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Adaptation to Short-Term Stress Exposures Prevents Poststress Dysfunction of Calcium-Activated Potassium Channels in Coronary Vessels

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Adaptation to short-term stress exposures prevents immobilization stress-induced decrease in functional activity of BK_{Ca} channels in smooth muscle cells of coronary vessels developing against the background of NO overproduction and increase in the relative content of oxidized glutathione (change in redox state of the glutathione system). The protective antistress mechanisms of adaptation probably include prevention of NO overproduction by inducible NO synthase and maintenance of dependent glutathione redox state functional activity of BK_{Ca} channels in smooth muscle cells of coronary vessels.

Key Words: *endothelium; nitric oxide; BK_{Ca} channels; stress; adaptation*

The myogenic tone of small coronary vessels provided by smooth muscle cells under stretching due to intravascular pressure is essential for autoregulation of coronary blood flow and vasodilation under the effect of vasodilating agents. The level of myogenic tone and, therefore, the diameter of vessels can vary significantly at the same blood pressure under different physiological conditions. This situation occurs due to changes in the expression or functional activity of large-conductance calcium-activated potassium channels (BK_{Ca} channels) in smooth muscle cells that play a key role in the regulation of pressure-related myogenic tone [9].

Adaptation of animals by short-term stress exposures has a strong effect on the production of nitric oxide (NO), main stage in the pathogenesis of poststress hypotonia of coronary vessels [2,3], abolishes the decrease in myocardial contractility under stress conditions [1,4], attenuates the stress-induced activation of LPO [5], and prevents dysfunction of Ca^{2+} pumps [4] and K_{ATP} channels [1]. Functional activity of BK_{Ca} channels increases under the influence of cyclic nucleotides (cGMP and cAMP) and depends on the oxidized/reduced glutathione ratio (redox state of cells). These data suggest that BK_{Ca} channels are involved in the effect of NO and redox regulation of smooth muscle cell function in coronary vessels. However, the effect of NO formation during adaptation to short-term stress exposures on functional activity of BK_{Ca} channels in smooth muscle cells of coronary vessels remains unknown.

This work was designed to study functional activity of BK_{Ca} channels in smooth muscle cells of the coronary vessels during long-term immobilization and adaptation to short-term stress exposures. The role of NO in this process was evaluated.

MATERIALS AND METHODS

Coronary flow velocity (CFV) and myocardial contractility were studied on the isolated hearts from fe-

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male rats under constant-pressure perfusion. A constant-volume latex balloon was inserted into the left ventricle of the heart. During stage 1, the heart was perfused with Krebs–Henseleit solution. During stage 2, the heart was perfused with Krebs–Henseleit solution and 1 mM tetraethylammonium (TEA). The concentration of TEA was selected as a selectively blocking dose for BK_{Ca} channels. Perfusion pressure was gradually increased from 40 to 120 mm Hg with an increment of 20 mm Hg (coronary autoregulation). Functional activity of calcium channels was estimated as the percentage of variations in CFV after addition of TEA to the perfusate.

The rats were divided into the following four groups: group 1, control (intact animals, n=8); group 2, animals after 6-h immobilization (n=7); group 3, adapted animals (n=8); and group 4, 6-h immobilization of animals after preadaptation to short-term stress exposures (n=8). Saponin (44 μg/ml coronary blood flow over 1 min; Merk) was used to induce damage to coronary vascular endothelium [10]. Constitutive NO synthase blocker N^{ω}-nitro-L-arginine methyl ester (L-NAME, 60 mM) was used to evaluate the role of NO in the effect of TEA. Inducible NO synthase was suppressed by a selective blocker *S*-methylisothiourea (S-MT) [8]. Stable products of NO degradation were assayed in rat blood plasma by the Griess method. The content of reduced and oxidized glutathione in erythrocytes was measured by HPLC [11]. Glutathione concentration was expressed in μg/ml blood (with correction for hematocrit).

The results were analyzed statistically (Microsoft Excel 2000, Statistica 6.0). Two quantitative values were compared by Student's t test. Data processing involved comparison of two independent samples (control/stress, adaptation/stress, *etc*.) and pairwise comparison test (control/control+TEA *etc*.). The data are expressed as the arithmetic mean and error of the mean. The differences were significant at $p<0.05$.

