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EXPERIMENTAL PAPERS

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# Unilateral Chronic Ischemia of the Carotid Bodies Alters Sympathetic Nervous System Activity

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**Abstract**—As is well known, activation of carotid body chemoreceptors that sense arterial blood oxygen can lead to sympathetic nervous system (SNS) activation, while chronic hypoxia-induced activation of the carotid bodies promotes the development of various cardiovascular diseases. It has been shown in the literature that bilateral activation of the carotid bodies reduces chronotropic baroreflex sensitivity, which is restored following their subsequent bilateral ablation. In this work, we demonstrate that unilateral chronic ischemization of the rat carotid bodies by ligation of the external carotid artery decreases chronotropic baroreflex sensitivity, whereas unilateral ablation of the carotid bodies, on the contrary, increases it. At the systemic level, the reduction in SNS activity is further confirmed by a decrease in the glucose level. Our results show that even unilateral hyperactivation or inactivation of the carotid bodies induce systemic alterations in SNS activity.

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**Keywords:** carotid bodies, sympathetic nervous system, rats, chronotropic baroreflex

## INTRODUCTION

Activation of carotid body chemoreceptors leads to a variety of considerable hemodynamic shifts. Long-term altered conditions of the carotid bodies can entail chronic cardiovascular diseases, e.g., arterial hypertension [1]. We have previously shown that patients with unilateral hemodynamically significant atherosclerotic plaque (obstructing more than 75% of the vascular lumen) in the carotid bifurcation area show the signs of pulmonary hypertension more frequently [2]. Moreover, these patients tend to have increased plasma glucose levels, which may indicate an increased activity of the sympathetic nervous system (SNS) in this condition. The possible causes include

comorbidity of these patients (i.e. the presence of concomitant diseases that may lead to the development of pulmonary hypertension and elevated glucose levels, such as arterial hypertension, diabetes mellitus, chronic heart failure), as well as cerebral ischemia, which may lead to SNS activation, and ischemia of carotid body chemoreceptors. Since it is necessary to differentiate these causes, the appropriate experiments were carried out on rats without comorbidity.

It is well known that the carotid bodies can rapidly alter the state of the SNS. They represent highly sensitive sensors of oxygen plasma levels, being activated when its partial pressure decreases. Since such autonomic reflexes are aimed to compensate the hypoxic state, the activation of the

carotid bodies increases SNS activity: heart rate (HR) and respiratory rate increase. The literature data show a chronic effect of carotid body hyperactivation and inactivation on the state of the SNS. Alzahrani et al. [3] demonstrated the role of chronic carotid body activation in the rat model of sleep apnea. Exogenous paroxysmal hypoxia (8 cycles by 15 s with 5% oxygen in the air, every hour for 8 h a day during three weeks) elicited carotid body activation, which in turn led to adrenaline release from the adrenals. Since the carotid bodies have  $\beta$ -adrenoreceptors, the adrenaline release closed a positive feedback loop: carotid body activation ensued not due to hypoxia, but as a result of SNS activity. Sleep apnea modeling against propranolol (a  $\beta$ -blocker) administration reduced SNS activity and markedly decreased the background firing rate in the isolated carotid bodies.

The effect of carotid body activation was also demonstrated in a study by Del Rio et al. [4] who found that bilateral selective removal of the carotid bodies alleviated the severity of the experimental chronic heart failure, which confirms the important role of the carotid bodies in diseases associated with SNS activation. It is important to note that in all cases, the models of chronic hypoxia with a bilateral chronic carotid body activation were studied.

Sugito et al. [5] assessed the effect of unilateral carotid body hypoxia on the state of pulmonary arteries in an acute experiment on rats. Under conditions of alveolar normoxia, the rat carotid bodies were stimulated unilaterally by injecting sodium cyanide which elicits local tissue hypoxia. This led to a significant transitory increase in pulmonary artery pressure. Meanwhile, the same stimulation of the carotid bodies following unilateral denervation (transection of glossopharyngeal nerve branches) led to no changes in the pulmonary arteries, which proves their reflex nature. However, this experiment only illustrated the acute effects of unilateral carotid body hyperactivation. In the literature, there are no data showing the long-term effect of unilateral carotid body ischemia on the state of the SNS, which makes the stated topic interesting and topical.

