SYSTEMIC AND REGIONAL HEMODYNAMICS IN CONSCIOUS RATS DURING 24-HOUR ANTIORTHOSTASIS

E. Yu. Bychkova, E. R. Martynova, O. S. Medvedev, V. P. Krotov, and F. E. Meertsuk

UDC 616.1-008.1-092.9.02: 612.014.477.064]-07

KEY WORDS: antiorthostasis; central hemodynamics; regional blood flow; microspheres

To simulate the effect of the hydrostatic component of weightlessness a model of antiorthostatic hypokinesia has been widely used [1], in which redistribution of the liquid media of the body takes place in the cranial direction. By means of such a model the state of the systemic hemodynamics has been investigated during a period of seven days in the antiorthostatic position, starting with the end of the 1st day, and during the subsequent recovery period [7]. However, we could find no information in the literature on changes in the parameters of the systemic hemodynamics during the first hours of a stay in antiorthostatic hypokinesia. There are likewise no data on the character of the blood flow in the organs and tissues at different times of a stay in antiorthostasis.

The aim of this investigation was to study the dynamics of the cardiac output and its redistribution among the organs during 24-h antiorthostasis.

EXPERIMENTAL METHOD

Under pentobarbital anesthesia (30 mg/kg) PE10 polyethylene catheters ("Portex," England) were inserted into the left ventricle of male Wister rats weighing 300-400 g through the right carotid artery, and into the abdominal aorta through the femoral artery. The free ends of the catheters were brought out subcutaneously in the dorsal region and fixed in the interscapular region. The experiment began 48 h after implantation of the catheters. An antiorthostatic position was created by hanging the rats by their tails by means of a staple so that the body made an angle of 30° with the horizontal. The staple was fixed to the tail with adhesive tape [1]. Blood flow was measured in 23 zones of the body with the aid of microspheres, 15 μs is diameter and labeled with ^{57}Co, ^{46}Sc, ^{113}Sn, and ^{85}Sr [2]. A single injection of about 100,000 microspheres was given in the form of a suspension of physiological saline containing 0.05% Tween-80. The microspheres, in a polyethylene coil, were shaken and irradiated in an ultrasonic bath for 5 min immediately before injection in order to prevent aggregate formation. Blood began to be taken from the abdominal aorta at the rate of 0.6 ml/min, 5 sec before injection of the microspheres. The total time during which blood was taken was 1.5 min. Blood was mixed with 0.6 ml of a 13.4% solution of Ficoll-70, creating an osmotic pressure equivalent to that of the proteins of the blood sample taken. The suspension of microspheres was injected over a period of 20 sec. The order of the experiments included four consecutive injections of microspheres: 1st injection) basic (rat in the horizontal position), 2nd) after suspension of the rat for 2 h, 3rd) for 5 h, and 4th) for 24 h, in the antiorthostatic position. During the first 2 h of the experiment the heat rate (HR) and blood pressure (BP) in the abdominal aorta were recorded continuously. After the end of the experiment the rats were killed by an overdose of pentobarbital and the organs were removed, weighed, and transferred to plastic tubes for counting. The number of microspheres in the specimens was determined on a gamma-counter ("Compu Gamma 1282," Sweden). The cardiac output and regional blood flow were calculated om a "Labtam 3015" microcomputer (Australia) by the equations given in [5]. The results were subjected to statistical analysis by Student's test for paired samples.

EXPERIMENTAL RESULTS

Switching to the antiorthostatic position caused quickening of the pulse of all the animals. On average for the group HR rose by 70 beats/min (Fig. 1, $p < 0.01$). However, HR

Institute of Experimental Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR. Institute for Medico-biological Problems, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR I. P. Ashmarin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 1, pp. 20-23, January, 1990. Original article submitted June 20, 1989.

