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**INFLUENCE OF CHRONIC STRESS ON HEMODYNAMIC INDICES, DIURNAL FLUCTUATION OF
CATECHOLAMINES PLASMA CONCENTRATIONS AND REMODELLING OF VARIOUS ORGANS IN
RATS**

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**ქრონიკული სტრესის ზეგავლენა ჰემოდინამიკურ მაჩვენებლებზე, კატექოლამინების
პლაზმური კონცენტრაციის დღე-ღამურ ფლუქტუაციაზე და სხვადასხვა ორგანოს
რემოდელირებაზე ვირთაგვებში**

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რეზიუმე

სტრესული მდგომარეობა აერთიანებს სხვადასხვა ტიპის სტრესორის საპასუხოდ განვითარებულ ემოციურ, ფიზიკურ და ფსიქოლოგიურ ცვლილებებს. სტრესის გამოსავალი შეიძლება კეთილსამედო იყოს ახალი ბიოლოგიური ბალანსის ჩამოყალიბებით, ან კიდევ შესაძლებელია გამოავლინოს დამაზიანებელი, სახიფათო ეფექტი კარდიოვასკულური სისტემის ფუნქციის ცვლილების სახით. ამის გამო თანამედროვე კარდიოლოგიაში დიდი მნიშვნელობა ენიჭება სტრესის თანხლები გულ-სისხლძარღვთა და სომატო-ვეგეტატიური კორელატების ფუნქციის ცვლილებისადმი ინდივიდუალურ მიდგომას.

აღნიშნული კვლევის ძირითად მიზანს წარმოადგენდა კარდიოჰემოდინამიკური პარამეტრების, კატექოლამინების პლაზმური დონის ცვლილებისა და სხვადასხვა ორგანოების რემოდელირების შესწავლა ქრონიკული იმობილიზაციური სტრესის და მისი იზოლაციურ სტრესთან კომბინირების დროს. ექსპერიმენტები ჩატარდა ვისტარის ჯიშის 75 ვირთაგვებზე წონით 200-250გ, რომლებიც დაყოფილი იყო 3 ჯგუფად: I - სტრესისგან თავისუფალი ცხოველები - საკონტროლო ჯგუფი (ს); II - ქრონიკულ იმობილიზაციურ სტრესს (ქიმს) დაქვემდებარებული ვირთაგვები; III - ქიმს+იზოლაციური სტრესის (ქიმს) გავლენის ქვეშ მყოფი ცხოველები. ვირთაგვები იმყოფებოდნენ მათი მოვლის ეთიკური სტანდარტების შესაბამისად, რომელიც დამტკიცებული იყო თსსუ-ის მიერ. ქიმს-ის მოდელირებას ვახდენდით ცხოველთა მოთავსებით სპეციალურ გამჭვირვალე პლექსიგლამის კამერაში ყოველდღიურად, 3 კვირის განმავლობაში დილის საათებში (10⁰⁰-12⁰⁰ სთ), სადაც მათ შენარჩუნებული ჰქონდათ თავის თავისუფალი მოძრაობის უნარი, სხეულისა და თათების მოძრაობის შეზღუდვით. III ჯგუფის თითოეული ვირთაგვა სტრესთან დაქვემდებარების შემდეგ II ჯგუფის ცხოველებისგან განსხვავებით თავსდებოდა იზოლირებულად, ცალკე გალიაში. კარდიოვასკულური პარამეტრები მოიცავდა: სისტოლურ (ს), დიასტოლურ (დ) არტერიულ წნევას (ან) მმ.ვწყ.სვ და გულის რითმს (გრ), რომელთაც ვზომავდით არაინვაზიური სფიგმომანომეტრული მეთოდით ვირთაგვას კუდიდან (tail-cuff). კატექოლამინების ნორადრენალინისა (ნ) და ადრენალინის (ა) პლაზმური დონის დღიური ფლუქტუაციის შესასწავლად და ამ პერიოდში მათი აკროფაზების დასადგენად ვიყენებდით დროის 5 წერტილს: 9:00, 13:00, 17:00, 21:00, 01:00 სთ. სხეულის წონაზე და სხვადასხვა ორგანოს რემოდელირებაზე სტრესის ზეგავლენას ვსწავლობდით მათი მასის ცვლილებებით.