The aim of the present work was to evaluate systemic effects—baroreflex sensitivity and plasma glucose level—in rats with chronic hyper-

activation and inactivation of the carotid bodies.

## MATERIALS AND METHODS

### *Animals*

All procedures with the involvement of animals complied with the ethical standards approved by the legal acts of the Russian Federation, principles of the Basel Declaration, and recommendations of the Bioethics Committee at the Lomonosov Moscow State University. The study was carried out on outbred male white rats ( $n = 25$ ) weighing 150–200 g at the time of carotid body surgery and 300–400 g during the in vivo experiment. The animals were kept under vivarium conditions with a regulated 12 h light/12 h dark light regime and ad libitum access to water and standard rodent food. Rats were randomized by weight into the following groups: intact (control) rats (IR,  $n = 9$ ), external carotid artery ligation (ECA,  $n = 8$ ), and carotid body ablation (CB,  $n = 8$ ).

### *Surgery protocol*

Rats were anesthetized with chloral hydrate (400 mg/kg). The drug (50 mg/mL) was injected intraperitoneally (i.p.). An incision was made in the neck at the site of the left common carotid artery (CCA) projection, parallel to the trachea. After the isolation of the common carotid artery, the same was done with the external carotid artery (ECA). Next, the surgery proceeded in different ways, depending on the experimental group:

Variant 1: rats with external carotid artery ligation (ECA group); the ECA was ligated immediately after its departing from the CCA and above the carotid body area; its smaller branches departing between the two ligatures were ligated as well. Thereby, the carotid bodies, while remaining physically intact, got into ischemic conditions.

Variant 2: rats with carotid body ablation (CB group); the ECA was first ligated as in variant 1, including all its branches departing therefrom between the ligatures; then, the ECA segment situated between two ligatures was ablated with vascular scissors.

### *Evaluation of hemodynamic parameters and baroreflex sensitivity*

The baroreflex sensitivity was determined in

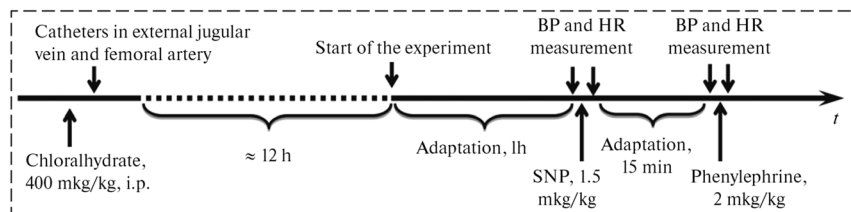


Fig. 1. Experimental protocol of determining baroreflex sensitivity and measuring hemodynamic parameters.

awake rats. One day before the experiment, two catheters were inserted into a rat: a venous to infuse drugs, and an arterial to measure hemodynamic parameters. To do this, the rats were anesthetized with chloral hydrate (400  $\mu\text{g}/\text{kg}$ , i.p.), and the femoral artery, as well as the right external jugular vein, were isolated. The catheter was inserted into the femoral artery in such a way that its tip was in the abdominal part of the aorta. Free ends of both catheters were pulled under the skin, brought out on the withers and fixed thereupon.

During the experiment, the free end of the arterial catheter was connected to a strain gauge pressure transducer (STATHAM, USA), while that of the venous catheter was connected to insulin syringes containing drug solutions. Using an analog-to-digital converter, the signal was recorded on a computer, using a digitization frequency of 100 Hz. Pulse pressure waves were used to automatically calculate HR.

Sodium nitroprusside (SNP), a nitric oxide donor, and  $\alpha 1$ -adrenomimetic phenylephrine were used as agents to evaluate cardiobaroreflex.

To avoid catheter-related arterial and venous thrombosis, 20  $\mu\text{L}$  of sodium heparin solution were injected at a dose of 50 IU/mL (Sintez, Kurgan, Russia, 5000 IU/mL). The commercial agent was diluted with 0.9% NaCl.