 0007-4888/90/0001-0024$12.50

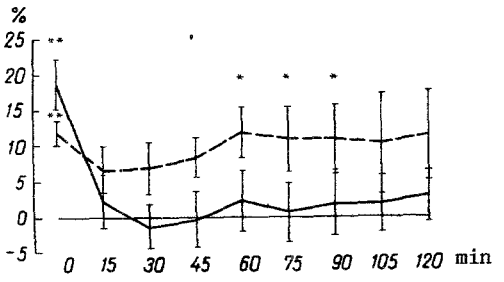

Fig. 1

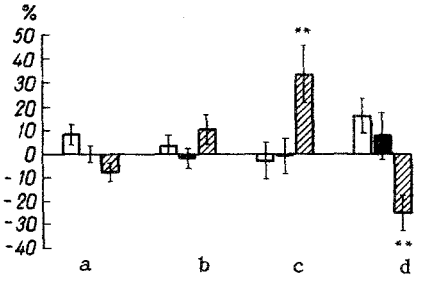

Fig. 2

Fig. 1. Dependence of change (in % of basic level) in HR (continuous line) and BP (broken line) on time spent by animal in antiorthostatic position. Here and in Figs. 2 and 3: *p < 0.05, **p < 0.01 compared with background (M ± m; n = 11).

Fig. 2. Changes in parameters of systemic hemodynamics (in % of basic level) after rat had spent 2 h (unshaded columns), 5 h (black columns), and 24 h (obliquely shaded columns) in antiorthostatic position. a) BP; b) HR; c) cardiac output; d) total peripheral resistance.

returned to its initial level after 15 min. The response of BP to suspension did not exceed 10 mm Hg compared with the initial level ($p < 0.01$) and lasted longer than the changes in HR. Toward the end of the period of 2 h BP remained high, the cardiac output was maintained at the basic level, but the peripheral resistance had a tendency to increase. After the rats had remained in the antiorthostatic position for 5 h parameters of the systematic hemodynaics reverted to their initial values, but, as Fig. 2 shows, after 24 h BP was 12 mm Hg lower than initially ($p < 0.05$) the cardiac output was increased by 40 ml/min ($p < 0.05$), due equally to increases in HR and the stroke volume; the peripheral resistance was 0.7 unit lower than initially ($p < 0.01$).

The effect of movement of the liquid compartments of the body from the direction of the gravitational vector on the state of the regional blood flow may be greatest in diametrically opposite regions of the body. Examples of these are muscle groups of the fore- and hind limbs. After a stay of 2 h in antiorthostasis, the blood flow in the gastrocnemius muscles of nine of 11 animals was reduced, to 8.17 ± 24.5% of the basic level for the group ($p < 0.05$); after 5 h it continued to fall, reaching 65.6 ± 14.9% of the basic level ($p < 0.04$), and remained at low level, namely 75.6 ± 6.9% after 24 h (Fig. 3). Diametrically opposite changes were found in the blood flow in the muscle group of the forelimbs (biceps and triceps): it was increased almost threefold after 2 h and remained at the same level after the end of 5 h of the rats' stay in antiorthostasis, and was almost 8 times higher than the initial blood flow after a stay of 24 h, namely 765 ± 122.7% of the basic level ($p < 0.01$). Such striking differences in the character of the blood flow in muscles of the fore- and hind limbs were evidently connected less with the antiorthostatic position of the animal and more with changes in their function: removal of the load from the gastrocnemius muscle, which is antigravity in its function, and increasing the load on the locomotor system of the forelimbs. The dynamics of the blood flow of the animal in the antiorthostatic position can thus be judged more effectively by analysis of data on its level in the neck muscles, whose activity in the course of the experiment underwent a much smaller change than that of the forelimb muscles. No significant increase in the blood flow was found after 2 and 5 h in this region, but after 24 h it was 3.5 times greater than initially ($p < 0.01$).

It will be clear from Table 1 that the blood flow both in the left and in the right cerebral hemisphere was unchanged during antiorthostasis, although after the end of the first day there was a tendency for it to increase in seven of the 11 animals. Meanwhile, the blood flow in the cerebellum was significantly increased after a stay of 24 h in antiorthostasis, to 130 ± 10% of the initial value ($p < 0.01$). It can be tentatively suggested that this increase in the blood flow in the cerebellum is evidence of increased activity of that part of the brain under conditions of antiorthostasis. The blood flow in the stomach and pancreas fell during the first 2 h of suspension by 35.5 ± 8% ($p < 0.01$) and 51.3 ± 3.3%, respectively; in the latter, moreover, it remained low after 5 h, at 57.7 ± 8% ($p < 0.01$) of the initial level. The blood flow in the pancreas did not return to the initial value until the end of a 24-h period in antiorthostasis. In the liver, on the other hand,

