Adaptation to Periodic High-Altitude Hypoxia Inhibits Baroreflex Vagal Bradycardia in Rats

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A 3-week course of adaptation to high- altitude hypoxia (4500 m above sea level) inhibited baroreflex vagal bradycardia induced by a rapid rise of systemic blood pressure in conscious rats. Bradycardic responses to electrical stimulation of peripheral end of the right vagus nerve and methacholine (M_2 muscarinic receptor agonist) in hypoxia-adapted rats did not differ from the control. It is concluded that hypoxia inhibits baroreflex vagal bradycardia by acting on a central element of the baroreceptor reflex arch.

Key words: *hypoxia adaptation*; *baroreceptor reflex*; *vagal bradycardia*

Adaptation to hypoxia produces a variety of cardiovascular changes. For instance, cardiac output in rats adapted to hypobaric hypoxia increased [1], probably, due to inhibition of parasympathetic tonic influences. Our findings indicated that blockade of peripheral cholinergic receptors in rats adapted to hypoxia induced a less pronounced rise in heart rate (HR) compared to the control [11]. Moreover, spectral analysis showed a decrease in pulse interval variability within the range of 0.02-0.75 Hz [11]. In rats, these fluctuations in HR are predominantly caused by parasympathetic influences [10]. Our previous study indicated diminished parasympathetic influences on the heart in rats adapted to periodic high-altitude hypoxia.

Arterial baroreflex (BR) is a fundamental mechanism, which regulates parasympathetic outflow to the heart. Rapid rise of systemic blood pressure (BP) is accompanied by a 90% decrease in HR due to activation of the parasympathetic nervous system [3,5]. Little is known about the effect of chronic hypoxia on BR, but the above-mentioned data suggest reduced functional activity of BR in rats adapted to hypoxia. The inhibitory effect of acute hypoxia on BR also confirms this hypothesis. The inhibition of baroreflex vagal bradycardia was observed in anesthetized animals ventilated with a hypoxic mixture [8,13], in humans after 60 min in a high-altitude chamber at 4300 m above sea level [13], and in patients with obstructive sleep apnea syndrome [12,15].

Our aim was to study reflex bradycardia induced by BP rise in rats adapted to periodic high-altitude hypoxia. To clarify the mechanisms underlying this phenomenon we studied the efferent link of BR.

MATERIALS AND METHODS

Experiments were performed on 10-week-old inbred rats weighing 190±15 g. The animals were randomly divided into two groups. Group 1 animals (controls) were at normal atmospheric pressure, while group 2 rats were adapted to 6-h periodic hypoxia in a high-altitude chamber (4500 m above sea level) 6 times per week for 3 weeks. It is well known that periodic hypobaric hypoxia markedly inhibits the development of tissue hypoxia [2] and causes a number of structural and functional changes in the cardiovascular system improving organism's resistance to O_2 deficiency [1]. Therefore, we did not analyze the development of long-term adaptation in our experiments. One day after completion of the adaptation course, the animals were anesthetized with hexenal (120 mg/kg, intraperitoneally). Polyethylene catheters were inserted into the

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TABLE 1. Effects of Adaptation to Periodic Hypoxia (APH) on Arterial Blood Pressure (BP), HR, and Sensitivity of Baroreflex Chronotropic Component (*M±m*, *n*=6-9)

Dose of phenylephrine, μg/kg	ΔBP, mm Hg		ΔHR, beats/min		ΔHR/ΔBP	
	control	APH	control	APH	control	APH
0.5	15.9±4.4	13.8±4.3	-31.90±6.05	-11.70±2.55*	-2.30±0.24	-1.10±0.18*
1	23.70±3.22	26.50±5.33	-42.60±7.76	-21.40±2.82	-1.80±0.32	-0.9±0.2*
1.5	28.00±2.65	31.70±5.98	-54.70±8.67	-28.90±4.26*	-1.90±0.29	-1.10±0.31+

Note: *$p<0.05$, +$p=0.06$ compared to the control.

femoral artery for BP recording and into the femoral vein for drug infusion. After surgery, the rats were housed individually with free access to food and water.

Experiments were carried out on conscious freely moving animals one day after surgery. BP was measured with a Statham 8200 P23AA transducer; the arterial catheter was permanently rinsed with heparinized (50 U/ml) physiological saline at 0.2 ml/h flow rate. The venous catheter was connected to a 50-cm polyethylene tube filled with phenylephrine in 0.9% NaCl. After 60-min adaptation to experimental conditions, baseline BP was recorded during 30 min, and then sensitivity of the chronotropic component of BR was evaluated. To this end, phenylephrine was successively injected in doses of 0.5, 1.0, and 1.5 mg/kg body weight in a random manner with 10-min intervals (volume of injected drug was 5-15 μl).