After catheterization, the rats were allowed to adapt for a while to experimental conditions. One hour later, prior to drug administration, initial levels of arterial blood pressure (BP) and HR were recorded. After that, a vasodilator SNP was infused (1.5  $\mu\text{g}/\text{mL}$  in 0.9% NaCl solution, 50  $\mu\text{L}$ ), and the values of a maximal decrease in BP and a maximal increase of HR due to activation of the sympathetic part of the baroreflex were recorded. Following SNP infusion, the initial values of BP and HR were allowed to be restored. Fifteen min after SNP infusion, BP and HR were

recorded again. Next, phenylephrine was infused (2  $\mu\text{g}/\text{mL}$  in 0.9% NaCl solution, 50  $\mu\text{L}$ ), and an increase in BP in response to its effect, as well as a reflex decrease in HR due to parasympathetic nervous system (PNS) activation was recorded. The experimental protocol is shown in Fig. 1. The baroreflex sensitivity was calculated as the ratio of the differences in mean BP and HR before and after drug infusion ( $S = |\Delta\text{HR}|/|\Delta\text{BP}_m|$ ).

#### Measuring plasma glucose levels

To measure glucose levels, blood was sampled a day before catheter placement surgery. Prior to sampling, the rats were deprived of food for 6 day-time hours, i.e. when the animals were least active.

Plasma glucose levels in rats were measured using a glucometer OneTouch Select Plus (Lifescan Europe, Switzerland). The tip of the awake rat's tail was incised with scissors to collect blood from the caudal artery bed. A drop of blood was applied onto a glucometer sensor.

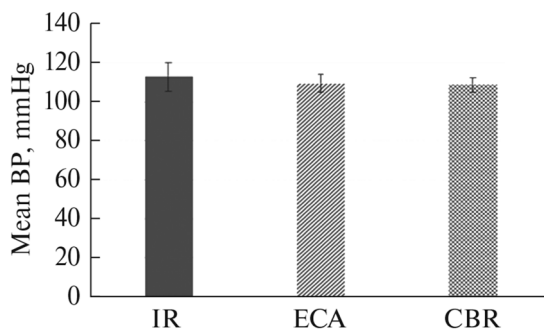
#### Statistical data analysis

Statistical data processing was carried out using STATISTICA 10 (Stat Soft Inc.). The data were presented as medians and quartiles (upper and lower), because the distribution in all groups was not normal. The Kolmogorov–Smirnov test was used to check distributions for normality. For statistical analysis, the Mann–Whitney test was applied to compare independent groups. Differences were considered significant at  $p < 0.05$ .

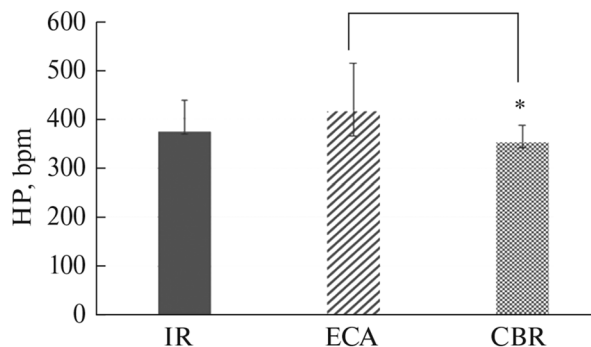
## RESULTS

#### Measuring hemodynamic parameters

To assess systemic manifestations of unilateral ischemization and ablation of the carotid bodies, mean BP and HR values, as well as baroreflex



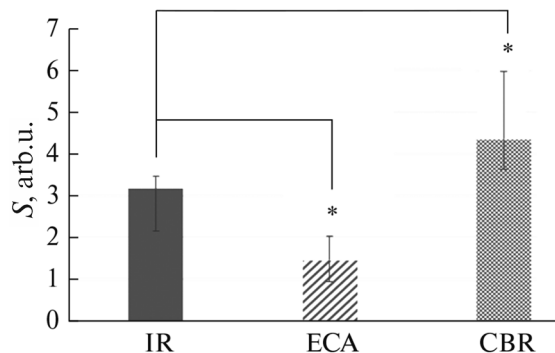
**Fig. 2.** Mean arterial pressure in awake rats at rest: intact rats (IR), rats with the ligated external carotid artery (ECA), and rats with ablated carotid bodies (CB).



