sampling was conducted during daytime hours [24–27]. It was also shown by some researchers that there might be significant differences in cortisol levels between blood sampling during day- and nighttime, which is consistent with the circadian rhythm [11,28].

The importance of all these findings has translational aspects on humans with PTSD, particularly cortisol level variations as a function of time when blood sampling is performed. The results of some recent animal studies of

Table 3 Nonparametric Friedman test shows differences within the experimental group.

Group		Mean rank	*N*	Chi-square	*df*	*p*
Group 1—Stress	Cortisol 0—prior to exposition	1.50	4	8.000	2	0.018
	Cortisol 1—at time of exposition	3.00				
	Cortisol 2—after exposition	1.50				
Group 2—Stress and dexamethasone	Cortisol 0—prior to exposition	1.63	4	2.000	2	0.368
	Cortisol 1—at time of exposition	2.38				
	Cortisol 2—after exposition	2.00				
Group 3—Control	Cortisol 0—prior to exposition	1.25	4	5.143	2	0.076
	Cortisol 1—at time of exposition	2.75				
	Cortisol 2—after exposition	2.00				

PTSD showed a blunted glucocorticoid response to acute stress in animals that have developed PTSD-like behaviors [14].

Our results showed enhanced negative feedback sensitivity to synthetic glucocorticoids such as dexamethasone as one of the most important findings of the present experiments, which occurs as a result of exposure to chronic psychosocial stress, involving two acute stress exposures and daily social instability. Based on the results of the studies that tested the presence of enhanced negative feedback inhibition of the HPA axis, rats exposed to a single prolonged stress paradigm subsequently exhibited a blunted restraint stress-induced increase in ACTH levels followed by the administration of cortisol [29]. Kohda et al. [30] reported that rats exposed to a single prolonged stress paradigm subsequently exhibited a blunted restraint cortisol stress level increase following the administration of dexamethasone. This suggests that stress paradigm produces changes in major endocrine axis function that resemble enhanced negative feedback inhibition and are comparable to the present set of data.

In conclusion, we can say that trauma induces an enhancement of the negative feedback inhibition of the HPA axis in predator exposure models of PTSD. Cortisol levels are increased after exposure to psychosocial paradigm and can be suppressed after dexamethasone application prior to stress exposure. The development of appropriate animal models is crucial for studying the specific and different mechanisms that underlie the development and persistence of PTSD psychopathology; it also needs to relate to its validity for studying the human disorder.

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