Effect of Unilateral Inactivation of Brain Hemispheres on Hypoxic Resistance in Mice

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The effects of unilateral cortical spreading depression on the resistance to acute hypoxia with hypercapnia were studied in male SHR mice. The life-span of mice with active right hemisphere was significantly longer than that of intact, but not of sham-operated (active control) animals. Mice with active left hemisphere differed significantly from intact and active control animals. It seems that the right hemisphere supports the optimal level of resistance to hypoxia with hypercapnia, while the left hemisphere performs the "antihypoxic" function. Additional analysis found no differences between the hemispheres in mice with low hypoxic resistance. The differences between the hemispheres increased with increasing the resistance to hypoxia.

Key Words: *interhemispheric asymmetry; hypercapnia; spreading depression; hypoxic resistance; SHR mice*

The hypoxic factor is one of the most important mechanisms in the development of many pathological processes (strokes, crush syndrome, ischemia, cerebral edema/swelling, *etc.*) [2,4], and hence, study of the mechanisms of resistance to different types of hypoxia remains an important problem. Of hypoxic states emerging during work, the most incident is hypoxia with hypercapnia, because it is associated with in fact all kinds of work in an enclosure, *e.g.* in submarines, in ship bilges, in mines, in space orbital stations, *etc.* [11]. We can speak not just about maintenance of working capacity under conditions of this kind of hypoxia, but largely about saving human life. In these cases, death is a result of respiratory center depression and discontinuation of its activity. Some studies of the bilateral organization of the brain demonstrated functional asymmetry of the respiratory center in frogs, rats, and guinea pigs. The effects of the olfactory lobes, organized asymmetrically, are essential for the respiratory center [12]. It has been demonstrated that dissimilar involvement of dopamine D_1 and D_2 receptors in the triggering function of the respiratory center is the neurochemical mechanism of functional asymmetry of this most important formation of the brain [5]. On the other hand, the role of the brain cortex in the regulation of hypoxic resistance remains little studied.

We studied the effects of inactivation of the left or right brain cortex on the resistance of SHR male mice to acute hypoxia with hypercapnia.

MATERIALS AND METHODS

Experiments were carried out on 100 adult male SHR mice. Hypoxia with hypercapnia was modeled in glass flasks (200 ml) with hermetic lids [13]. After the lids were closed, the flasks were turned upside down and placed into cuvettes with water to rule out air leakage into the flasks. The life-span of animals was recorded. The mice were divided into 4 groups (25 per group): intact animals (group 1), sham-operated animals (active control, group 2), and animals with inactivated left and right hemispheres (group 3 and 4, respectively).

Experiments were carried out in series (16-20 animals per day). All four groups were equally rep-

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resented in each series. Experiments were carried out during the same period from 13.00 to 15.00, because it is known that hypoxic resistance in albino rats and mice varies throughout 24 h. Temporary inactivation of the cortex of one of the hemispheres was induced by epidural application of filter paper impregnated with 25% potassium chloride [1,8] through a 1-mm hole, drilled in ether-narcotized animals above one of the hemispheres 2 days before the experiment. Application was carried out 15 min before testing. The same procedures without trephination and application were carried out in sham-operated mice.

The data were statistically processed using nonparametric Mann–Whitney U test at $p<0.01$ minimal level of significance.

RESULTS

Sham operation (group 2) negligibly prolonged lifespan of mice (by 2.2 min; Table 1). This was presumably due to preconditioning phenomenon receiving great attention in recent studies [14]. In our experiments, the animal was fixed for application of filter paper during several seconds by the left thumb and index finger, which inevitably led to clamping of the carotid arteries and hence, to short-term bilateral cerebral ischemia. This treatment served as preconditioning for the brain, presumably increasing its hypoxic resistance. Inactivation of the left hemisphere (under conditions of isolated functioning of the right hemisphere) led to still greater significant prolongation of the life-span (by 4 min) in comparison with intact animals. However, the increase was negligible (1.8 min) in comparison with sham-operated animals. Similar, but more expressed changes in the hypoxic resistance of mice were observed during inactivation of the right hemisphere (with the left hemisphere remaining active). The difference between experimental and control animals in that case was 5.8 min, between experimental and intact animals 8 min, both differences being significant. The life-span (Table 2) of mice with active left and right hemispheres increased by 10 and 33%, respectively, in comparison with the control and differed more than 3-fold. We think that these data suggest that the right hemisphere is dominant in supporting the initial hypoxic resistance, while the left hemisphere performs a sort of "antihypoxic" function. Traditionally we consider dominant the hemisphere, which is active when the studied parameter is closer to that in intact and/or sham-operated animals [9,10]. In that case it was the right hemisphere. The left hemisphere could be considered performing the antihypoxic function, because during its isolated functioning the life-span of mice increased significantly in comparison

TABLE 1. Effect of Unilateral Cortical Spreading Depression on Survival of Outbred Albino Male Mice under Conditions of Hypoxia with Hypercapnia ($M\pm m$)

Group	Hypoxic resistance, min			
	entire sample (n=25)	low (n=8)	medium (n=9)	high (n=8)
Intact	17.40±4.47	13.70±0.73	16.70±1.12	22.7±3.93
Sham-operated (active control)	19.60±3.65	15.80±1.09	19.10±0.77	24.10±1.87
Active right hemisphere	21.40±4.83*	17.60±1.85*	20.90±1.26*	27.30±3.86*
Active left hemisphere	25.40±6.81*°	19.00±1.21*	24.50±2.47*°+	33.0±6.1*°+

Note. $p<0.01$ compared to: *intact animals, °sham-operated animals; +animals with active right hemisphere.

