Importance of the control of catecholamines levels in the scientific experiment

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ABSTRACT

The stress syndrome includes ample events characterized by complex neuroendocrinological alterations. These alterations, at least partially, are part of an "adjustment mechanism" of the tissues. The effects of the manipulation of rats on plasma catecholamines (norepinephrine, epinephrine, dopamine and L-dopa) levels are investigated. The results show that increase on the epinephrine and norepinephrine levels are resulting of alert reaction caused by animal manipulation. Thus this possibility of obtaining altered response in function of stress leads to the need of special care in the prevention and control of stressogenic stimuli in the process of research. Taking care of preventing stressogenic stimuli, it becomes possible to determine a profile of trustworthy replies, considered as standard.

KEY WORDS: Catecholamines, Plasma, CLAE**.**

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INTRODUCTION

The nervous system, as well the endocrine system, is responsible for the majority of control functions in the body. The transmission between neurons is made by neurotransmitters, neuroactive chemical substances released in the pre-synaptic junction between two neurons.

Catecholamines are important neurotransmitters and circulating hormones (BENEDICT, 1987, PEREIRA, 1998).

Epinephrine, norepinephrine, dopa and dopamine are hormones that have the properties of active catecholamines presenting the compound catechol and are synthesized from tyrosine.

The complex interaction of the hipotalamus-hipophysis-adrenal axis and the sympathetic-adrenal used by the organism as a modulator of homeostasis, results mainly in the release of the adrenocorticotrophic hormone from the anterior hipophysary glandule, glucocorticoid from the adrenal cortex, epinephrine and nor-epinephrine in the sympathetic nerve endings (FIGURE 1).

The evaluation of the physiological modifications that follow some pathologies may be done by quantification (dosage) of catecholamines, glucocorticoids and gonadothrophines, among others (MILLS, 1985).

These physiological modifications are known as stress or general adaptation syndrome (GAS), a name proposed by Hans Selye, a researcher that first studied this issue.

Complex interactions are involved in the regulation of such hormones. Glucoroticoids regulate the biosynthesis of catecholamines in the adrenal cortex and stimulate the release of adrenocorticotrophine from adrenohipophysis (AXEROLD, 1984).

The levels of catecholamines and steroids may vary according to the stressogenic stimulus such as in animals for scientific experiments. In this way, the stress may be induced by the researcher aiming to evaluate the influence of such stressogenic agents. However, these stimuli may be at random when the conditions of the experiment are not properly controlled such as conditions of the animal farm and handling of animals.

Taking into consideration that the alterations of serum levels of catecholamines include a phenomenon of wide physiopathological repercussion and that such hormonal alterations, which characterize the "Stress Syndrome" may modify the activity of many physiological systems leading to pathological situations, it is important to evaluate the serum levels of catecholamines in animal preserved from a pre-established model of stress in which the experimental conditions varies according to the manipulation of animals.

MATERIAL AND METHOD

Animals

In this study were used adult, male, albinos Wistar rats (UNESP strain) with average 90 days, weighing circa 300g, provided by the animal farm of UNESP. Animals were kept at the animal farm of the Department of Pharmacology of the Institute of Biosciences of the Universidade Estadual Paulista (UNESP) for a minimum period of 10 days under controlled conditions of feeding, temperature, noise and light-dark cycle for adaptation. Animals were maintained in collective cages for 5 animals with free access to water and food.

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Experimental groups

Groups I and II included rats preserved from stress, anesthetized with sodium pentobarbital (50mg. Kg^{-1}). A blood sample was collected 50 minutes after anesthesia in group I and 5 minutes in group II. It is important to note that catecholamines released during stress are metabolized within 50 minutes.

Sampling, storage and transport of blood serum

Blood samples of 5 and 6mL were collected in flasks with heparin through insertion of a cannula in the abdominal aorta. Immediately after collection blood was centrifuged at 2000 rpm for 20 minutes at 2˚C and the obtained serum was stored in dry ice at –70˚C. Flasks with serum were stored in an isothermic container in order to keep a stable temperature during transportation from the Department of Pharmacology of the Institute of Biosciences (UNESP-Botucatu) to the Department of Chemistry of the School of Sciences (UNESP-Bauru).

