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Regulation of Contractile Responses of Vascular Smooth Muscle Cells under Conditions of Hypoxia—Reoxygenation

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We analyzed the effects of hypoxia and reoxygenation on changes in contractile activity in rat aortic smooth muscles. Both hypoxia and reoxygenation induced relaxation of smooth muscle cells precontracted with high-potassium Krebs solution (30 mM KCl) or α_1-adrenoceptor agonist phenylephrine. Vasodilation resulted from enhancement of potassium permeability of smooth muscle cell membranes caused by activation of voltage-gated potassium channels (triggered by both precontracting agents) or by opening of ATP-sensitive potassium channels (phenylephrine). In isolated smooth muscle cells, both hypoxia and inhibition of Na^+,K^+-ATPase with ouabain led to depletion of intracellular store of macroergic substances, reduced potassium concentration, and elevated the content of sodium ions.

Key Words: *smooth muscle cells; hypoxia; reoxygenation, Na^+,K^+-ATPase*; *rat aorta*

Disturbances in oxygen homeostasis affect numerous physiological functions [2,3] including the contractile activity of smooth muscles forming the walls of blood vessels [5,10]. There are data that vascular smooth muscle cells (VSMC) work as myogenic sensors of low oxygen tension [6,12]. It has been demonstrated that hypoxia induces relaxation of VSMC and decreases the force of their contraction [12,14] due to activation of ATP-sensitive potassium channels (K_{ATP} channels) in the plasma-membrane against the background of deficiency of macroergic substances [9,11]. However, there are virtually no published data on the effect of reoxygenation on contractile responses of VSMC.

Our aim was to examine the effect of hypoxia and reoxygenation on the mechanisms of regulation of contractile activity of vascular smooth muscles.

MATERIALS AND METHODS

The experiments were carried out on denuded segments of the thoracic aorta from male Wistar rats. The animals were sacrificed by cervical dislocation under deep intraperitoneal narcosis (Nembutal, 70 mg/kg). The force of isometric contraction (FC) of aortic ring was recorded in isometric mode with a Myobath II mechanographic system. The smooth muscle preparation was mounted in aerated chambers filled with Krebs solution (in mM): 120.4 NaCl, 5.9 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 5.5 glucose, and 15 $NH_2C(CH_2OH)_3$. pH was adjusted to 7.35-7.40 at 37°C. After a 40-50-min incubation in Krebs solution, contractions of aortic rings were evoked with a high-potassium Krebs solution, in which 30 mM Na was equimolarly replaced by KCl. In some experiments, the contractions were triggered by 1 μM phenylephrine (PHE). The amplitude of FC evoked by any of these stimulants was taken for the control values (100%). To examine VSMC contractile responses under hypoxia, the rings were placed in hypo-oxygenated Krebs solution containing 10.0±0.2 %vol. O_2. Oxygen level was controlled with an HI 9146-04 dissolved oxygen meter (HANNA). Reoxygenation was modeled by replacement of hypo-oxygenated solution for the physiological one with normal oxygen concentration.

Measurements were performed in VSMC culture derived from rat aortic cells (Lonza) grown in

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DMEM medium within specialized incubator (37°C, 5% CO_2). Depending on experimental approach, the cells were cultured in control DMEM medium ($[Na^+]_o/[K^+]_o$=140.1/5.4 mM), DMEM with 3 mM ouabain, and potassium-free Sp-DMEM medium. Hypoxia was modeled by changing the incubator atmosphere from 5% CO_2/air to 5% CO_2/N_2. The intracellular potassium $[K^+]_i$ and sodium $[Na^+]_i$ ion concentrations were calculated from the stationary cell/medium distributions of ^{86}Rb and ^{22}Na, correspondingly [7]. To this end, the radioactivity levels of culture medium and cell lysate were assessed with the help of a liquid scintillation analyzer. The total cation content (V, nM/mg over 10 min) was calculated as V=A/a×m, where *A* stands for sample radioactivity (disintegration/min), *a* is the total radioactivity of isotopes in medium (disintegration/min/nM), and *m* is protein content in the lysates (mg). Intracellular $[ATP]_i$ was assessed by luciferase-dependent luminescence according to Sigma's protocol guide.