TABLE 2. Lifespan (%) of Mice under Different Experimental Conditions Exposed to Hypoxia with Hypercapnia ($M\pm m$)

Group	Hypoxic resistance, min			
	all sampling	low	medium	high
Intact	100	100	100	100
Sham-operated (active control)	113.0	115.3	114.4	106.2
Active right hemisphere	123.0	128.5	125.1	120.3
Active left hemisphere	146.0	138.7	146.7	145.4

intact and even (which is the most important) with sham-operated animals.

Hence, we consider that we revealed a species-specific (according to V. L. Bianki) functional interhemispheric asymmetry of the brain in the resistance of albino mice to hypoxia with hypercapnia.

Since the entire sampling was heterogeneous, we additionally analyzed the differences between the hemispheres in mice with different hypoxic resistance. This was an important step, because previous studies had shown that highly resistant and low resistant animals differently reacted to extreme factors of different nature [3]. The data were distributed into 4 parallel series by the life-span, starting from the shortest values, and divided into 3 equal subgroups. The first values for each of the subgroups were regarded as intrinsic of animals with low hypoxic resistance, the medium values of the series were considered characteristic of mice with medium hypoxic resistance, and the last (lowest, the highest) values were considered typical of highly resistant animals. This distribution pattern is often seen in published reports, though other methods of animal distribution by resistance to acute hypoxia are sometimes used [3]. The data on mouse resistance to hypoxia are presented in Tables 1 and 2.

The life-span of intact animals with low resistance was 3.7 min shorter than in the entire sample (Table 1). Sham operation had a certain preconditioning effect similarly as in the entire sampling, prolonging the life-span by 2.1 min in comparison with intact animals. The increment in animals with active right hemisphere was 13.2% in comparison with sham-operated ones and 28.5% in comparison with intact ones; under conditions of active left hemisphere, the corresponding values were 23.4 and 38.7%, respectively. The differences between the "right-hemispheric" and "left-hemispheric" animals were negligible (10.2%; Table 2). We think this fact indicated that the mice with low hypoxic resistance had no interhemispheric asymmetry.

The life-span of mice with medium resistance to hypoxia was virtually the same as in the entire sample in all groups. In animals with active right hemisphere, the differences were significant in comparison with intact, but not sham-operated animals. Animals with active right hemisphere differed significantly from intact, but not sham-operated mice. In animals with active left hemisphere the differences were significant in comparison with all the rest groups. The differences between animals with active left or right hemispheres were significant in comparison with the entire sample (Table 1). It seemed to indicate higher degree of functional interhemispheric asymmetry under conditions of inactivation of one of the hemispheres than in the entire sampling. The differences in the life-span increment in animals with active left and right hemisphere in the group of animals with medium hypoxic resistance reached 21.6% *vs.* 10.2% in animals with low resistance (Table 2).

The life-span was 5-7 min longer in mice highly resistant to hypoxia in all groups in comparison with the entire sampling. Animals with active right hemisphere differed significantly from intact, but not control animals. Animals with active left hemisphere differed significantly from all rest groups, that is, the picture resembled greatly that in mice with medium hypoxic resistance. The difference between the life-spans of mice in groups 3 and 4 reached 25.1% (Table 2).

Hence, increase of hypoxic resistance is associated with greater functional interhemispheric asymmetry of the brain.

Asymmetrical reduction (up to exhaustion) of dopamine and norepinephrine in symmetrical damage of the brain cortex can explain our data. For example, vacuum extraction of the right frontal cortex led to hyperactivity of animals and to reduction of catecholamine concentrations in the cortex of both hemispheres and in the blue spot. A similar operation on the left neocortex caused no effects of this kind. Reduction (asymmetrical) of dopamine level in the cerebral hemispheres explains our results. It was previously shown [6] that stimulation of the respiratory center dopaminergic structures led to its depression. It was logical to hypothesize that reduced dopamine level would lead to an increase of the respiratory center functional potential and hence, to prolongation of animal life-span.

The prospects of further studies consist in use of inbred animals, including the pharmacogenetic analysis, as we have shown previously that inbred animals can have quite different lateralization of the brain for one and the same function [6,7,9,10]; use of female mice, as interhemispheric asymmetry of males and females differ and depends on the estral cycle phase [15]; biochemical analysis of left and right hemispheric structures involved in the respiratory function.

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