გამოვლენილ იქნა ქიმს გამოწვეული მნიშვნელოვანი ცვლილებები ჰემოდინამიკური მაჩვენებლების მხრივ, სან, დან და გრ-ის თანდათანობითი მომატებით, სტრესიდან ექსპოზიციის მე-

14 დღეს, მათი პიკური მნიშვნელობებით სტრესიდან 21-ე დღეს (138 ± 4 მმ ვწყ.სვ ($P < 0,01$), 99 ± 4 მმ ვწყ.სვ ($P < 0,05$) და 472 ± 6 დარტყმა/წთ-ში ($P < 0,01$), შესაბამისად, ს ჯგუფის ცხოველების იგივე მონაცემებთან შედარებით: 106 ± 9 მმ ვწყ.სვ., 72 ± 7 მმ ვწყ.სვ. და 414 ± 6 დარტყმა/წთში, შესაბამისად. ქიმი+ქიმი ექსპოზირებულ ვირთაგვებში ეს ცვლილებები უფრო მკვეთრად იყო გამოხატული ს და ქიმი ჯგუფის ცხოველებთან შედარებით, რაც მიუთითებდა ჰეტეროგენული სტრესის ინტერვენციის მნიშვნელოვან ზეგავლენაზე ჰომოტიპურ სტრესთან მიმართებაში. ქიმი-თან დაკავშირებული ჰემოდინამიკური ცვლილებები ასოცირდებოდა სისხლის პლაზმაში კატექოლამინების კონცენტრაციის მნიშვნელოვან ცვლილებებთან, რომელთა საშუალო დღიური დონე ($6-456,5 \pm 28,6$ პგ/მლ, $5-563 \pm 25,2$ პგ/მლ, შესაბამისად) სარწმუნოდ სჭარბობდა ს ვირთაგვების იგივე მაჩვენებლების დონეს ($6-287,7 \pm 15,6$ პგ/მლ, $p < 0,01$; $5-255 \pm 14,9$ პგ/მლ, $p < 0,01$), კატექოლამინების აკროფაზების ცდომით დღის საათებიდან (9:00-17:00 სთ) საღამოს და გვიანი ღამის საათებისკენ (21:00-01:00). ამავე დროს, კატექოლამინების პლაზმური საშუალო დონე ქიმი მქონე ვირთაგვებში სარწმუნოდ სჭარბობდა ქიმი-ისას დადგენილ პლაზმურ კონცენტრაციებს. ზემოაღწერილთან ერთად, ორივე ტიპის სტრესი ხასიათდებოდა სხეულის წონის, თირკმლისა და ლიმფოიდური ორგანოების (ელენთა, თიმუსი) მასის დაქვეითებით და თირკმელზედა და ფარისებრი ჯირკვლების წონის მომატებით.

პოსტულირებულია, რომ ქრონიკული ჰომოტიპური სტრესი ხასიათდება სხეულის წონის შემცირებით, სხვადასხვა ორგანოების რემოდელირებით და კარდიოვასკულური პარამეტრების ცვლილებით, რაც ვლინდება სისხლის არტერიული წნევისა და გულის რითმის მომატებით, კატექოლამინების პლაზმური დონის გაზრდით და მათი აკროფაზების ცდომით დღის საათებიდან საღამოს და გვიანი ღამის საათებისკენ, რაც უფრო გამოხატულია კომბინირებული სტრესის დროს ამ ცვლილებების მიმართ ჰაბიტუაციის გარეშე.

Introduction: Stress can be considered as body response to retain homeostasis against irritation caused by various stressors, creating hazard to individual physiologic or psychologic condition [1]. Stress involves the different type of alterations resulting in emotional, physical or psychological strain [2]. Strong intensity or prolong stress may lead to favourable outcome by formation of a new biological balance or because of maladaptation can exert damaging effect [3,4].