Aiming to keep the chemical stability of catecholamines, the serum samples were stored in liquid nitrogen in the Department of Chemistry of the School of Sciences (UNESP-Bauru)

Afterwards, the samples were stored in isothermic containers with dry ice at –70˚C to transport samples from the Department of Chemistry to the Department of Nephrology of the Escola Paulista de Medicina (São Paulo).

Dosage of serum catecholamines

The determination of the serum concentration of catecholamines was made by liquid chromatography of high efficiency (NAFFAH-MAZZACORATTI, 1992).

Separation of catecholamines was done at 22ºC with mobile phase flow adjusted to 0.80mL.mim^{-1}. The mobile phase was composed by 0.02M of dibasic sodium phosphate, 0.02M of citric acid, pH 2.64, methanol 10%, 0.12mM of sodium EDTA and 556mg.L^{-1} of heptanossulphonic acid. The potential of the electrochemical detector utilized was adjusted to +0.5V.

When necessary the samples were measured twice. Results are presented in pg.mL^{-1} of serum.

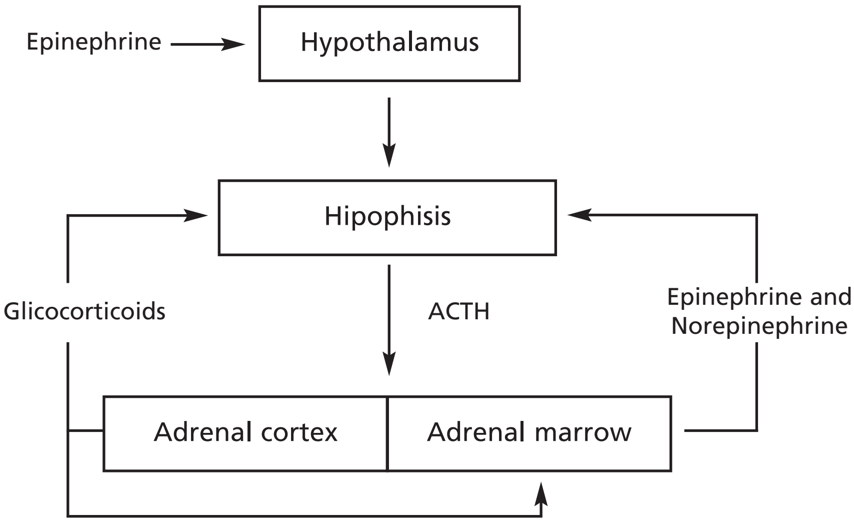

FIGURE 1: The interaction of the hipotalamun-hipophysis- adrenal axe.

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RESULTS

Group I included animals preserved from stress, which blood samples were collected 50 minutes following the anesthesia – period enough to the metabolisation of catecholamines released due to an alert condition. In its turn, group II is similar from control group I except from the fact that blood sampling was done 5 minutes following anesthesia, period not sufficient for metabolization of catecholamines.

Results in TABLE 1 and FIGURE 2 shows the basal level of catecholamines and the difference among groups.

Values related to the serum concentration of catecholamines are shown in pg.mL^{-1}.

TABLE 1: The interaction of the hipotalamun-hipophysis- adrenal axe.

Group	NA	ADR	LD	DA
I	29.05±5.63	116.26±24.87	34.64±6.35	45.54±2.23
II	50.24±8.32*	162.94±18.02*	36.24±3.84	79.77±9.27*

Values with mean ± standard deviation of average 5 experiments by group.
* $p < 0.05$, ANOVA.