RESULTS

CFV increased by 26% ($p<0.05$ compared to the control; Fig. 1, *a*), while the autoregulation index decreased by 29% after 6-h immobilization (Table 1). The developed intraventricular pressure under these conditions was 29% lower than in the control. These changes were followed by a decrease in the efficiency of coronary blood flow, which reflects the development of myocardial hyperperfusion due to poststress hypotonia of coronary vessels [6,7]. Adaptation by short-term stress exposures was shown to abolish poststress changes in coronary blood flow and myocardial contractility (Fig. 1, *a*). Autoregulation of coronary blood flow and developed intraventricular pressure in animals of the adaptation group and adaptation+stress group did not differ from the control (Table 1).

The study of the isolated hearts from control animals showed that blockade of BK_{Ca} channels with TEA is followed by an 18% decrease in CBF at the perfusion pressure of 40-120 mm Hg (Fig. 2). The index of autoregulation was elevated by 42% (Table 1). A significant decrease of coronary blood flow was observed only in the range of autoregulation (aortic pressure 80-120 mm Hg). In the group of immobilized animals, TEA caused a less significant decrease in CFV (by 9% at the perfusion pressure of 100 or 120 mmH, $p<0.05$; Fig. 2) and increase in the autoregulation index (by 31%, $p<0.05$ compared to the control). The decrease in CFV and increase in the autoregulation index in adapted specimens after treatment with TEA did not differ from the control (by 22 and 42%, respectively). Administration of TEA to the coronary vessels of the isolated hearts from animals of the adaptation+stress group was followed by a more significant decrease in CFV (as compared to the control, by 26%; Fig. 2) and increase in the autoregulation index (by 49%; Table 1). Only in stressed rats, the developed intraventricular pressure was low before and after addition of TEA. Contractile function did not change in animals of other groups.

Saponin-induced deendothelialization was followed by a decrease in CFV by 29% ($p<0.05$ compared to intact rats; Fig. 1, *b*). Significant differences were revealed between these samples and coronary vessels with normal endothelium ($p<0.05$). The autoregulation index was reduced by 35% (Table 1). Myocardial contractility did not change under these conditions. Poststress changes in CFV (Fig. 1, *b*) and autoregulation index were not found after endothelium removal from the heart of stressed animals. The test parameters were comparable with those observed after endothelial injury in the coronary vessels of control heart samples. Adaptation did modify the effect of saponin on autoregulation of coronary blood flow and myocardial contractility before or after stress exposure (Fig. 1, *b*).

After saponin-induced endothelial injury in the hearts of animals from various groups, TEA had the most significant effect on CFV. The degree of TEA-induced increase in coronary vascular tone in the hearts was shown to increase significantly in animals exposed to stress before and after adaptation (by 10-25%). Between-group differences in blood flow were not observed under these conditions.

Administration of L-NAME into the coronary vessels was accompanied by a significant decrease in CFV in animals of various groups. Perfusion of the isolated heart from stressed animals with an L-NAME-containing solution abolished the stress-induced increase in CFV, which did not differ from that in the heart of rats

TABLE 1. Effect of Treatment with TEA Alone or in Combination with Saponin, L-NAME, and S-MT on the Autoregulation Index in Adapted Rats before and after Stress (*M*±*m*)