Chronic stress facilitates to vascular hypertrophy and endothelium dysfunction becoming as a risk factors for the development of cardiovascular diseases: arterial hypertension, myocardial infarction, atherosclerosis and etc., characterized by high mortality [5,6,7,8,9,10,11,34]. For this reason, individual approach to somato-vegetative complications accompanied stress situation represent important direction in modern cardiology. However, despite of experimental and clinical data regarding hemodynamic changes in acute stress, lack of information exists concerning prolong, chronic stress associated cardiovascular changes and about influence of isolation stress on the hemodynamic indices registered in habitual homotypic immobilization stress.

In the organism the most leading circadian biorhythm is associated with sleep – wake cycle, which orchestrates many systems biorhythm. This includes autonomic nervous system function and production of agents participating in the fluctuation of vascular tone and hemodynamic parameters. One of such biomarkers are catecholamines [12,13,14,15,16,17], which diurnal rhythm during stress is not completely elucidated as well as morphometric changes in different organs after their possible remodelling in chronic stress.

The objective of this study was to investigate the modulatory action of chronic immobilization stress and coincident isolation stress on hemodynamic parameters, circadian alterations of catecholamines plasma level and remodelling of various organs.

Materials and Methods: In vivo study. Experiments were carried out on 75 male Wistar rats weighing 200-250 g. The animals were handled in compliance with ethical standard approved by the Animal Care Committee of Tbilisi State Medical University. All rats were divided into 3 groups: I – Stress

– naive animals without to any restraint, constituted control (C) group, n=25; II – rats with chronic immobilization stress (CIZS), n=25 and III – CIZS rats with associated isolation stress (CISS), n=25. CIZS model was created by placing animals in a special Plexiglas transparent camera for 120 minutes every day in the morning hours (10⁰⁰-12⁰⁰ hr) during 21 days [18,19]. Animals retained ability to turn a head with restriction of body and paws movement. Each rat from III group after exposure to CIZS vs. II group of animals was maintained in case separately. Cardiovascular parameters including systolic, diastolic blood pressure (SBP, DBP) – mm Hg and heart rhythm (HR) – beat minute (Bpm) – were obtained by non-invasive “tail-cuff” sphygmomanometric method. Hemodynamic parameters in I group of animals were determined 3 times in 3-5 minutes intervals after 10 minutes of their adaptation to experimental condition, while in rats exposed to CIZS – after 120 minutes.

In vitro experiments sample collection and storage. On the day before blood sampling (after 21 days of CIZS) a cannula was implanted into the tail artery under anesthesia [19] using - ketamine 87 mg/kg +xylazine 13 mg/kg intraperitoneally. Plasma was collected using sterile tubes containing 1% heparin and centrifuged for 15 minutes at 1000 x g, 2-8°C within 30 minutes of blood collection. Samples were stored at 20°C. The next steps were associated with determined plasma catecholamines level (Norepinephrine and Epinephrine) according manufacturer instruction using Elisa Kits (Cusabio, USA). The analysis of catecholamines plasma concentration was performed by using microplate reader, at 450 nm wavelength, investigated 5 time points: 9:00, 13:00, 17:00, 21:00 and 01:00 hours to determine the acrophases in this period (time when catecholamines plasma levels were attained peak values).

Morphometric analysis. Stress influence on various organs remodeling was analyzed by alterations in body and organ masses after 21 days when rats were sacrificed by overdose of anesthesia. Thyroid and adrenal glands, thymus, spleen and kidneys removed, dried on filter paper and weight by using analytical precision balance.

Statistical analysis. The SPSS Software was used for statistical analysis measurement data to mean standard deviation (SD) using t-test and single factor analysis of variance for group comparison. P<0,05 indicates significant differences using Student’s test.