The data were analyzed statistically using SPSS Statistics 17.0.1 software, Mann—Whitney *U* test for two independent samples, and Wilcoxon's *T* test for dependent samples. The data are summarized as median (Me) and interquartile range (Q_1-Q_3).

RESULTS

Incubation of vascular preparations in hypo-oxygenated Krebs solution for 60 min produced no effect on their basal tone. However, this incubation decreased the amplitudes of VSMC contractile responses induced by high potassium solution or PHE by 13% (n=11, p<0.05) and by 21% (n=11, p<0.05), correspondingly, relatively to the control values obtained under normoxic conditions. A 15-min reoxygenation produced no effect on initial FC of aortic rings. During reoxygenation, the amplitude of potassium- and PHE-induced contractile responses significantly decreased relatively to the control normoxic values by 20% (n=11, p<0.05) and by 30% (n=11, p<0.05), correspondingly. Thus, both hypoxia and reoxygenation inhibited the contractile responses of aortic rings evoked by K^+ or PHE. It is noteworthy that α_1-adrenoceptor agonist PHE produced the greatest inhibitory effect. Probably, the difference in FC caused by K^+ and PHE relates to various mechanisms of induced contractures. In contrast to potassium trigger employing passive ionic transport, PHE activates the receptor-operated entry of Ca^{2+} ions, which mobilizes the intracellular signaling cascades mediated by membrane phosphoinositides and protein kinase C [1,12].

Down-regulation of contractile function of vascular smooth muscles during hypoxia and reoxygenation can be caused by the changes in membrane permeability of VSMCs. Actually, the disturbances of oxygen homeostasis in VSMCs inhibit Ca^{2+} channels, which regulate the entry of calcium ions into cytosol and therefore control the muscle contraction [4,5,12]. In addition, potassium permeability of VSMCs can contribute into the development of vasodilation [8,9,13].

Application of 1 mM 4-aminopyridine (4-AP), a blocker of voltage-gated K^+ ionic channels, during potassium- or PHE-induced contraction of aortic ring under normoxia resulted in a significant increment to the developed FC (p<0.05, Table 1). Similarly, 4-AP significantly augmented FC during hypoxia and reoxygenation (Table 1). Under normoxia, glibenclamide (10 μM), an ATP-sensitive potassium channel (K^+_{ATP}) blocker, produced no significant changes in FC of aortic rings precontracted with high potassium solution or PHE (p>0.05). In contrast, the same concentration of glibenclamide significantly increased FC of PHE-precontracted aortic rings under hypoxia or reoxygenation (relatively to the control contraction) to 114 (111-116)% (n=8, p<0.05) or to 113 (109-115)% (n=8, p<0.05), correspondingly. When applied to potassium-precontracted aortic rings, glibenclamide produced no significant increase in FC.

TABLE 1. Combined Effects of Hypoxia and Reoxygenation on Contraction of Smooth Muscle of Rat Aorta under conditions of Ionic Channel Blockade with 4-AP (Me, Q_1-Q_3)

Group	Amplitude of FC, %			
	KCl (30 mM) (n=11)	+4-AP (1 mM) (n=9)	PHE (1 μM) (n=11)	+4-AP (1 mM) (n=9)
Normoxia (control)	100	107.76+ (104.59-110.72)	100	117.64+ (115.86-118.96)
Hypoxia	87.18* (85.74-89.63)	115.05*+ (112.29-118.41)	78.61* (76.64-80.48)	125.44*+ (121.89-127.82)
Reoxygenation	80.48* (76.91-86.32)	114.41*+ (111.96-119.6)	70.13* (69.4-71.55)	122.88*+ (119.14-125.14)

Note. p<0.05 in comparison with *normoxia and +contraction in 4-AP-free solution.

