Influence of Hypodynamic Stress on the Endothelial Function of Thoracic Aorta

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In order to establish the influence of the intensity of hypodynamics (as a risk factor of ischemic heart disease) on the function of the endothelium, we investigated the endothelium-dependent relaxation in response to acetylcholine. Hypodynamic stress that lasted 48 days was provoked, with permanent and periodically recurrent intervention (hypodynamics periodically atternated with physical activity) in Chinchilla rabbits (n = 19) by placing them in metal hutches according to Fiodorov B. M. (1991). Rabbits (n = 10) of the control group which had no intervention were kept under vivarium conditions. The relaxing effect of endothelium-dependent vasodilator agonist (acetylcholine at a concentration of 10^{-8} to 10^{-4} M) on the smooth muscles of thoracic aorta (treated with prostaglandin $F_{2\alpha}$) was detected by means of mechanotron 6MXIC in an isometric regime. The response is expressed as the percent relaxation to prostaglandin $F_{2\alpha}$ (2.10^{-5}M)-induced precontraction. The endothelium-dependent relaxation to acetylcholine was more reduced in the smooth muscles of thoracic aorta in permanent hypodynamic stress lasting 48 days. The endothelial function was insignificantly impaired as compared with the control group when the hypodynamic state was alternated with physical activity.

Key words: endothelium-dependent relaxation, smooth muscle, acetylcholine, hypodynamic stress

INTRODUCTION

In the coronary circulation, vasoconstrictive response occurs in variant angina, following myocardial infarction and at the site of coronary artery stenosis (1). Dysfunction of the endothelium is a part of the pathophysiology of these conditions (1). Decreased endothelium-dependent vasodilation can be induced by hypertension, diabetes and/or atherosclerosis (2). On the other hand, physical inactivity is also an important risk factor for the heart disease, like hypertension, high serum cholesterol and smoking (3). Thus it is important to know the changes of endothelium-dependent relaxation of the smooth muscles in the case of hypodynamic stress of various intensity.

MATERIAL AND METHODS

Hypodynamic stress that lasted 48 days was provoked, with permanent and periodically recurrent intervention (hypodynamics periodically alternated with physical activity) in Chinchilla rabbits (n = 19) by placing them in metal hutches according to Fiodorov B. M. (1991). Rabbits (n = 10) of the control group without intervention were kept under vivarium conditions. The relaxing effect of the endothelium-dependent vasodilator agonist (acetylcholine in concentration 10^{-8} M, 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M) on the smooth muscles of thoracic aorta (treated with the vasoconstrictor prostaglandin $F_{2\alpha}$) was detected by means of a 6MXIC mechanotron in isometric regime. Response was expressed as the percent relaxation to prostaglandin $F_{2\alpha}$ (2.10^{-5} M)-induced precontraction. Student's paired t test was used in performing statistical analysis and establishing differences in p values; $p < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

The endothelium-dependent relaxation to acetylcholine was more significantly (p 0.05–0.01) reduced in the rabbits affected by permanent hypodynamic stress than in those after periodically recurrent hypodynamic stress (Figure). The relaxation (%) of the smooth muscles of thoracic aorta after permanent stress (**c**) under the influence of acetylcholine (10^{-6},

10^{-5}, 10^{-4} M) was significantly ($p < 0.05–0.01$) decreased as compared with this index in the control group (**a**) and in the case of periodically recurrent hypodynamic stress (**b**). On the other hand, the relaxation of the smooth muscles of thoracic aorta rabbits after periodically recurrent intervention (**b**) statisticaly did not ($p > 0.05$) change as compared with the control group (**a**). Thus, permanent hypodynamic stress that lasted 48 days caused strongly expressed conractility of smooth muscles which was associated first of all with an ultrastructural damage of the endothelium (4). In the group of rabbits after permanent hypodynamic stress, if compared with the control (4), an increased number of endothelial cell nuclei, a marked tallness of endothelial cells, as well as winded and fragmented internal elastic lamina were found ultrastructurally in the intima of thoracic aorta. According to literature data, endothelium-derived NO is involved in the regulation of the blood vessel tone, the interaction of the endothelium with blood cell elements. *Absence of the endothelium prevents a vessel regulating its tone (5).*