Results. The mean baseline values of cardiovascular parameters (Table 1) in C (I group) rats did not show any marked changes after their measuring in 1, 7, 14 and 21 days intervals, while CIZS subjected animals revealed significant alterations in hemodynamic indices after 14 and 21 days to stress exposure. SBP in this group of rats was gradually increased attaining its peak level (138±4 mmHg) after 21 days subjected to immobilization stress condition markedly exceeded the same value in C group of rats (106±8 mmHg, P<0,01), as well as after 14 days of measurement of SBP (120±5mmHg) vs I group of rats (100±4mmHg, P<0,05), respectively. Along with SBP, DBP also revealed significant alterations achieving maximal values (99±4mmHg) like SBP after 21 days to stress exposure, with marked differences as compared to C rats (72±7 mmHg, P<0,05) in corresponding period and after 14 days subjected to CIZS (87±5 mmHg) vs. significances obtained in this time interval in C rats (76±6 mmHg, P<0,05). Such changes in arterial pressure were associated with peak acceleration in HR (472±6 beat minute after 21 days to stress exposure and with marked differences in comparison to C rats in corresponding period (414±6 beat minute as well as after 14 days of HR measurement, (434±4 beat/minute) vs (422±8 beat/minutes) in C rats.

In CIZS + CISS subjected rats vs CIZS animals cardiovascular parameters were changed earlier (after 7 days) and more expressively in comparison with C animals data. SBP was significantly increased after one week exposure to combine stress. (130±6mmHg, P<0,01) with respect to C rats same values (105,7±6mmHg), reaching its peak level by stepwise fashion (152±4mmHg) after 21 days to stress exposure, pronouncedly exceeded SBP indices in C rats also after 14 days to combine stress subjection (132±5mmHg,

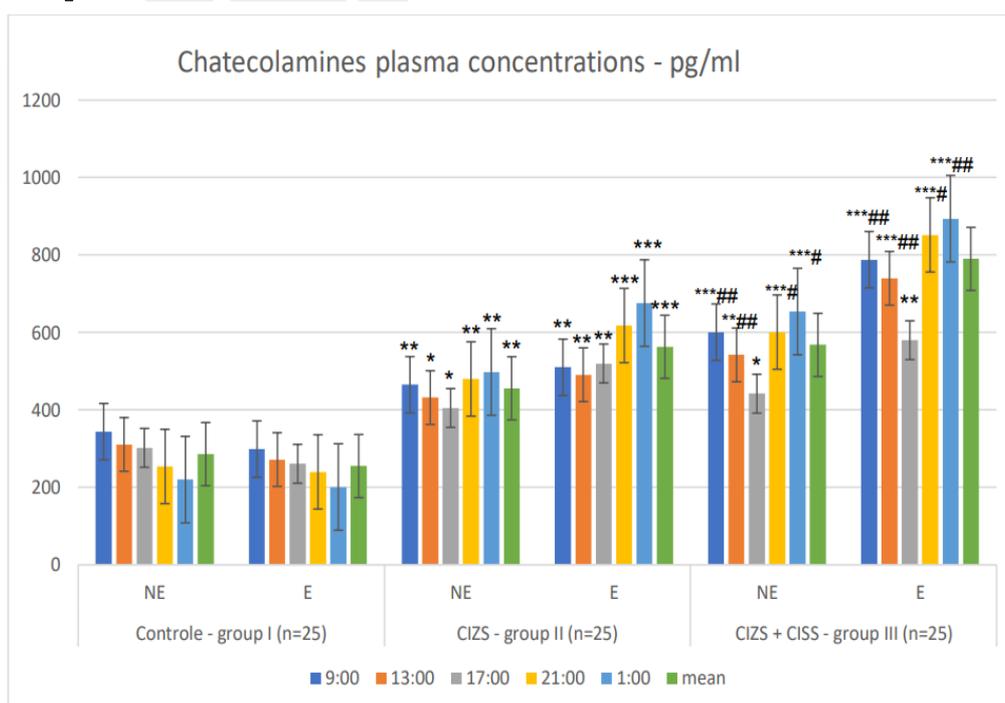
$P < 0,05$) vs C rats (100 ± 4 mmHg). Such changes were correlated with marked alterations in DBP already revealed after 7 days (86 ± 8 mmHg, $P < 0,05$) of stress influence, achieving peak values (100 ± 4 mmHg) that exceeded the same indices in C rats (72 ± 7 mmHg, $P < 0,01$). HR in combine stress also underwent to marked changes vs C animals after elapsing 7 days to stress exposure, reaching its maximal value (like arterial pressure) after 21 days subjected to this type of stress (498 ± 8 beat/minute), markedly outnumbered the identical significances in C rats after the 7 (448 ± 8 beat/minute, $P < 0,05$) and 14 days (455 ± 5 beat/minute, $P < 0,05$) to stress exposure. Such hemodynamic alterations induced by CIZS and CIZS+CISS were accompanied with diurnal changes of catecholamines plasma levels (Fig.1.) in all animals.