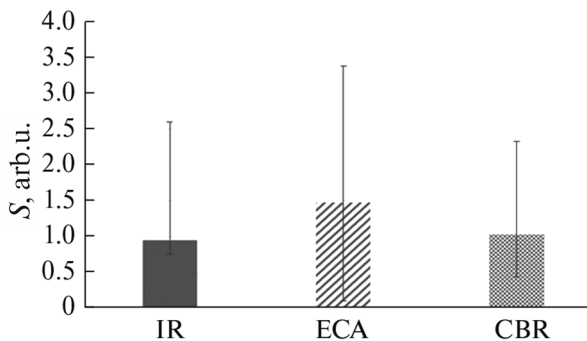
**Fig. 3.** Heart rate in intact rats (IR), rats with the ligated external carotid artery (ECA), and rats with ablated carotid bodies (CB) at rest. \*  $p < 0.05$  vs ECA rat group.

indices, were measured. The median BP in intact rats was 113.2 mm Hg (lower quartile—105.6, upper quartile—120.2), in operated rats with ligated ECA—109.4 mm Hg (lower quartile—105.1, upper quartile—114.4), in rats with ablated carotid bodies—109.1 mm Hg (lower quartile—104.9, upper quartile—112.2). No significant differences were found between these groups, although some tendency toward a decrease in mean BP ( $BP_m$ ) was traced in both groups of operated rats (Fig. 2).

Median HR in intact rats was 374.0/min (lower quartile—370.0/min, upper quartile—435.0/min), in rats with the ligated ECA—416.0/min (lower quartile—365.5/min, upper quartile—464.4/min), in rats with ablated carotid bodies—351.8/min (lower quartile—341.5/min, upper quartile—377.6/min). There were also no significant differences in HR between intact and operated rat groups, although there was some tendency toward



**Fig. 4.** Baroreflex sensitivity ( $S$ ) to sodium nitroprusside administration in intact rats (IR), rats with the ligated external carotid artery (ECA), and rats with ablated carotid bodies (CB). \*  $p < 0.05$  ECA and CBR vs IR.



**Fig. 5.** Baroreflex sensitivity ( $S$ ) to phenylephrine administration in intact rats (IR), rats with the ligated external carotid artery (ECA), and rats after ablated carotid bodies (CB).

an increase in HR in rats with ligated ECA and a decrease in HR in the group with ablated carotid bodies. Therefore, the difference between the groups with ligated ECA vs. ablated carotid bodies was statistically significant (Fig. 3).

Thus, the main hemodynamic parameters of the systemic circulation in the operated rats remained practically intact compared to the control.

After SNP administration, mean BP in the intact rat group dropped by an average of 13.45%, while HR increased by 12.40%. The median baroreflex sensitivity ( $S$ ) to SNP in this group was 3.16 (lower quartile—2.15; upper quartile—3.47). In the group with the ligated ECA, BP dropped by an average of 21.45%, while HR increased by 9.61%. In this case, the median baroreflex sensitivity was 1.45 (lower quartile—0.94; upper quartile—2.03). The difference in baroreflex sensitivity to SNP between these two groups was significant ( $p <$

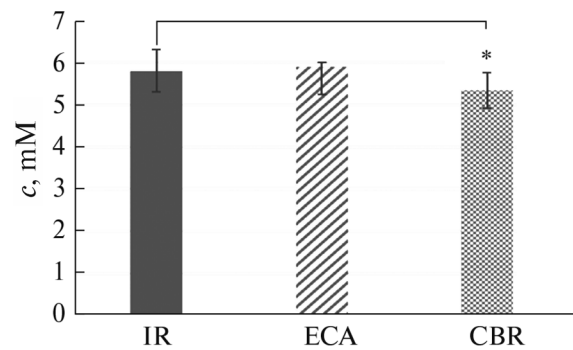
0.05), i.e., the response (as a change in HR) to a drop in BP was more pronounced in the intact group than in that with the ligated ECA. In the group with ablated carotid bodies, mean BP dropped by an average of 14.14%, while HR rose by an average of 19.20%. The median baroreflex sensitivity in this group was 1.03 (lower quartile—0.44; upper quartile—2.32). The difference in baroreflex sensitivity between this group and the intact control group was also significant ( $p < 0.05$ ), i.e., the response (as a change in HR) to a drop in BP was less pronounced in the intact group compared that with ablated carotid bodies (Fig. 4).

