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Electrolyte and Water Contents in Organs and Tissues of Rats during and after Exposure to Prolonged Restriction of Motor Activity

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Sodium and potassium concentrations in organs and tissues of rats have been determined during hypokinesia (HK) and posthypokinesia (post-HK) periods. The study was performed on 180 male Wistar rats with an initial body weight of 370 to 390 g. They were divided into control and experimental groups: the 1st group of rats was placed under ordinary vivarium conditions (control) and the 2nd group was subjected to 90 days of HK (hypokinetic animals). The animals were kept in small individual wooden cages which restricted their movements in all directions without hindering feed and water intake. On the 30th, 60th and 90th day of the HK period and on the 5th, 10th and 15th day of the post-HK period animals were decapitated, and potassium and sodium concentrations were measured in the myocardium, skeletal muscles, erythrocytes and plasma. Fluid content was also determined in the myocardium and skeletal muscles. During both the HK period and initial stages of the post-HK period the concentration of sodium and potassium in the myocardium, skeletal muscle, plasma and erythrocytes as well as water content in the myocardium and skeletal muscle changed significantly.

Key words: Electrolytes, Organs, Tissues, Rats, Hypokinesia.

Functional impairment of several systems and dystrophic alterations in different organs and tissues have been repeatedly demonstrated as a result of analysis of experimental material dealing with general and special aspects of the effect of longterm restriction of motor activity (hypokinesia) on the animal and human organism (2, 4). Thus, substantial disturbances have been observed following

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exposure to prolonged restriction of motor activity in the concentration of electrolytes (10, 11) in different organs and tissues of animals. Prolonged restriction of motor activity, also has an adverse effect on fluid-electrolyte metabolism in animals and man, which is characterized by a significant increase in the rate of excretion of fluid and electrolytes (5, 7, 9), with its consequent reduction in different organs and tissues (9-11).

Several experimental studies, as well as some pathological processes, have demonstrated that distinctions of development of disturbances in the concentration of electrolytes in different organs and tissues are manifested against the background of prolonged restriction of motor activity (5, 7, 9-11). However, the prime pathogenic mechanism of reduction of concentration of electrolytes in different organs and tissues still remain unclear.

For this reason, we have undertaken an attempt to investigate the influence prolonged restriction of motor activity has on the content of potassium and sodium in blood plasma, erythrocytes, myocardium, and skeletal muscles of rats during and after exposure to prolonged restriction of motor activity.

Materials and Methods

Experiments were performed on 180 male Wistar rats obtained from National Breeding Animal Laboratories. At the start of the study the animals weighed 370 to 390 g and all of them were about 75 to 95 days old. They were housed in individual cages with light (07:00 to 19:00 h), temperature (28 ± 1.6 °C) and relative humidity (75 to 80 %) automatically controlled and allowed free access to food and water. The studies were approved by the Committee for the Protection of Animals.

The experimental studies were preceded by a series of clinical and biochemical examinations, training, testing and conditioning of rats to new type of laboratory conditions. This period which aimed at preparing the animals and minimizing the effect of hypokinetic stress, which is inherent to prolonged restriction of motor activity (2, 4), lasted a period of about 460 to 590 hours.

The control animals were housed under ordinary vivarium conditions, and for the simulation of the hypokinetic effect the animals (hypokinetic group) were kept for 90 days in small individual wooden cages, with good insulation, whose dimensions (195 x 80 x 95 mm) allowed for restricting movements in all directions without hindering food and water intake. At the same time, the animals could assume a postural position and groom different parts of its body. The cages were constructed in such a way that their size could change in accordance with the size of individual animals so that the degree of restriction of movements could be maintained at a relatively constant level throughout the study.

During the complete experimental time all animals were paired-fed laboratory food, they received homogeneous biologically complete laboratory chow with all essential nutrients: 14-16 % protein, 19-22 % cellulose, 1.4 % potassium, 0.91 % calcium, 0.54 % phosphorus, 0.4 % sodium, 0.33 % chloride, and vitamins A, D, and E. The constant chemical composition of the food, its homogeneous, pasty consistency which ruled out the possibility of food loss when, feeding the animals, made it possible to maintain a relatively constant dietary intake throughout the experimental period.

Each animal was fed once a day in the morning at the rate of 30 to 50 g and 120 to 150 ml water. Body weight and the amount of food consumed was determined at regular intervals.