Table 1. Influence of chronic immobilization stress (CIZS) and CIZS coincident with chronic isolation stress (CISS) on cardiovascular parameters in rats

Animal group	Hemodynamic indices	Days of measurement			
		1	7	14	21
I – Control (C), n=25	SBP	102,5±5	105±6	100±4	106±8
	DBP	70±4	74±5	76±6	72±7
	HR	415±10	420±8	422±8	414±6
II - Chronic immobilization stress (CIZS), n=25	SBP	108±4	112±3	120±5*	138±4**
	DBP	76±2	81±4	87±5*	99±4*
	HR	424±7	430±6	434±4*	472±6**
III – Chronic isolation stress (CISS) +CIZS, n=25	SBP	106±5	130±6**,#	132±5*,#	152±5**,#
	DBP	80±4	86±8	94±8*	100±4**
	HR	430±10	448±7*	455±5*,#	498±8***,#

Note: SBP, DBP – systolic and diastolic blood pressure – mmHg, HR – Heart rhythm – beat/minute; Significant differences between; *-C and stress groups corresponding values, # - between CIZS and CIZS+CISS groups; n=animal number, one symbol – $P < 0,05$, two – $P < 0,01$, three – $P < 0,001$.

Fig.1. Influence of chronic immobilization stress and its coincident with chronic isolation stress on catecholamines plasma levels in rats



Note: CIZS – Chronic immobilization stress; CISS – chronic isolation stress; N-Norepinephrine, E-Epinephrine, n=animals number in group; Significant differences as compared: *-with corresponding values of control group, #-with CIZS group; one symbol – $P < 0,05$, two – $P < 0,01$, three- $P < 0,001$.

The analysis of norepinephrine (NE) and epinephrine (EPN) plasma content of C rats in studied periods (9:00, 13:00, 17:00, 21:00 and 00:01 hr) showed their peak values at 9:00-17:00 hr as compared to CIZS and CIZS+CISS groups of rats where catecholamines peak significances (especially EPN) shifted at late night time with acrophases registered at 00:01 hr. The mean daily plasma concentrations of catecholamines in CIZS group of animals (NE-456,5±28,6 and EPN – 563,5±25,2 pg/ml) significantly exceeded the same values in C rats (NE-286,7±15,6 and EPN – 255,0±14,9 pg/ml, $P<0,001$), respectively, with 1,5 and 2,2 times higher plasma content of NE and EPN, respectively as compared to C animals. The highest mean plasma levels of catecholamines were identified in CIZS+CISS group of rats in comparison to C animals. Eventually the significant alterations of hemodynamic indices with increased blood pressure and HR in CIZS and especially CISS+CIZS were associated with marked elevation in catecholamines plasma level indicating about prevalence of vascular sympathetic tone and possible disbalance between production of vasoconstrictive and vasodilating agents resulting in changes of diurnal fluctuation of NE and EPN by shifted their acrophases to late night hours as compared to C rats.