Lesser injuries of the endothelium were observed in the group of rabbits with periodically recurrent intervention. The internal elastic lamina was found to be a little puffy, and the surface of the endothelium was wavy (4). Endothelial cell injury is one of the initiating events in the development of atherosclerosis (6, 7). Studies in experimental models and humans have shown that atherosclerosis induces an early and selective impairment of endothelium-derived, NO-mediated relaxation (6, 8). In the coronary microcirculation, impaired NO-mediated relaxation can occur without structural changes associated with early atherosclerosis (9). In an experimental model of atherosclerosis, using cholesterol-enhanced diets, modulation of endothelium-derived relaxation occurs before any visible change in the structure of the vessel wall has occurred (9). The proposed mechanism of this defect is a decrease in NO release from the endothelium through alterations in a certain receptor-mediated function and an associated interaction of NO with oxidized low-density lipoproteins, leading to NO inactivation (2). According to our data (4), in the case of permanent hypodynamic stress lasting 48 days the concentration of cholesterol in plasma was also increased. Besides, in the case of this hypodynamic stress a decreased level of manganese (as an activator of manganese–superoxide dismutase) in thoracic aorta and blood plasma may lead to vasoconstriction (10). The half-life of NO and the relaxation of aortic rings are enhanced by superoxide dismutase. Manganese potentiates the activity of NO both *in vivo* and *in vitro*. Manganese relaxation of aortic segments was endothelium- as well as concentration-dependent. Cyclic GMP concentrations in the segments were increased 2- and 4-fold with 5 and 300 microM manganese, respectively. N-monomethyl-L-arginine pretreatment of aortic rings abolished the relaxation and cyclic GMP accumulation mediated by manganese (11).

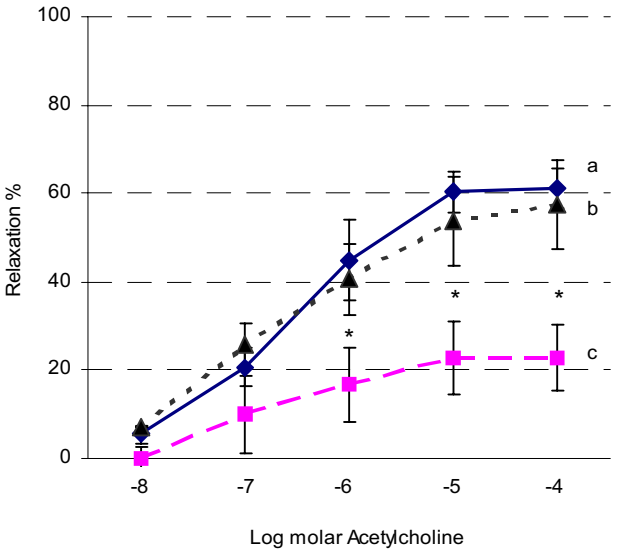

Figure. Concentration–response curves to the endothelium-dependent vasodilator acetylcholine: *a* – relaxation of smooth muscles of thoracic aorta preparations of control rabbits; *b* – relaxation of smooth muscle preparations after 48 days of periodically recurrent hypodynamic stress; *c* – relaxation of smooth muscle preparations after 48 days of permanent hypodynamic stress;
*$p < 0.05–0.01$, *c* compared with *a* and *c* compared with *b*.

Thus, the impairement of endothelium-dependent relaxation is a result of structural, functional and biochemical changes in the blood vessels.

CONCLUSIONS

Our study has shown that endothelium-dependent relaxation of the smooth muscle of thoracic aorta in response to acetylcholine is related to the intensity of hypodynamics. Permanent long-standing (48 days) hypodynamic stress, contrary to the periodically recurrent stress also lasting 48 days, caused a strong impairement relaxation of smooth muscles.

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HIPODINAMINIO STRESO ĮTAKA KRŪTINĖS AORTOS ENDOTELIO FUNKCIJAI

S a n t r a u k a

Norėdami nustatyti hipodinamijos intensyvumo (kaip vieno išeminės širdies ligos veiksnio) įtaką endotelio funkcijai, mes tyrėme nuo endotelio priklausomą lygiųjų raumenų relaksaciją, veikdami juos acetilcholinu. 48 parų hipodinaminį stresą su pastovia ir periodiškai pasikartojančia intervencija (kai hipodinamiją periodiškai keitė fizinis aktyvumas) sukėlėme B. Fiodorovo metodu inšilos veislės triušiams, juos imobilizuodami metaliniuose narveliuose. Endotelio funkciją tyrėme veikdami krūtinės aortos lygiųjų raumenų preparatus didėjančiomis acetilcholino koncentracijomis (nuo 10^{-8} iki 10^{-4} mol/l ir užrašydami preparatų atsipalaidavimą dėl mechanotrono 6MXIC izometriniue režimu. Atsipalaidavimą išreiškėme procentais nuo prostaglandino $F_{2\alpha}$ (2.10^{-5} M) sukeliamo susitraukimo. Gauti tyrimo duomenys rodo, jog pastovus 48 parų hipodinaminis stresas statistiškai patikimai ($p < 0,05–0,01$) mažina krūtinės aortos lygiųjų raumenų atsipalaidavimą dėl acetilcholino, lyginant šiuos duomenis ne tik kontrolės, bet ir periodiškai kintamos hipodinamijos atveju. Kai hipodinamiją periodiškai keitė fizinis aktyvumas (periodiškai kintamo hipodinaminio streso atveju), krūtinės aortos lygiųjų raumenų atsipalaidavimas statistiškai patikimai nesiskyrė nuo kontrolės.

Raktažodžiai: nuo endotelio priklausoma relaksacija, lygieji raumenys, acetilcholinas, hipodinaminis stresas