During the hypokinetic period 15 rats from each group were decapitated on the 30th, 60th and 90th days, and during the post-HP period other 15 rats on the 5th, 10th and 15th day. The concentrations of potassium and sodium were determined by the method of flame photometry in blood plasma, erythrocytes, myocardium and femoral extensor muscles. The content of fluid in the myocardium and skeletal muscle was determined by the method of drying tissues to a constant weight.

Statistical analysis. – Each measure was analyzed separately with a two factor mixed ANOVA design with replications on the same sampling intervals factor. A probability of P < 0.05 was considered to be statistically significant.

Results

General conditions .- During the initial 15 days of the HK period the rats become restless, striving to escape and when they were removed for the cleaning of their cages they did not want to return to them. During this period of time the appetite was considerably decreased. As a result of this reaction 20 % - 30 % of their food remained uneaten every day. After the 15th day of the HK the animals started to adapt and by the 30th day they had adapted well, as to behavioral reactions and eating patterns, however, by the end of the experimental period they manifested an impairment in their general condition and a reduction in their motor activity. During the post-HK period, the animals readapted well to ordinary motor activity conditions and their appetite, behavior, and general conditions improved progressively.

Body weight changes (table I).- The control animals gained body weight progressively throughout the experimental period reflecting prevalence of assimilation processes. Rats subjected to prolonged HK displayed significant decreases in the body weight, reflecting prevalence of catabolic processes. Body weight decreased drastically by the 30th day of the HK period, then increased somewhat, and by the 60th to 90th day it stabilized somewhat at the same level, but it remained significantly lower than the control level. During the post-HK period body weight increased progressively but it remained significantly below the control level.

Organ and tissue potassium and sodium concentration (tables II and III).- During the HK period potassium and sodium concentrations in plasma and erythrocytes of the hypokinetic animals increased significantly by the 30th day, decreased somewhat on the 60th day, and increased again by the 90 day. Potassium concentration in the skeletal muscles and myocardium decreased significantly by the 30th day, increased somewhat on the 60th day and decreased again by the 90th day. The same results occurred for sodium concentrations on the myocardium.

During the post-HK period potassium and sodium concentration in plasma and erythrocytes of the hypokinetic animals decreased progressively and by the 15th day it approached the control levels, although during the initial 5 to 10 days it remained significantly different. In myocardium, and skeletal muscles potassium again increased progressively, but at the initial 5 to 10 days it remained significantly different and by the 15th day it reverted back to the control levels.

Myocardium and skeletal muscle water content (table IV).- In the hypokinetic

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Ē	Initial Body Weight	Hypokineti	Hypokinetic period in days (n = 15)	n = 15)	Posthypokine	Posthypokinetic period in days (n = 15)	; (n = 15)
Group	(n = 90)	30th	60th	90th	5th	10th	15th
Control Hypokinetic	376 ± 12.6 387 ± 10.4	464 ± 15.6 310 ± 10.5*	538 ± 16.4 328 ± 14.0*	615 ± 10.6 343 ± 11.7*	620 ± 19.4 379 ± 11.3*	632 ± 13.8 438 ± 16.6*	648 ± 15.9 524 ± 16.0*
p < 0.05 significan	*p < 0.05 significant differences compared to the control group.	to the control group.					
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			Values are the means ± SEM.	ieans ± SEM.	:		1
	Baseline	Hypokineti	Hypokinetic period in days (n = 15)	n = 15)	Posthypokin	Posthypokinetic period in days (n = 15)	s (n = 15)
Tissues	(n = 90)	30th	60th	90th	5th	10th	15th
Control							
Plasma	5.0 ± 1.3	5.0 ± 1.5	5.1 ± 1.3	5.0 ± 1.2	5.1 ± 1.4	5.1 ± 1.2	5.0 ± 1.3
Erythrocytes	77.5 ± 0.42	2 76.5 ± 0.55	77.1 ± 0.46	76.4 ± 0.52	77.0 ± 0.33	76.5 ± 0.49	77.0 ± 0.35
Myocardium	31.2 ± 0.38	3 31.5 ± 0.26	32.0 ± 0.30	31.3 ± 0.48	31.5 ± 0.50	31.0 ± 0.28	30.7 ± 0.49
Skeletal Muscle	cle 41.5 ± 0.24	t 41.6 ± 0.32	41.5 ± 0.46	42.0 ± 0.22	41.3 ± 0.43	41.6 ± 0.27	41.3 ± 0.50
Hypokinetic							
Fiasma	4.8 ± 1.1 75 2 ± 0 55	0.0 ± 1.4 ⁻ 5 85 7 ± 1 48*	• 810±125*	01.1 ± 1.3 ⁷ * 80 8 ± 1 €0*	2.8 ± 1.4 ⁻	70.6 ± 1.42*	2.1 ± 5.0 75.0 ± 0.48
Mvocardium	1 +		27.0 ±		$35.1 \pm 1.35^*$	$27.4 \pm 1.29^{*}$	29.6 ± 0.53
Chalatal Milecia	201	33 5	35.1 +	313+1	+	35 8 + 1 45*	49 4 + 0 41