Morphometric analysis of body weights and masses of various organs. Investigation of body weight and masses of different organs before and after animals euthanasia elicited marked changes in body weights of stress-exposed animals vs. C rats (Table 2). Body weights (BW) in CIZS and CIZS+CISS rats after the 21 days of stress influence significantly decreased by 20,5% ($P<0,05$) and 27,6% ($P<0,001$), respectively as compared to C group of rats. BW such alterations were accompanied by marked changes in masses of various organs. As it was revealed, thyroid gland weight significantly increased in CIZS (7,8%, $P<0,05$) and especially in CIZS+CISS group of animals (13,2%, $P<0,01$) vs C rats, along with adrenal gland weight which mass in CIZS and CISS rats (12,7%, $P<0,05$) and (18,65%, $P<0,01$) pronouncedly outnumbered the same value in C rats. Lymphoid organs such as thymus and spleen also underwent to significant morphometric changes in stress submitted rats. Thymus mass in CIZS animals markedly reduced (15,4%, $P<0,01$) as well as in CISS rats (28,9%, $P<0,001$), while spleen mass in the same group of animals decreased by 22,7% ($P<0,01$) and 27,7% ($P<0,001$), respectively relative to C rats. Kidney weight also underwent to significant reduction in both group of stress exposed rats: CIZS (7,7%, $P<0,05$) and CISS (9,5%, $P<0,05$) as compared to C rats. It should be noted that all alterations in organs weights were more expressed in rats submitted to combine stress. Above-described alterations illustrate the high sensitivity of lymphoid organs (thymus, spleen), adrenal and thyroid glands and kidney, to morphometric changes and remodelling.

Table 2. Influence of different type of chronic stress on mean body weights and the various organs masses in rats

Organs mean weight in g	Control (C)	Chronic immobilization stress (CIZS)	CIZS+chronic isolation stress (CISS)
Body weight	226±12 (n=25)	180±11* (n=25)	164±14** (n=25)
Thyroid gland	0,059±0,001	0,064±0,0016*	0,068±0,002***,#
Adrenal gland	0,048±0,015	0,056±0,005*	0,059±0,008***,#
Thymus	0,305±0,012	0,258±0,014**	0,217±0,008***,#
Kidney	0,598±0,026	0,552±0,045*	0,541±0,036*
Spleen	0,675±0,018	0,522±0,026**	0,488±0,016***,#

Note: n=animal number in groups. Other symbols are the same as in Fig.1.

Discussion. Immobilization stress is expressed in complex changes involving central and autonomic nervous system, production of catecholamines and corticosteroids, associated with alterations

of immune reactions, hemodynamic indices and remodelling of various organs [20,21,23]. In present study we investigated the modulatory effect of chronic immobilization stress (CIZS) and its coincident with isolation stress on hemodynamic parameters, plasma catecholamines daily level fluctuation, body weight and organ masses alterations in rats. According our data during 21 days CIZS submitted animals revealed significant changes of hemodynamic parameters vs. control ones with significant increased systolic, diastolic arterial pressures and cardiac rhythm after 14 days to stress exposure, attaining their peak values especially on 21 days of CIZS influence indicating about absence of hemodynamic adaptation to habitual prolong homotypic stressor. Adding to CIZS the permanent heterotypic stressor component, like chronic isolation, increased sensitivity to combine stress manifested already in more expressing similar changes of hemodynamic parameters after 7 days to stress exposure, which proved the impact of the heterotypic stress stimuli to homotypic habitual stress condition.

Concerning chronic immobilization stress related hemodynamic alterations there are controversial data demonstrating by different authors [24,25,26,27,28,29]. It was shown that strain specific adaptation in rats to chronic homotypic stress is associated with cardiovascular autonomic and endocrine changes of basal values in freely moving rats [18,24]. The repeatedly restrained WKY rats reveal hemodynamic and autonomic adaptation to chronic homotypic stress only during post stress recovery period, whereas SHR (Spontaneously hypertensive rats) in such cases showed reduction in stress associated tachycardia with enhanced parasympathetic tone and diminished sympathetic activity. According authors explanations, less effective adaptation of WKY rats vs SHR animals to cardiovascular alterations during exposure to stress condition is related to reduce corticosterone production expressing the severity of uncontrollable stress [27] and possible increased sensitivity to repeated restraint stress in WKY rats.