After phenylephrine administration, mean BP in the intact rat group increased by an average of 19.41%, while HR dropped by 7.20%. The median baroreflex sensitivity to phenylephrine in this group was 0.95 (lower quartile—0.75; upper quartile—2.60). In the group with the ligated ECA, BP increased on average by 13.36% and HR dropped by 4.77%. The median baroreflex sensitivity in this group was 1.48 (lower quartile—0.10; upper quartile—3.38). In the group with ablated carotid bodies, mean BP after phenylephrine administration increased on average by 32.85%, while HR decreased by 13.07%. The median baroreflex sensitivity of phenylephrine in this group was 1.03 (lower quartile—0.44; upper quartile—2.32). The difference in baroreflex sensitivity to phenylephrine between operated and control groups was nonsignificant, i.e., a change in HR due to pressure rise was almost the same in the intact group and those with ablated carotid bodies or their ischemization (Fig. 5).

Thus, changes in chronotropic baroreflex sensitivity were only found when HR increased in response to an imposed decrease in BP.

#### *Measuring plasma glucose levels*

Another systemic effect associated with a unilateral change in carotid body activity was revealed when measuring glucose levels. The median fasting plasma glucose level in intact rats was 5.8 mmol/L (lower quartile—5.3 mmol/L, upper quartile—6.3 mmol/L), in rats with the ligated ECA—5.9 mmol/L (lower quartile—5.3 mmol/L, upper quartile—6.0 mmol/L), and in rats with ablated carotid bodies—5.4 mmol/L



**Fig. 6.** Fasting plasma glucose levels (molar concentration,  $c$ ) in intact rats (IR), rats with the ligated external carotid artery (ECA), and rats with ablated carotid bodies (CB). \*  $p < 0.05$  in CB vs IR.

(lower quartile—4.9 mmol/L, upper quartile—5.8 mmol/L). The results of measurements in the intact control group and that with the ligated ECA did not differ, but statistically significant differences were found between the intact control group and that with ablated carotid bodies: the plasma glucose level in the group with ablated carotid bodies was significantly lower than in the intact control group (see Fig. 6).

## DISCUSSION

According to our data [6], unilateral ischemization of the rat carotid bodies may be an independent factor in the development of pulmonary hypertension, which raises the question of what mechanisms underlie this process. It is possible that unilateral ischemization of the carotid bodies entails SNS activation, which in turn affects the state pulmonary arteries. It is extremely important to emphasize that it is only a unilateral exposure that is concerned. In other words, the effects triggered unilaterally with the contralateral carotid bifurcation remaining intact suffice for the implementation of the process. In the present work, we focused on seeking other possible effects of SNS activation during a unilateral impact on the carotid bodies.

In vivo experiments on awake rats aimed at baroreflex assessment showed that, in response to SNP administration, the SNS is activated significantly less in the group with the ligated ECA than in the control, as reflected in the lower sensitivity of the baroreflex sympathetic component in oper-

ated rats. At the same time, the parasympathetic component persists at the level of intact control, since no changes in the response of HR to a phenylephrine-induced increase in BP were revealed, i.e., in the case of chronic ischemization of the carotid bodies, there is a suppression of SNS effects in the systemic circulation. Perhaps, the observed systemic effect relates to secondary changes caused by chronic SNS activation. It is possible, at the level of the systemic circulation, the intensity of inhibition of the SNS effects increases, e.g., due to presynaptic  $\alpha_2$ -adrenoreceptors of postganglionic neurons. In our experiments, we disregarded the possibility of cerebral ischemization effects or reduced excitation of carotid sinus mechanoreceptors, since the blood flow in the internal carotid artery remained intact.