Group of animals	Addition of substances to the perfusate	Perfusion pressure, mm Hg				
		40	60	80	100	120
Control (*n*=8)	without TEA	–	0.40±0.08	0.51±0.08	0.71±0.05	0.72±0.07
	with TEA	–	0.51±1.00	0.84±0.05*+	0.91±0.10+	0.92±0.05*+
Stress, 6 h (*n*=7)	without TEA	–	0.27±0.03*	0.46±0.1	0.43±0.07*	0.80±0.10
	with TEA	–	0.21±0.05*	0.46±0.10	0.79±0.10	0.60±0.10*
Adaptation (*n*=8)	without TEA	–	0.31±0.08	0.42±0.07	0.61±0.08	0.88±0.10
	with TEA	–	0.30±0.06	0.61±0.10+	0.74±0.10+	0.88±0.10
Adaptation+stress (*n*=8)	without TEA	–	0.41±0.08	0.44±0.05	0.61±0.07*	0.81±0.10
	with TEA	–	0.40±0.06	0.41±0.10	0.91±0.05+	0.93±0.10*
L-NAME (*n*=8)	L-NAME	–	0.40±0.09	0.71±0.10*	0.71±0.07	0.87±0.07
	L-NAME+TEA	–	0.6±0.1*+	0.90±0.03*+	0.84±0.07*+	0.97±0.02*+
Stress (6 h)+L-NAME (*n*=8)	L-NAME	–	0.27±0.08*	0.53±0.10	0.41±0.10*	0.91±0.10
	L-NAME+TEA	–	0.40±0.07+	0.50±0.07	0.50±0.04*	0.90±0.07*
Adaptation+L-NAME (*n*=8)	without TEA	–	0.31±0.04*	0.40±0.06*	0.71±0.08	0.81±0.06
	with TEA	–	0.41±0.10	0.70±0.07*+	0.63±0.06+	0.87±0.08
Adaptation+stress+L-NAME (*n*=8)	without TEA	–	0.41±0.06	0.45±0.08	0.51±0.08*	0.86±0.05
	with TEA	–	0.37±0.10	0.70±0.07*+	0.80±0.07+	0.81±0.07
Control+saponin (*n*=8)	without TEA	–	0.20±0.06*	0.21±0.05*	0.52±0.10*	0.76±0.10
	with TEA	–	0.61±0.10*+	0.30±0.05*	1.0±0.2*+	1.0±0.1*+
Stress (6 h)+saponin (*n*=8)	without TEA	–	0.32±0.05	0.33±0.09*	0.58±0.10*	0.67±0.10
	with TEA	–	0.30±0.09	0.4±0.1+	1.0±0.2*+	0.6±0.1
Adaptation+saponin (*n*=8)	without TEA	–	0.32±0.02	0.21±0.10*	0.53±0.10*	1.0±0.5*
	with TEA	–	0.62±0.10*+	0.22±0.10*	0.92±0.10*+	1.0±0.3*
Adaptation+stress+saponin (*n*=8)	without TEA	–	0.52±0.04	0.51±0.10	0.70±0.07	0.93±0.04*
	with TEA	–	0.86±0.09*+	0.61±0.08	1.0±0.2*+	1.0±0*+
S-MT (*n*=6)	S-MT	–	0.41±0.03	0.43±0.05	0.72±0.05	0.71±0.04
	S-MT+TEA	–	0.41±0.02	0.51±0.01	0.91±0.06*	0.82±0.06
Stress (6 h)+S-MT (*n*=6)	S-MT	–	0.51±0.06*	0.50±0.06	0.71±0.11	0.71±0.03
	S-MT+TEA	–	0.61±0.09*	0.51±0.10	0.91±0.08*	0.80±0.10

Note. $p<0.05$: *compared to the control (without TEA); +compared to the corresponding group without TEA. The autoregulation index was calculated as follows: $AI=\Delta Q_1-\Delta Q_2/\Delta Q_1$, where ΔQ_1 is the change in the initial coronary blood flow during elevation of perfusion pressure; and ΔQ_2 is the difference between the initial blood flow and level of blood flow during the autoregulatory response at a new pressure level. The autoregulation index of 1 was considered to reflect "ideal autoregulation". The autoregulation index of 0 was considered to reflect the absence of autoregulation.

with normal endothelium (Fig. 1, *c*). The autoregulation index remained below the control value (Table 1). The developed intraventricular pressure was reduced by 13-15% ($p<0.05$). L-NAME abolished the stress-induced increase in CFV, which did not differ from that in the control hearts with normal endothelium. However, CFV in these specimens remained higher than that in control animals whose hearts were treated with L-NAME. These differences were not revealed after addition of S-MT to the perfusate (Fig. 1, *d*). The developed intraventricular pressure returned to normal under these conditions. After treatment with L-NAME the autoregulation of coronary blood flow and myocardial contractility in animals of the adapta-