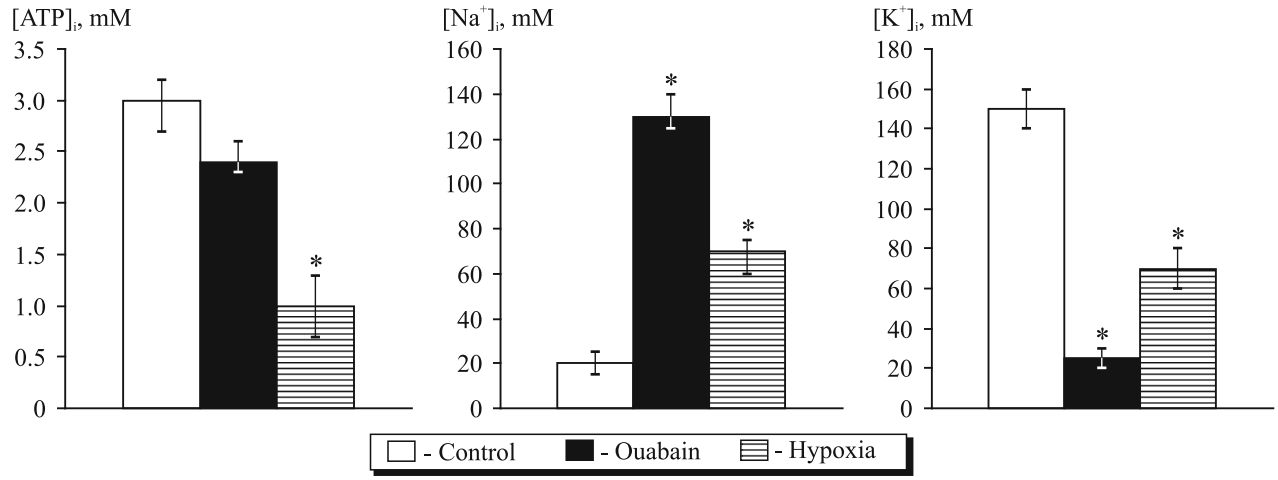

Fig. 1. Effect of hypoxia and ouabain on intracellular ATP, Na^+, and K^+ in rat aortic VSMC. The cells were incubated for 24 h under control normoxia or experimental hypoxia with and without 3 mM ouabain. *p<0.05 in comparison with the control.

The present findings conclude that during hypoxia and reoxygenation, regulation of VSMC contractile activity is mediated via the voltage-dependent component of potassium permeability in plasmalemma of these cells. The role of K^+_{ATP} channels, which are considered by many researchers as the leading actors in disturbances of oxygen homeostasis, is manifested only during stimulation of vascular rings with PHE.

The use of ouabain or incubation of rat aortic VSMCs in potassium-free medium can reproduce the effects of hypoxia on the ion transport systems such as Na^+,K^+-ATPase [7]. Specifically, a 6-h inhibition of VSMCs with ouabain significantly increased $[Na^+]_i$ from the control level of 15-20 to 130 mM and decreased $[K^+]_i$ from the control value of 150 to 25 mM. Similar increase in $[Na^+]_i/[K^+]_i$ ratio was revealed in experiments with a 6-h inhibition of Na^+,K^+-ATPase in potassium-free solution.

In our study, a 24-h incubation of VSMCs under hypoxia decreased $[ATP]_i$ by 3 times in comparison with the control level, while ouabain decreased $[ATP]_i$ by <20% (Fig. 1). Hypoxia increased $[Na^+]_i$ 3-fold and decreased $[K^+]_i$ by 2 times relatively to the control levels.

The present findings showed that under oxygen deficiency, the reduced store of intracellular macroergs is possibly accompanied not only with inhibition of Na^+,K^+-ATPase, but also with down-regulation of other ATP-dependent processes that underlie the contraction-relaxation cycle of smooth muscle. Probably, the concurrent disbalance of intracellular monovalent cations indirectly affects the performance of ion transport systems in VSMCs.

Thus, examination of the mechanism regulating the contractile activity of VSMCs during disturbances of oxygen homeostasis is especially important, because VSMCs play the leading role in maintaining the tone of the blood vessels [5]. This study showed that hypoxia- and reoxygenation-triggered effects are caused not only by alterations of calcium permeability of VSMC membrane activated with precontracting agents of diverse nature, but also by modulation of activity of potassium channels and the systems responsible for balance of monovalent ions.

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