In other experiments chronic daily submission of rats to relatively mild homotypic stressor like restraint was accompanied by rapid habituation development to homotypic stress stimuli after six exposures, while stronger stressor like immobilization requires a greater number to stress exposure for occurring habituation [25]. Additionally, intervention of novel heterotypic stressor may lead to increased sensitivity and response with reduction of habituation [30]. These results are consistent with our findings when hemodynamic indices including arterial pressure and cardiac rhythm reached their peak values in 14-21 days interval during immobilization stress submission and underwent to the same changes more early after 7 days exposure to isolation stress coincident to immobilization stress, showing lack habituation to strong stressor with increased sensitivity to heterotypic stress stimuli.

According our data cardiovascular alterations in chronic stress situation were associated with significant daily fluctuation of NE and EPN daily plasma levels in comparison to C rats. In CIZS animals mean values of NE and EPN significantly exceeded the same significances in C rats. The acrophases of catecholamines plasma content in C group of rats were established in light phase of daily hours (9:00-17:00 h), while in animals submitted to CIZS and especially to CIZS+CISS stress condition such acrophases were shifted to evening (17:00-01:00 h) and late (21:00-01:00 h) night hours, respectively. Our results concerning diurnal variations of NE and EPN plasma concentration in C rats are consistent with findings showing highest catecholamines concentrations during daylight hours and low levels reached a nadir 5 hours after the onset of darkness in stress naive rats [31]. Elevation in plasma catecholamines level in stress condition is associated with increased activity of sympathoadrenomedullary system and high production of NE and EPN [27] especially in response to new stressor, like isolation [19].

Some data demonstrated that habituation to a homotypic stressor may developed rapidly, which is associated with the intensity of the stress stimuli, such as minor restraint, when habituation can occur after a few exposure [25]. However, habituation concerning catecholamines plasma level did not reveal in

our experiments after exposure to CIZS and CIZS+CISS, which is in agreement with data of other authors postulated that in more intense stress stimuli, for example CIZS, habituation to catecholamines plasma content requires a greater number of daily exposures to stress [29]. In our findings was established distinction in catecholamines plasma level daily fluctuation between C and stress exposed animals with acrophases in 9:00-17:00 and 17:00-01:00h, respectively, which are consistent with evidence that rats being crepuscular, not nocturnal animals, are most active at dusk and dawn and sleep at night or during the day often accompanied with blood pressure decreased, while chronic stress situation associated to increased sympathoadrenomedullary activity vs its basal level facilitates to disruption of circadian rhythm with shifted catecholamines plasma level acrophases to late night hours [4].

The endocrine system plays significant role in stress management. Under chronic stress body weight and endocrine organs masses, such as adrenal and thyroid glands, as well as lymphoid organs (spleen, thymus) and kidneys may undergo to marked morphometric alterations. In our experiments body weight (BW) of CIZS and CIZS+CISS rats were significantly decreased ($P < 0,05$ and $P < 0,01$), respectively in comparison with C group of animals. Such reduction in BW in stress submitted animals was revealed in both (II and III) group of rats associated with significantly increased masses of adrenal and thyroid glands and marked decreased weights of spleen, thymus and kidneys. This changes more pronouncedly expressed in rats exposed to CIZS+CISS.

The different physiological alterations accompanied the various stress conditions involves hypothalamic-pituitary-adrenal axis participating in relax of trophic hormones – TSH and CRH resulting eventually in greater production of corresponding hormones including thyroid hormones, epinephrine and steroid hormones involving in thermogenesis, energy expenditure for muscle activity, facilitating to increase masses of thyroid and adrenal glands. Reduction in kidneys weight in stress exposure may due to morphological changes including reduction in glomerular volume density and lower number of glomeruli per kidney [32]. Our findings are consistent with results of other authors [33] that showed in rats exposed during 2, 4 and 8 weeks to restraint stress significant alterations in lymphoid organs (spleen, thymus), leading to their involution and weight reduction, the severity of which was associated with period to stress submission.