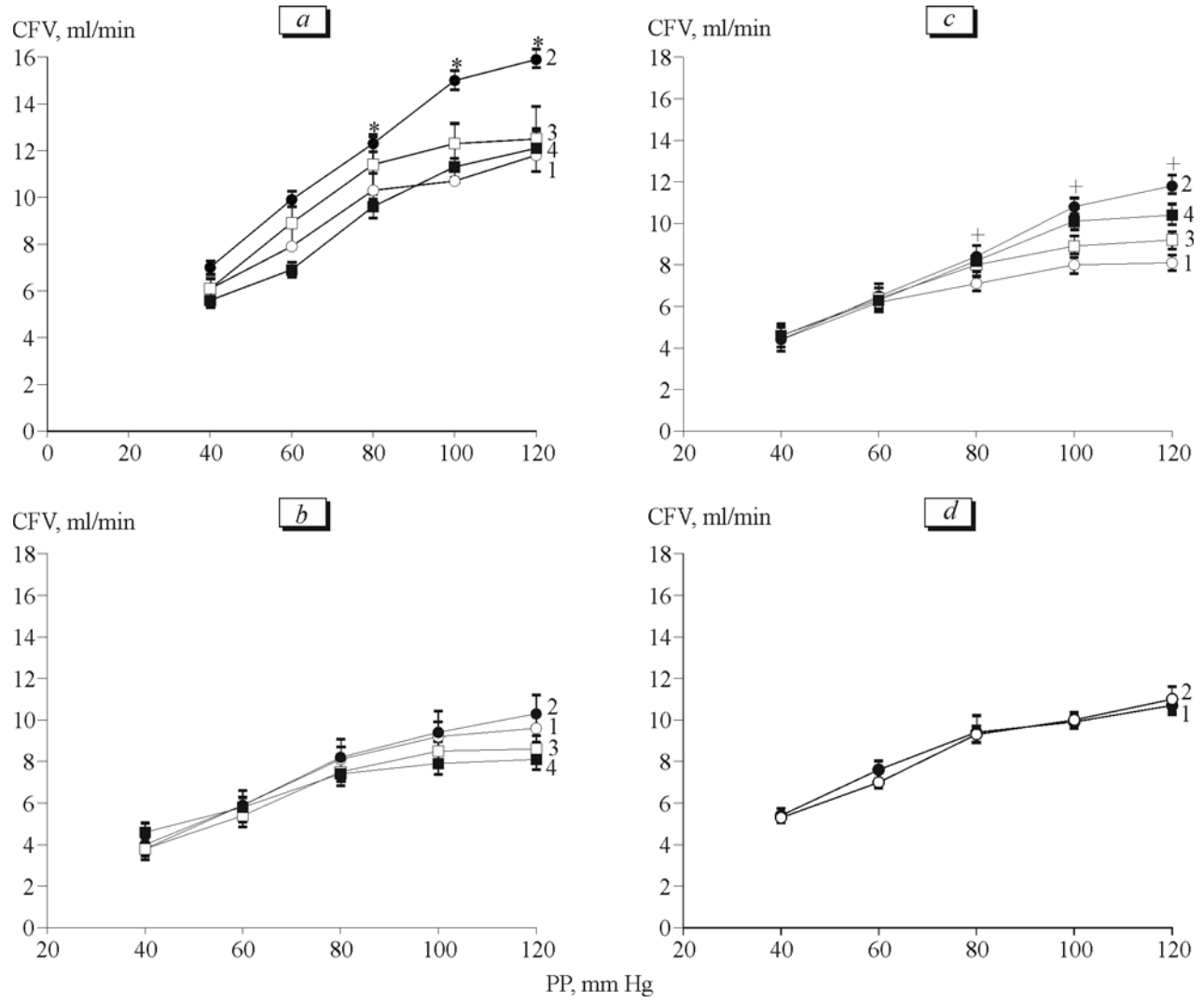

Fig. 1. Effect of adaptation on poststress changes in CFV in the rat heart during perfusion with Krebs–Henseleit solution (*a*) containing saponin (*b*), L-NAME (*c*), or S-MT (*d*). *a*: Control (*1*); stress (*2*); adaptation (*3*); stress+adaptation (*4*). *b*: Saponin (*1*); saponin+stress (*2*); saponin+adaptation (*3*); saponin+adaptation+stress (*4*). *c*: L-NAME (*1*); L-NAME+stress (*2*); L-NAME+adaptation (*3*); L-NAME+adaptation+stress (*4*). *d*: S-MT, control (*1*); S-MT, stress (*2*). PP, perfusion pressure. $p<0.05$: *compared to the control; +compared to L-NAME.

tion group and adaptation+stress group did not differ from the control (Table 1).