Our results on changes in baroreflex sensitivity are consistent with those obtained by del Rio et al. [1]. This group of authors showed that frequent hypoxic episodes over a month when modeling sleep apnea lead to a decrease in chronotropic baroreflex sensitivity in response to SNP administration. At the same time, subsequent ablation of the carotid bodies restored baroreflex sensitivity. It is very important that, as in our experiments, it is the chronic activation of the carotid bodies that is under discussion. However, in the above experiments, it is the carotid bodies located in the bifurcation area of both carotid arteries that were exposed to hypoxia and ablation. In our study, however, we dealt with unilateral manipulations with the carotid bodies. Based on the data by del Rio et al. [1], we assume that in our case (during unilateral ischemization of the carotid bodies), the systemic hemodynamic effects (changes in baroreflex sensitivity) also result exactly from carotid body activation against the background of severe hypoxia, which is a direct consequence of ECA ligation, but not from their possible subsequent inactivation due to an extremely limited blood supply.

The reduction in the manifestation of SNS effects does not seem to be reflected in the formation of pulmonary arterial hypertension, since the pathological feedback can be closed already at the level of pulmonary arteries. It is assumed that the initial SNS activation leads to an increase in basal tone of pulmonary arteries and initiates their remodeling. This effect can be implemented via

different types of adrenoreceptors. In the case of  $\alpha_1$ -adrenoreceptor activation, intracellular calcium levels increase and calcineurin is activated. The latter targets the nuclear factor of activated T-cells (NFAT), mediating a decrease in the expression of potassium voltage-gated channels in the smooth muscle cell sarcolemma. This also decreases membrane repolarization, resulting in an increase in intracellular calcium levels, closing thereby a positive feedback loop. The mitogenic effect of calcium ions enhances remodeling of pulmonary arteries [7]. Another factor that may influence the formation of pulmonary hypertension is the hypoxia-induced factor (HIF), which is phosphorylated by protein kinase A whose activity increases upon activation of  $\beta$ -adrenoreceptors. The HIF's mechanism of action in this case is similar to that of the nuclear factor of activated T-cells [8, 9].

In the rat group with ablated carotid bodies, the opposite effect is observed. The sensitivity of the parasympathetic baroreflex component again remains intact compared to the intact control, but the sensitivity of the sympathetic component turns out to be significantly higher than in the intact control group and much higher than in the group with ECA ligation, which suggests that in the chronic experiment, there is a higher SNS sensitivity to changes in BP in the systemic circulation than in the norm. Thus, the effect of unilateral carotid body ablation is also related to the SNS, but is opposite to the effect of carotid body ischemization in the bifurcation area of one of the carotid arteries.

Systemic shifts in SNS activity are also noticeable when assessing plasma glucose levels. It should be noted that even this index, which is extremely dependent on various parameters, turned out to be significantly reduced in the group with ablated carotid bodies. Overall, the evaluated systemic effects of neural and humoral nature indicate a decrease in SNS activity after an unilateral ablation of the carotid bodies (although, at the same time, the sensitivity of the effector component, the heart, to the action of mediators of sympathetic nerve endings increases). On the other hand, we observed some tendency toward an increase in the plasma glucose level in the group with ischemized carotid bodies, and this coincides with our initial assumption on an

increase in SNS activity in this group based on the results of measuring hemodynamic parameters. An increased SNS activity in the group with ischemized carotid bodies is further indicated by a higher basal level of HR compared to the group with ablated carotid bodies). The results obtained in the rat group with ischemized carotid bodies are consistent with those obtained in patients: if an atherosclerotic plaque is present in the CCA bifurcation area, there is a tendency toward an increase in plasma glucose levels compared to those who lack this plaque [2].

#### AUTHORS' CONTRIBUTION

The basic idea and experimental design (M.P.D., M.A.M.); data collection (M.A.M.); data processing (M.P.D.); writing and editing a manuscript (M.A.M., M.P.D.).

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#### CONFLICT OF INTEREST

The authors declare that they have neither evident nor potential conflict of interest associated with the publication of this article.

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