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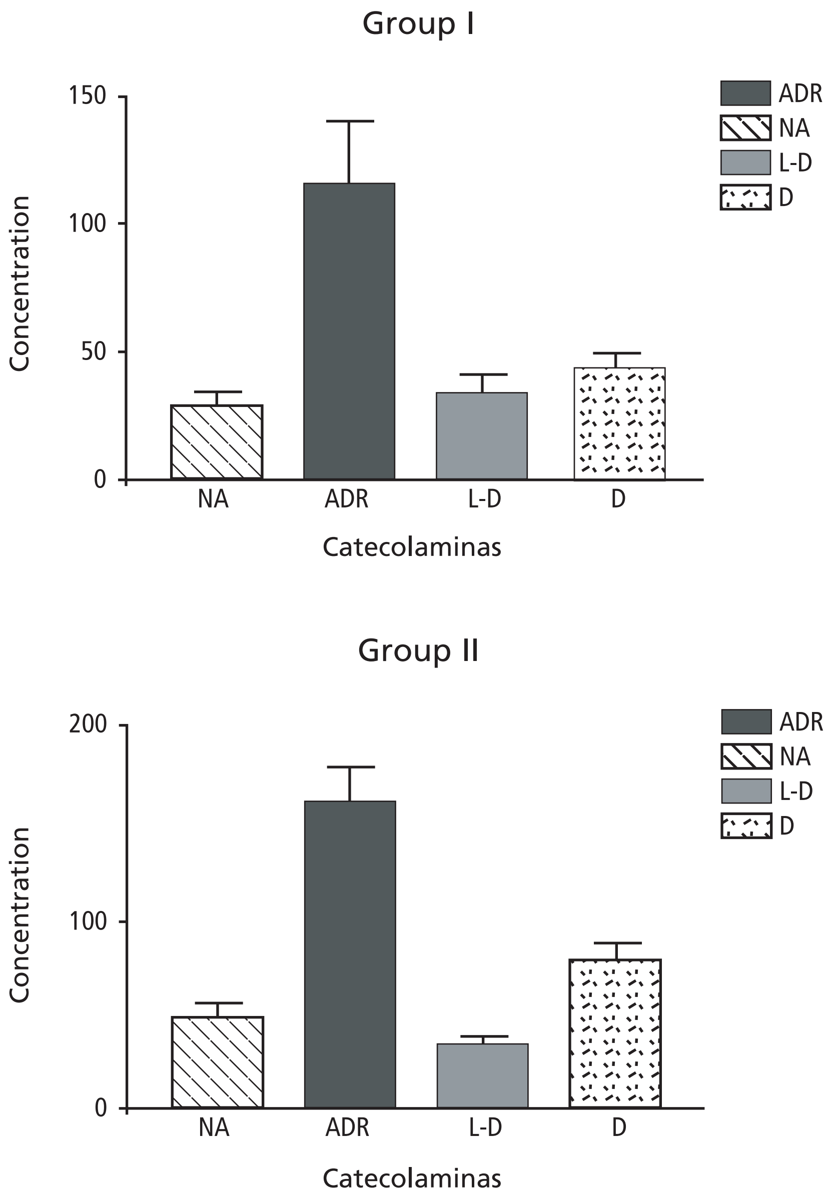

FIGURE 2: Graphic of the serum levels of catecholamines (pg.mL^{-1}), norepinephrine (NE), epinephrine (EPN), L-dopa (L-D) and Dopamine (D) in individuals of groups I and II.

DISCUSSION

Adrenals are the main source of substances lavishly released during stress. These glands are important components of both the hypothalamic-hipophysis-adrenal axe and sympathetic-adrenal axe. The adrenal gland shows a cortical portion that releases glucocorticoid and related substances as a response to the adrenocorticotrophic hormone (ACTH) released during stress. In addition, it has a soft marrow-like center portion that releases catecholamines (basically epinephrine) in response to threatening situations to the body.

Therefore, the adrenals take part in the neuroendocrine mechanism of stress. It is important to stress that the release of catecholamines by adrenals is evoked by nervous impulse through the sympathetic fibers that innervate this gland (SCHEURINK et al., 1989, FERRARESE et al., 1993). Moreover, epinephrine from adrenals modulates the release of catecholamines from the adrenergic endings, through the pre-synaptic adrenoceptors. In these endings the epinephrine may be taken and later on released as co-transmitter (FERRARESE et al., 1993, SCHEURINK et al., 1989, COOPS et al., 1993).

Stressogenic stimulus is any stimulus capable of provoking organic, mental, psychological and/or behavioral responses related to physiological modifications that lead to a hyper-function of the adrenal gland and of the sympathetic autonomous nerve system. In this regard, result for serum levels of catecholamines shows that levels of epinephrine and norepinephrine in group II are higher than in group I, characterizing the presence of a state of alert as a result of the manipulation of animals during anesthesia.