TABLE 1. Dynamics of Regional Blood Flow (in ml/min/g) in Rats after Spending Different Times in Antiorthostasis

Organ	Initial values	Length of stay in antiorthostasis		
		2	5	24
Stomach	1,18±0,13	0,72±0,09**	0,98±0,12	0,87±0,08
Pancreas	1,90±0,21	0,91±0,10**	1,06±0,15**	1,71±0,20
Small intestine	2,49±0,29	2,38±0,24	2,72±0,29	2,71±0,20
Lungs	3,33±0,82	2,33±0,87	1,43±0,20	2,96±0,98
Spleen	2,25±0,24	1,30±0,14**	1,28±0,22**	1,54±0,24*
Liver	0,25±0,06	0,53±0,06**	0,48±0,10	0,05±0,01**
Brain:				
left hemisphere	1,73±0,10	1,78±0,09	1,76±0,13	1,88±0,12
right hemisphere	1,57±0,13	1,70±0,09	1,72±0,17	1,82±0,11
cerebellum	1,80±0,09	1,79±0,12	1,95±0,11	2,23±0,12**
brain stem	1,21±0,11	1,01±0,07	1,14±0,12	1,34±0,08
Heart:				
left ventricle	8,10±0,72	7,52±0,63	8,28±0,82	11,35±1,15*
right ventricle	6,26±0,82	5,88±0,56	6,75±0,99	9,33±0,90*
septum	8,05±0,84	7,63±0,64	8,55±1,21	12,14±1,19*
Kidneys	5,37±0,47	4,78±0,46	4,77±0,62	3,78±0,26*
Testes	0,29±0,02	0,40±0,02**	0,39±0,05*	0,43±0,09**
Adrenals	8,56±0,94	4,08±0,48**	3,93±0,59**	4,94±0,64**

Legend. *p < 0.05, **p < 0.01 compared with initial value.

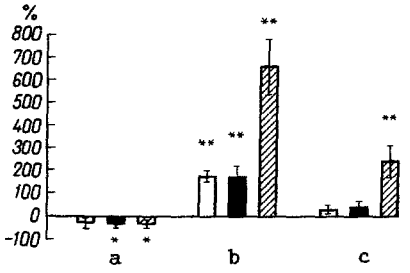

Fig. 3. Changes in blood flow (in % of basic level) in limb and neck muscles after rat had spent 2 h (unshaded columns), 5 h (black columns), and 24 h (obliquely shaded columns) in antiorthostatic position. a) Blood flow in muscles of hind limbs; b) of forelimbs; c) in neck muscles.

the blood flow increased during the first few hours of antiorthostasis, after 362 ± 72% of the initial value ($p < 0.01$), but after 24 h it was only 22.7 ± 5.7% ($p < 0.01$). The blood flow in the spleen remained low throughout the experiment. In the kidneys the blood flow fell progressively to reach 75 ± 9% of the basic value after 24 h ($p < 0.01$), possibly due to a decrease in the intensity of metabolism in the kidney tissue due to a decrease in reabsorption of fluid under the influence of antiorthostatic tilting [3]. During the first few hours of the experiment the blood flow in the myocardium of the right and left ventricles and in the septum remained unchanged, but it increased by 1.5 times after 24 h. The blood flow of the adrenals fell during the first few hours of suspension and remained lower than initially throughout the period of exposure.

Changes in the hemodynamics during orthostasis have been studied in the greatest detail in experiments on conscious animals. The typical response to a short-term orthostatic test includes reduction of the cardiac output and elevation of the total peripheral vascular resistance [4]. As a rule BP is unchanged [4, 6]. In the present experiments, under the conditions of antiorthostasis, the appearance of hemodynamic responses of the opposite sign might be expected. However, we did not measure the cardiac output during the first minutes after switching the animal into the antiorthostatic position. Significant differences in the parameters of the systemic hemodynamics were not observed until the 24th hour of antiorthostasis, and these changes are in good agreement with results obtained previously [7]. The reasons why the increase in cardiac output of the rats were delayed until the end of the first day in the antiorthostatic position are not known. It can be postulated that the response of the cardiac output takes place in "phases": an increase on the 1st and 2nd days of antiorthostasis followed by normalization toward the end of the 3rd day [7].