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	Baseline	Hypokinet	Hypokinetic period in days (n	(n = 15)	Posthypoki	Posthypokinetic period in days (n = 15)	tys (n = 15)
Tissues	(n = 90)	30th	60th	90th	5th	10th	15th
Control							
Myocardium	14.54 ± 0.27	14.55 ± 0.30	14.53 ± 0.47	14.56 ± 0.35	14.52 ± 0.51	14.54 ± 0.25	14.56 ± 0.40
Erytrocytes	23.40 ± 0.33	23.44 ± 0.42	23.41 ± 0.36	23.45 ± 0.25	23.43 ± 0.46	23.45 ± 0.39	23.40 ± 0.24
Plasma	143.56 ± 0.50	141.22 ± 0.22 1	145.43 ± 0.46	143.59 ± 0.54	145.96 ± 0.48	143.77 ± 0.35	141.65 ± 0.56
Hypokinetic							
Myocardium	14.56 ± 0.44	12.88 ± 0.27*	$13.24 \pm 0.83^*$	12.83 ± 0.69*	13.30 ± 0.45*	13.14 ± 0.19	15.61 ± 0.40
Enytrocytes	23.42 ± 0.60	25.51 ± 0.62*	24.40 ± 0.71*	26.68 ± 0.95*	25.47 ± 0.60*	23.76 ± 0.42	22.59 ± 0.33
Plasma	141.39 ± 0.58	160.35 ± 1.77* 1	154.75 ± 1.44*	158.80 ± 1.73*	146.91 ± 1.58 [*]	142.66 ± 1.60	140.27 ± 1.46
$^{\circ}$ p < 0.05 significant differences compared to the control group.	ifferences compared t	to the control group.					
Table IV. Water co	intent (mi/kg wet	Table IV. Water content (ml/kg wet tissue) in the myocardium and skeletal muscle of rats during and after prolonged restriction of motor	cardium and skel	letal muscle of re	its during and af	ter prolonged res	striction of motor
			activity. Values are the means ± SEM.	y. eans ± SEM.			
	Baseline	Hypokinetic	Hypokinetic period in days (n = 15)	1 = 15)	Posthypokin	Posthypokinetic period in days (n = 15)	s (n = 15)
Tissues	(n = 90)	30th	60th	90th	5th	10th	15th
Control			1				
Myocardium	66.59 ± 0.48	66.65 ± 0.34	66.66 ± 0.58	65.44 ± 0.45	66.90 ± 0.53	65.77 ± 0.49	66.65 ± 0.42
Skeletal Muscle	€ 62.48 ± 0.39	63.27 ± 0.56	62.75 ± 0.43	63.69 ± 0.61	63.48 ± 0.47	62.93 ± 0.54	62.56 ± 0.34
Hypokinetic							
Myocardium	65.33 ± 0.25		60.0 ±	53.46 ±	55.61 ± 1.47*	+1	H-
Skeletal Muscle	9 61.74 ± 0.46	55.80 ± 1.49*	57.4 ± 1.56*	48.08 ± 1.62*	51.55 ± 1.59 *	55.74 ± 1.46*	59.57 ± 1.52
$r_{\rm D} < 0.05$ significant differences compared to the control group of rate.	fferences compared t	to the control group of	rats.				

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and in the post-HK periods water content in the skeletal muscle and myocardium, significantly decreased in the hypokinetic animals.

Discussion

The demonstrated changes in the behavioral and eating pattern of the hypokinetic animals as well as the changes in their general condition are inherent to prolonged restriction of motor activity (2, 4). However, since all of these changes disappeared during the post-HK period they may be characterized as adaptational reactions to prolonged hypokinesia.