Addition of TEA to the perfusate in the presence of L-NAME was followed by a decrease in CFV (except for stressed animals). The changes were similar to those observed after saponin-induced endothelial injury (Fig. 2). TEA had a smaller effect in stressed animals after blockade of NO synthesis with L-NAME (by 11% as compared to endothelial injury). However, this effect was restored after addition of S-MT (Fig. 2). Hence, adaptation to short-term stress exposures (similarly to endothelial injury or inhibition of inducible NO synthase) abolished the poststress decrease in functional activity of BK_{Ca} channels. This effect was not observed after blockade of NO synthase with L-NAME.

The concentration of NO degradation products in blood plasma from animals exposed to stress or adaptation was elevated by 56% ($p<0.05$; Fig. 3, *a*) or 24%, respectively. At the same time, no between-group differences were found in the contents of reduced and oxidized glutathione. However, the ratio between these forms of glutathione decreased by 46%. Adaptation to short-term stress exposures had no effect on the reduced/oxidized glutathione ratio in the blood, but abolished the poststress decrease in this ratio (Fig. 3, *b*).

We showed that the effect of TEA in control specimens is observed only at the perfusion pressure of more than 80 mm Hg (range of autoregulation of coronary blood flow). Therefore, functional activity of BK_{Ca} channels occurs only during smooth muscle cell

contraction. This state is induced by an increase in intracellular Ca^{2+} concentration and develops in response to stretching of the vascular wall due to elevation of perfusion pressure. The constricting effect of TEA on the coronary vessels was 9% lower after stress exposure ($p<0.05$). Moreover, the TEA-induced decrease in CFV in stressed animals was observed in a narrower range of perfusion pressure (100 and 120 mm Hg). We conclude that functional activity of BK_{Ca} channels triggered by smooth muscle contraction is reduced under stress conditions. These data are consistent with the results of our previous experiments. We revealed a poststress decrease in functional activity of another group of potassium channels, K_{ATP} channels in smooth muscle cells of the coronary vessels [7]. Six-hour immobilization probably results in nonspecific poststress dysfunction of the channels.

After endothelium removal, the effect of TEA in control heart samples was observed at a lower level of perfusion pressure (40 mm Hg). Activity of calcium-activated channels was elevated by 12% ($p<0.05$ compared to animals with normal endothelium). Therefore, endothelium-derived vasodilatory substances decrease the vascular tone and reduce activity of BK_{Ca} hearts. It should be emphasized that the effectiveness of TEA after blockade of NO synthesis in normal endothelium did not differ from that in deendothelialized animals. These data suggest that among a variety of endothelium-derived metabolites, NO plays a particular role in the regulation of BK_{Ca} channel function under normal conditions.

As differentiated from control animals, saponin-induced deendothelialization in stressed rats was accompanied by a significant increase in functional activity of BK_{Ca} channels (by 9 and 22%, respectively). This effect was not observed under conditions of NO blockade with L-NAME. Therefore, endothelium removal by saponin (but not blockade of constitutive NO synthase with L-NAME) completely restores functional activity of BK_{Ca} channels in smooth muscle cells of stressed rats. NO, which is produced by constitutive NO synthase, does not contribute to the stress-induced decrease in functional activity of BK_{Ca} channels. Poststress changes in functional activity of BK_{Ca} channels were completely abolished by selective blocker of inducible NO synthase. The data indicate that NO synthesis by this enzyme plays a pathogenetic role.

After blockade of endothelial NO production in the heart of adapted animals, the effectiveness of TEA and activity of BK_{Ca} channels in smooth muscle cells of the coronary vessels were 10% lower than under conditions of endothelial injury. Hence, NO production decreases activity of BK_{Ca} channels. The remaining

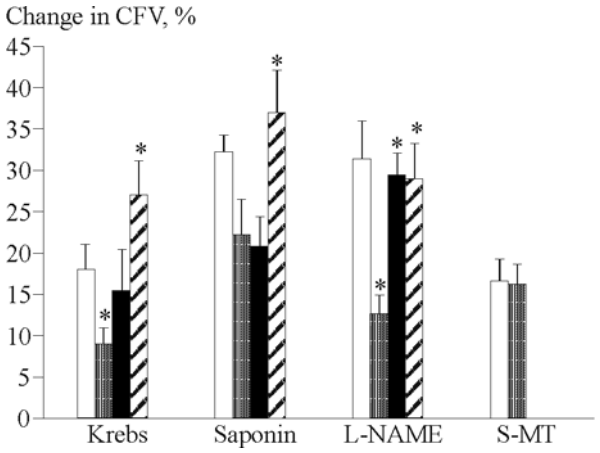

Fig. 2. Effect of TEA on CFV in the group of adapted animals before and after stress. Perfusion of the isolated heart: normal endothelium (Krebs); removal of the endothelium (saponin); and after blockade of NO synthase with L-NAME or S-MT. Light bars, control; vertical shading, stress; dark bars, adaptation; horizontal shading, adaptation+stress. Here and in Fig. 3: *$p<0.05$ compared to the control.