LITERATURE CITED

1. E. A. Il'in and V. E. Novikov, Kosmich. Biol., No. 3, 79 (1980).
2. O. S. Medvedev, A. N. Murashev, F. E. Meertsuk and S. F. Dugin, Fiziol. Zh. SSSR, 72, No. 2, 253 (1986).
3. V. P. Krotov and V. I. Bogdanov, Current Problems in Space Biology and Medicine [in Russian], Moscow (1980), pp. 39-40.

4. Kh. Khaike, B. I. Tkachenko, G. Tseshke, and A. V. Samoilenko, Vest. Akad. Med. Nauk SSSR, No. 6, 16 (1987).
5. R. P. Hof, Triangle, 21, No. 1, 29 (1982).
6. C. H. Lee, A. M. Strosberg, A. P. Roszkowski, et al., J. Pharm. Meth., 7, 15 (1982).
7. V. Popovic, Physiologist, 24, No. 6, Suppl. 15 (1981).

EFFECT OF THE CRUSH SYNDROME ON INSULIN-RECEPTOR INTERACTION IN CELLS

N. P. Mikaelyan

UDC 616.001.36-092:[612.014.467:577.175.722]-092.9

KEY WORDS: insulin receptors; blood; rat liver; crush syndrome

During prolonged crushing of the soft tissues profound metabolic disturbances arise, which affect energy, carbohydrate, and other types of metabolism [1, 3]. Local injury, as a factor determining the state of the body as a whole, has quite often been regarded as less important in the dynamics of development of the soft tissue crush syndrome than disturbances of various stages of the neuroendocrine system, which can give rise to widespread metabolic changes. These changes include, in particular, changes in hormone-receptor relations. The sensitivity of tissues to a hormone is mediated through specific receptors in the cells. Accordingly, the study of insulin-receptor interaction in various tissues during the crush syndrome is of great interest, more especially because the state of the insulin receptors during prolonged trauma has not been investigated at all.

This paper describes a study of insulin-receptor interaction in blood and liver cells.

EXPERIMENTAL METHOD

Altogether 36 male Wistar rats weighing 180-220 g were used, and under open ether anesthesia clips were applied to the right hind limb for 6 h; the investigation was conducted both with the clips applied (1 and 6 h of compression) and after their removal (2 h after decompression).

The control group consisted of 10 rats. Blood was taken from the animals after decapitation. The immunoreactive insulin (IRI) concentration was studied by radioimmunoassay using kits, and glucose was determined by the glucose oxidase method. Pure suspensions of mononuclears (MN) were obtained from blood in a one-step Ficoll-Verografin gradient, with density of 1.077 g/ml [5]. The protein concentration in preparations of the plasma membranes was determined by the method in [8], which is a modification of that in [7]. To study insulin-receptor interaction the method of displacement of ^{125}I-insulin from its complex with receptors by increasing amounts of unlabeled hormone under equilibrium conditions was used [6]. Porcine ^{125}I-insulin (MI-47, Poland), with specific radioactivity of 7.3 ± 1.0 GBq/mg was used as the labeled compound. Specific binding of insulin was calculated as the difference between total and nonspecific (in the presence of unlabeled insulin in a concentation of 0.4×10^{-6} M) binding and expressed as a percentage of total radioactivity of the incubation medium. Affinity was estimated by the concentration of unlabeled insulin inducing 50% inhibition of binding of ^{125}I-insulin. The total number of insulin-binding sites was determined with the aid of a Scatchard plot (where the curve interesects the abscissa) and affinity of free and maximally occupied binding sites was determined from the graph [9].

EXPERIMENTAL RESULTS

Table 1 shows that the serum insulin concentration was depressed only during the first few hours after compression; by the 6th hour it showed a sharp increase (171.8 ± 21.6 M compared with 69.4 ± 15.1 pM in the control. The insulin concentration still remained high 2 h after removal of the press (186.3 ± 27.4 pM).

Research Institute of Endocrinology and Pathology of Metabolism, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR B. T. Velichkovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 1, pp. 23-25, January, 1990. Original article submitted June 16, 1989.