The efferent link of BR was investigated one day after experiments on conscious animals. The rats were anesthetized with urethane (1.2 g/kg, intraperitoneally) and fixed in the supine position on a thermocontrolled table (37°C). The arterial catheter was connected to BP transducer, and the venous catheter to a polyethylene tube with methacholine in 0.9% NaCl. Methacholine (1, 2, 4, and 8 μg/kg) was bolus-injected in a random manner with 10-15-min intervals, and the chronotropic effect of activation of cardiac M_2 muscarinic receptors was evaluated. Twenty minutes after the last injection, the left and right vagus nerves were isolated, ligated, and cut. Peripheral end of the right vagus nerve was put on silver wire electrodes coated with mineral oil to protect the nerve from drying. Recordings were started 30 min after surgery. The vagus nerve was stimulated for 10-12 sec with trains of pulses (5 V, 0.2 msec) delivered at rates of 1, 2, 4, 8, and 16 Hz with 5-min intervals.

TABLE 2. Decrease in HR (% of Baseline) in Response to Electrical Stimulation of Peripheral End of the Right Vagus Nerve and Methacholine (*M±m*, *n*=7-9)

Experimental series	Control	APH
Electrical stimulation, Hz		
1	6.50±1.91	4.00±1.12
2	16.50±3.74	12.70±4.22
4	28.70±4.11	26.70±5.34
8	51.20±5.91	47.70±8.93
16	68.70±7.44	76.90±4.08
Methacholine, mg/kg		
1	2.40±0.83	2.00±0.35
2	19.70±5.25	25.00±6.99
4	74.80±1.95	72.50±4.31
8	77.20±2.61	75.00±2.89

The signals were recorded and analyzed with a computer using precise analog-to-digital conversion (12 bit) at a 250 Hz scanning rate. Cycle-by-cycle analysis of BP was performed: systolic, diastolic, mean BP, the duration of cardiac cycle, and HR were calculated. Basal BP and HR were averaged for 30-min recording.

The sensitivity of the chronotropic component of BR was calculated as ΔHR/ΔBP, where Δ is the difference between the peak and baseline (10 sec before injection of phenylephrine) values. Chronotropic responses to methacholine and electrical stimulation of the vagus nerve were evaluated by a decrease in HR from the baseline value (10 sec before stimulation).

The data were analyzed statistically using nonparametric Mann—Whitney test.

RESULTS

In conscious rats, adaptation to periodic hypoxia (APH) had no effect on BP (109.20±3.31 *vs.* 104.70± 3.11 mm Hg in the control), but significantly increased HR (456.50±13.63 *vs.* 408.80±9.52 beats/min, $p<0.05$). Moderate tachycardia after APH can be due to inhibition of parasympathetic tonic influences [11].

Similar rise in BP in control and adapted rats in response to the same doses of phenylephrine was accompanied in adapted rats by a less pronounced decrease in HR (Table 1). Consequently, the chronotropic component BR calculated for all three doses of phenylephrine was less sensitive after APH. Since

baroreflex bradycardia is mediated by parasympathetic influences [3,5], this result is consistent with our previous findings on inhibition of vagal influences on the heart after APH [11].

To study the mechanisms of inhibition of parasympathetic baroreflex influences on the heart, we tested the efferent link of BR. The negative chronotropic effects produced by electrical stimulation of the peripheral end of the right vagus nerve and methacholine were similar in control and adapted rats (Table 2). Hence, the inhibitory effect of APH on baroreflex vagal bradycardia is not related to impaired transmission of parasympathetic signals to the sinus node or modulation of cardiac muscarinic cholinergic receptors. These results agree with previous findings that inhalation of a low-oxygen mixture does not alter bradycardic response to electrical stimulation of the vagus nerve [8,13].

Previous studies demonstrated decreased density of muscarinic receptors in the sinus node of hypoxia-adapted guinea pigs, without changes in their affinity to acetylcholine [4]. These findings are at controversy with our results, probably, due to different animal species or different schemes of adaptation used.

Normobaric APH enhances acetylcholine-induced inotropic response of isolated rat heart [7]. This is consistent with previous reports on increased muscarinic receptor density in ventricles after adaptation to hypoxia [6,9] and inhibition of parasympathetic influences. We could not directly compare our results and these data [7], because we studied only the chronotropic effects. However, it is also important to study inotropic effects in rats adapted to periodic hypobaric hypoxia.

Some authors suppose that activation of aortal chemoreflex plays the key role in the inhibition of baroreflex vagal bradycardia during acute hypoxia, and that the interaction between chemoreflex and baroreflex influences occurs at the level the medulla oblongata [8,13]. Similar mechanism probably underlies the effect of chronic hypoxia. At the same time, hypoxia can directly affect brain structures associated with the central link of the baroreflex.

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