Conclusion: Eventually it can be concluded that chronic immobilization stress (CIZS) may lead to significant alterations in hemodynamic indices after 3 weeks to stress exposure, expressing in marked elevation of systolic and diastolic blood pressure and acceleration in cardiac rhythm, without habituation to homotypic stressor. Adding a novel stressor such as isolation stress showed that such heterotypic component enhanced abovementioned cardiovascular alterations, accompanied by changes in catecholamines (norepinephrine, epinephrine) daily plasma fluctuation with shifted their acrophases vs control group of rats towards evening and late-night hours. Hemodynamic and catecholamines plasma content alterations in rats exposed to CIZS and combine stress condition also revealed pronounced changes in animals body weight and organ masses with reduction in body weight, lymphoid organs (spleen, thymus) and kidney weights and increased thyroid and adrenal gland masses.

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INFLUENCE OF CHRONIC STRESS ON HEMODYNAMIC INDICES, DIURNAL FLUCTUATION OF CATECHOLAMINES PLASMA CONCENTRATIONS AND REMODELLING OF VARIOUS ORGANS IN RATS

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SUMMARY

Stress condition combines the different type of emotional, physical and psychological alterations in response to various stressor. Stress may result in favourable outcome by leading to a new biological balance or sometimes can exert damaging dangerous effect including changes in function of cardiovascular system. For this reason, individual approach to stress accompanied cardiovascular somatovegetative correlates alterations is one of the important directions in modern cardiology.

The goal of the present study was the investigation of cardiohemodynamic parameters, catecholamines plasma level alterations and remodelling of different organs in chronic immobilization stress and its coincident with isolation stress.

Experiments were performed on 75 male Wistar rats weighing 200-250g divided into three groups: I- Stress naive animals control (C) group; II – rats exposed to chronic immobilization stress (CIZS) and CIZS animals with coincident chronic isolation stress (CISS). Rats were handled in compliance with ethical standards approved by the Animal Care Committee of Tbilisi State Medical University. CIZS was created by placing rats in a special plexiglas transparent camera for 120 min every day in the morning (10⁰⁰-12⁰⁰) hours during 21 days, with retained their ability to turn a head with restriction body and paws movement. Each rat from III group after submitted to CIZS vs II group of animals was maintained in cage separately. Cardiovascular parameters including systolic (S), diastolic (D) blood pressure (BP) and heart rhythm (HR) were obtained by non-invasive „tail-cuff“ sphygmomanometric method. Catecholamines norepinephrine (NE) and epinephrine (EPN) blood level daily fluctuation was studied at 5 different points (9:00, 13:00, 17:00, 21:00, 01:00 h) to determine the acrophases in this period. Stress influence on body weight and remodeling of various organs was studied by changes of their masses.

It was established the CIZS induced significant changes in hemodynamic indices with gradual elevation in SBP, DBP and HR after 14 days to stress exposure, attaining peak values after 21 days to stress submission - 138±4 mmHg (P<0,01), 99±4 mm Hg (P<0,05) and 472±6 beat/min (P<0,01), respectively vs. the same values in C rats: 106±8 mm Hg, 72±7 mm Hg and 414±6 beat/min, respectively. In CIZS +CISS exposed rats revealed more pronounced changes with respect to C and CIZS group of animals indicated about marked influence of heterotypic stress intervention to homotypic ones. Cardiovascular alterations in CIZS animals were associated with significant increased mean plasma levels of NE-456,5±28,6 pg/ml and EPN-563±25,2, respectively vs. C rats with shifted their acrophases from light hours (19⁰⁰-17⁰⁰h) revealed in C rats to evening and late-night hours (17⁰⁰-01⁰⁰h) in stress exposed animals. Catecholamines plasma level elevation in combine stress condition outnumbered the same values in CIZS exposed rats. Along with abovementioned, both type of stress characterized with decreased: body weight, kidney and lymphoid organs (spleen, thymus) masses and increased in adrenals and thyroid organs weights.

It is postulated that prolong chronic homotypic stress characterized with significant alterations of cardiovascular indices expressed in increased BP and HR, associated with elevation in catecholamines plasma level and shifting their acrophases from light to evening and late-night hours. Such alterations are more revealed in combine stress condition without habituation concerning such changes.

Keywords: Immobilization stress, Cardiovascular parameters, Catecholamines, Diurnal rhythm