Increased serum levels of catecholamines may be taken as the main neuroendocrine alteration resulting from stress. In this connection, the manipulation and procedures for anesthesia of animals can be considered as a type of stress.

There are many studies being developed searching for an analysis of the physiological alteration due to different models of stress. However, in order to analyze such responses and to consider them as modified, it is necessary to establish a rigid standardization for experiments as well as a reliable “control” response.

One should consider the serum levels of catecholamines of Group I as the ones closer to the basal level. However, the values for Group II can be take as reference for potential comparison among animals submitted to a stress situation.

Emotional stress release epinephrine from the adrenal soft marrow-like center and physical activity releases nor-epinephrine from nerve endings of the sympathetic nervous system (SCHEURINK et al., 1989). In fact it was shown an increase in serum concentration of epinephrine and nor-epinephrine (KVETNANSKY et al., 1993) in stress situations induced by forced immobilization, in which the emotional component is predominant.

Significant alterations among the groups were detected only to serum levels of dopamine, nor-epinephrine and epinephrine showing the presence of physical and emotional components of stress. However, the serum levels of L-dopa were not altered in both groups. Therefore, such neurotransmitter may not participate of the studied alteration during stress.

According to Bispo and Pereira (1994) stress is a phenomenon of wide physiopathological repercussion and one should consider stress as

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an important feature while using animals for scientific experiments. The same authors also stress the importance of controlling the conditions of the experiment as well as the care of such animals in the vivarium.

CONCLUSION

The increased levels of serum catecholamines in animals of group II is due to stress situation of alert while handling animals. Animals of group II can be used as factor of comparison to other experimental groups in which it is aimed to test the influence of stress.

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BIBLIOGRAPHIC REFERENCES

1. AXEROLD, J. RESINE, T.D. Stress hormones: their interaction and regulation. *Science*, v. 224, p. 452-59, 1984.

2. BENEDICT, C. R. simultaneous measurement of urinary and plasma norepinephrine, epinephrine, dopamine, dihydroxyphenylalanine, and dihydroxyphenylacetic acid by coupled-column high-performance liquid chromatography on C_8 and C_{18} stationary phases. *J. Chromatogr*., v. 385, p. 369-375, 1987.

3. BISPO, D. L. N.; PEREIRA, O. C. M. Importância do conhecimento das alterações reduzidas pelo estresse, em animais domésticos. *Interciência*, v, 19, p. 72-74, 1994.

4. COOPES, R. P. et al. Strong activation of vascular prejunctional _$_2$ – adrenoceptors in freely moving rats by adrenaline release as a co-transmitter. *Eur. J. Pharmacol.*, v. 243, p. 273-279, 1993.

5. FERRARESE, C. et al. Benzodiazepine receptors and diazepam binding inhibitor: a possible link between stress, anxiety and the immune system. *Psychoneuroendocrinology*, v. 18, p. 3-22, 1993.

6. KVETNANKY, R. et al. Endogenous glucocorticoids restrain catecholamines synthesis and release at rest and during immobilization stress in rats. *Endocrinol.*, v. 133, n. 3, p. 1411-1419, 1993.

7. MILLS, F. J.; CHIR, M. A. The endocrinology of stress. *Aviat. Space Environ. Med.*, v. 56, p. 642-650, 1985.

8. NAFFAH-MAZZACORATTI, M. G. et al. Serum catecholamines levels determined by high performance liquid chromatography coupled with electrochemical detection. *Arquivos Brasileiros de Endocrinologia e Metabologia*, v. 36, n. 4, p. 119-122, 1922.

9. PEREIRA, O. C. M.; DEGA, M. R.; SOUZA, M. S. Noradrenergic response in vas deferens from rats submitted to acute and repeated stress. *Gen. Pharmacol.*, v. 30, p. 417-421, 1998.

10. SCHEURINK, A. J. W. et al. Adrenal and sympathetic catecholamines in exercising rats. *Am. J. Psysiol.*, v. 256, p. 155-160, 1989.

11. SCHEURINK, A. J. W. et al. Experience affects exercise-induced changes in catecholamines, glucose, and FFA. *Am. J. Physiol.*, v. 256, p. 169-173, 1989.

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