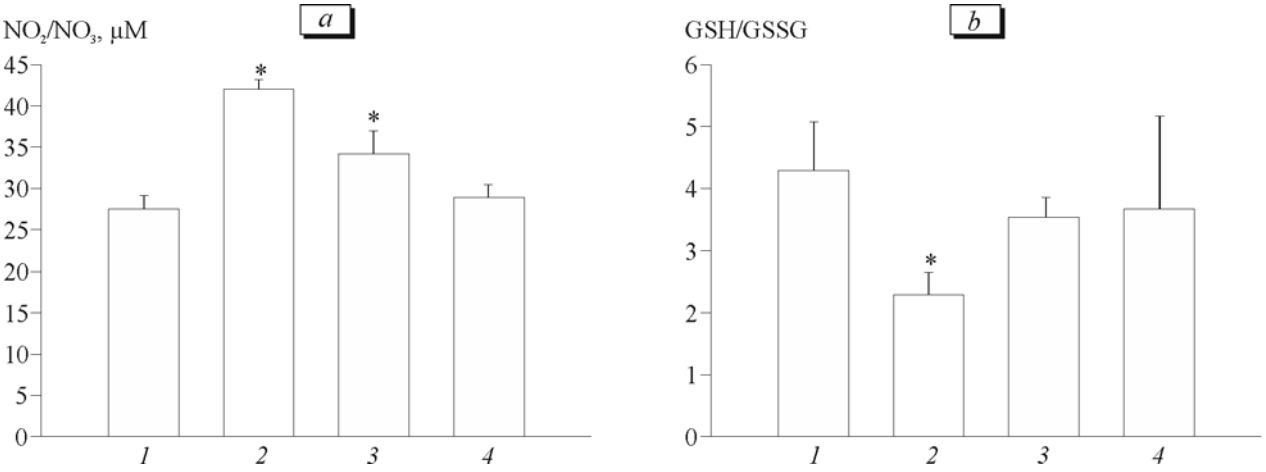

Fig. 3. Effect of adaptation by short-term stress exposures on the NO_2/NO_3 content (*a*) and thiol-disulfide ratio (*b*) in blood plasma before and after stress. Control (*1*); stress (*2*); adaptation (*3*); adaptation+stress (*4*).

endothelium-derived substances with vasoconstrictor activity (prostaglandins, leukotrienes, and products of the cytochrome P450 enzyme pathway) have a direct or indirect effect on smooth muscle contraction and can activate BK_{Ca} channels. It is important that adaptation to short-term stress exposures not only abolishes the poststress decrease in functional activity of BK_{Ca} channels, but also increase activity of channels under these conditions. Further studies are required to evaluate the mechanisms of these changes.

Adaptation to short-term stress exposures is accompanied by increased production of NO. However, the impairment of coronary vascular tone does not occur under these conditions (as differentiated from stress-induced changes with a similar increase in NO production). It probably results from the fact that adaptation is also accompanied by increased accumulation of NO [3]. This process is not accompanied by changes in the oxidized/reduced glutathione ratio, which determines the redox state and sensitivity of BK_{Ca} channels to signal molecule NO. The GSH/GSSG ratio decreases during stress, which reflects increased oxidation of glutathione and inactivation of the systems for reduction of oxidized glutathione (thioredoxin, glutathione reductase, *etc*.). The proteins of BK_{Ca} channels contain a variety of amino acids with SH groups. Oxidation of SH groups significantly decreases the permeability of BK_{Ca} channels. Similar changes are revealed under stress conditions, but not during adaptation. Therefore, adaptation by short-term stress exposures prevents the poststress decrease in functional activity of BK_{Ca} channels. The redox state and functional activity of NO in adapted animals do not differ from those in control specimens.

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