Body weight changes.- The body weight losses of the hypokinetic animals are typical to the losses of body weight which are developed during prolonged restriction of motor activity regardless of whether they are on controlled diets or are allowed free choice of food intake (2, 4). This lower growth may be attributable to the decreased metabolic processes in the direction of prevalence of catabolic processes over anabolic processes and mobilization of fat from fat depots (2, 4), as well as body dehydration due to intensification of excretion of fluid and electrolytes in urine during prolonged hypokinesia (9-11). The progressive increase of body weight during the postexperimental period may be attributable to the intensification of anabolic processes which are present during post-HK (2, 4); the body weight grew rapidly, coming very close to the values of the control animals after 15 days of recovery period without reaching however, the level of the control animals. Analogous results were obtained in previous studies (1, 3, 6, 8).

Organ and tissue water and electrolyte contents during HK.- The demonstrated

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significant changes in their concentrations were apparently induced as a result of water and electrolyte deficiency due to the impossibility of the body to retain adequate amounts of water and electrolytes during prolonged HK (5, 9-11). Our results also revealed that as the duration of the HK period increased the concentrations of electrolytes in plasma and erythrocytes increased further, while the water and electrolyte concentrations in the skeletal muscle and myocardium decreased further, depending on the duration of the HK period. The most clearly expressed changes in the contents of water and electrolytes in organs and tissues of rats were demonstrated during the initial and final stages of the HK period.

The water and electrolyte contents changes in organs and tissues of rats due to the effect of prolonged HK also develop in a wave-like pattern. Evaluation of the demonstrated alterations revealed that they reflect in essence the initial and final stages of adaptation to HK, and they are attributable primarily to diminished functional load on different organs and systems and the organism as a whole (5, 9-11), could also have affected water-electrolyte concentrations in different organs and tissues of rats. However, the rate of adaptation process does not appear uniformly in different tissues and organs during prolonged exposure to HK, and this is apparently caused by both the extent of decline in functional load during prolonged HK and intensity of their inherent metabolic processes (2, 4). These changes in organs and tissues of HK rats are a reflection of the disturbances in water-electrolyte metabolism. These significant changes constitute a typical feature of reactions to prolonged exposure to HK. This fact indicates that mechanism impairment for maintaining homeostasis at the level of the integral organism apparently does not occur during prolonged

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HK and especially at the initial and final stages (2, 4).

Organ and tissue water and electrolyte content during Post-HK.- The post-HK changes of water and electrolyte content in organs and tissues of hypokinetic rats were constantly manifested by progressive decreases of sodium and potassium in plasma and erythrocytes, and progressive increases of water and electrolytes in myocardium and skeletal muscle. However, the demonstrated changes in organs and tissues of HK rats were not minor during the initial stages of the post-HK period and although they progressively reverted back to the level of the control animals by the 15th day of the post-HK period, these changes usually indicated significant deficiencies of water and electrolytes in the body of animals (5, 9-11). It is known that the influence of the initial stages of post-HK on water-electrolyte concentration in organs and tissues of hypokinetic rats is among its specific effects (5, 9-11). These changes were apparently transient since all of them were reverted to the levels of the control animals during the post-HK period, and they could have developed due to changes in the systems of regulation of water and electrolytes and hormonal status during the HK and post-HK period. It is also possible that water and electrolyte contents in the hypokinetic animals were due to the decrease in water-electrolyte pool of cells as a result of metabolic changes in the muscular system (5, 9-11). It should also be mentioned that the changes in water and electrolyte contents, which were observed during the HK and initial stages of the post-HK periods, were probably related to inability of muscle tissue to retain water and electrolytes as a result of atrophic processes, which develop due to insufficient load on the skeletomuscular

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system during prolonged exposure to hypokinesia.

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Se determinan las concentraciones de sodio y potasio en órganos y en tejidos de rata durante períodos de hipocinesia (HK) y posthipocinesia (post-HK). El estudio se realiza en 180 ratas Wistar macho de un peso corporal inicial de 370-390 g, separadas en dos grupos: 1) grupo control de animales estabulados en condiciones ordinarias; y, 2) grupo experimental (hipocinético), sometido durante 90 días a HK. Los animales se mantienen en pequeñas jaulas de madera, individuales, que restringen sus movimientos en todas las direcciones sin impedir la ingesta de comida y agua. En los días 30, 60 y 90 del período HK y en los días 5, 10 y 15 del post-HK se decapitan 15 animales de cada subgrupo y se miden las concentraciones de potasio y sodio en el miocardio, músculo esquelético, eritrocitos y plasma. Se determina también el contenido de agua en el miocardio y en músculo esquelético. Durante el período HK y en los estadios iniciales del post-HK la concentración de sodio, potasio y agua cambia significativamente en miocardio y músculo esquelético.

Palabras clave: Electrolitos, Órganos, Tejidos, Rata, Hipocinesia.

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