Abstract

Aerobic exercise promotes beneficial effects on the prevention and treatment of diseases such as arterial hypertension, atherosclerosis, venous insufficiency, and peripheral arterial disease. β-adrenergic receptors are present in a variety of cells. In the cardiovascular system, β-adrenergic receptors promote positive inotropic and chronotropic response and vasorelaxation. Although the effect of exercise training has been largely studied in the cardiac tissue, studies focused on the vascular tissue are rare and controversial. This review examines the data from studies using animal and human models to determine the effect of physical exercise on the relaxing response mediated by β-adrenergic receptors as well as the cellular mechanisms involved in this response. Studies have shown reduction, increase, or no effect of physical exercise on the relaxing response mediated by β-adrenergic receptors. Thus, the effects of exercise on the vascular β-adrenergic sensitivity should be more deeply investigated. Furthermore, the physiopathology of the vascular system is an open field for the discovery of new compounds and advances in the clinical practice.

Keywords: β-adrenergic receptors, blood pressure, vascular smooth muscle, physical exercise.

Resumo

O exercício aeróbio promove efeitos benéficos na prevenção e tratamento de doenças como hipertensão arterial, aterosclerose, insuficiência venosa e doença arterial periférica. Os receptores β-adrenérgicos estão presentes em várias células. No sistema cardiovascular, promovem inotropismo positivo cardíaco e relaxamento vascular. Embora os efeitos do exercício tenham sido investigados em receptores cardíacos, escudos focados nos vasos são escassos e controversos. Esta revisão abordará os efeitos do exercício físico sobre os receptores β-adrenérgicos vasculares em modelos animais e humanos e os mecanismos celulares envolvidos na resposta relaxante. Em geral, os estudos mostram resultados conflitantes, onde observam diminuição, aumento ou nenhum efeito do exercício físico sobre a resposta relaxante. Assim, os efeitos do exercício na sensibilidade β-adrenérgica vascular merecem maior atenção, e os resultados mostram que a área de fisiopatologia vascular é um campo aberto para a descoberta de novos compostos e avanços na prática clínica.

Palavras-chave: Recepetores β-adrenérgicos, pressão arterial, músculo liso vascular, exercício físico.
Physical exercise promotes a direct impact on vascular function, with significant beneficial effects on the patient's quality of life. Studies report that patients with peripheral arterial occlusive disease start to feel less pain and increase walking distance without claudication in response to physical exercise, significantly reducing mortality among these patients. Although there are drugs that also improve walking ability without claudication, the results are still modest when compared to supervised exercise programs associated with smoking cessation. In post-surgical varicose vein patients, physical exercise appears to be able to restore microvascular endothelial function to levels observed in age-matched healthy controls, even in the first minutes after exercise.

In addition to acting on endothelial cells, physical exercise reduces sympathetic activity and increases parasympathetic activity, leading to an improvement in vascular tone. Physical exercise also contributes to morphological changes of the vessels, modulating the growth of vascular smooth muscle cells, the formation of endothelial cells, and apoptosis reduction and promoting angiogenesis. There are reports of improvement in muscle oxidative activity in patients with peripheral arterial occlusive disease via decreased concentration of short-chain acylcarnitine, an intermediate of oxidative metabolism, which contributes to increase the walking distance without claudication in patients with peripheral arterial occlusive disease performing exercise training.

Adrenergic receptors are also implicated in vascular activity. Stimulation of α and β receptors in response to exposure to their agonists promotes constriction or relaxation of arteries and veins. NO production by endothelial cells is partly mediated by activation of β-adrenergic receptors. However, little is known about the role that β-adrenergic receptors play in blood vessels and the influence of physical exercise on these receptors in healthy individuals or patients with different pathological conditions, such as atherosclerosis, hypertension and diabetes mellitus.

Therefore, this review approaches the involvement of β-adrenergic receptors in vasorelaxation, the effects of physical exercise on the relaxant response, and the molecular mechanisms involved. The study of β-adrenergic receptors offers an interesting field of study in the area of vascular physiology, which might open new perspectives in the prevention and/or treatment of vascular diseases of different etiologies.

Vascular smooth muscle and endothelium

Arterial vessels usually have three layers: the intima, which is in contact with blood elements and consists mainly of endothelial cells; the media, composed of smooth muscle cells; and the adventitia, composed of fibrous connective tissue, which is the outer coat of the artery. Smooth muscle cells are often spindle-shaped with larger diameters in the core region. The sarcoplasmic reticulum, less developed compared to reticula of other types of muscle cells, is closely associated with the plasma membrane, which explains its involvement in Ca$^{2+}$ signaling mechanisms and muscle contraction. The activation of this biochemical cascade of vascular smooth muscle contraction occurs through binding of contractile agents, such as norepinephrine, phenylephrine and endothelin, to specific membrane receptors present in the muscle cell. These receptors, in turn, activate a protein called G protein that stimulates phospholipase C, present in the cell membrane, which catalyzes the formation of second messengers from membrane phospholipids generating inositol-1,4,5-triphosphate (IP3) and diacylglycerol (DAG). IP3 binds to its receptors located in the sarcoplasmic reticulum, releasing to the cytosol Ca$^{2+}$ ions present within this organelle. The DAG molecule activates a protein called protein kinase C (PKC), which, in turn, phosphorylates proteins bound to L-type calcium channels, favoring the influx of extracellular Ca$^{2+}$ to the intracellular medium. These two messengers cause an elevation in Ca$^{2+}$ concentration, enabling actin-myosin interaction and producing contraction of vascular smooth muscle.

The relaxation of vascular smooth muscle is triggered by different agents produced by endothelial cells, including prostacyclin, endothelin-derived hyperpolarizing factor (EDHF), and NO. NO is considered the most potent vasodilator produced by the endothelium, and control of its production is directly related to various diseases, such as hypertension, atherosclerosis, and coronary artery disease. Several mediators and neurotransmitters can promote the release of NO by endothelial cells, such as acetylcholine, bradykinin and norepinephrine, through activation of its specific receptors.

More recently, hydrogen peroxide (H$_2$O$_2$) and hydrogen sulfide (H$_2$S) have been highlighted in vascular research as important mediators in the relaxant response of different vessels. Thus, the discovery of these molecules in vascular function opens a relevant field on the therapeutic potential of thromboembolic disease.

β-adrenergic receptors

Adrenergic receptors were initially divided into two broad categories, α and β. Subsequently, they were subdivided into subtypes α$_1$, α$_2$, β$_1$, β$_2$, and β$_3$ by using subtype-selective antagonists and sequencing of amino acids that participate in
Vascular β-adrenergic receptors

Early studies investigating vascular beds have shown the existence of two β-adrenergic receptor subtypes, β₁ and β₂, in different arteries and veins. It was observed that the vasodilator response was mediated predominantly by β₁-adrenergic receptors compared with β₂-receptor subtypes, with an order of potency of epinephrine > norepinephrine > phenylephrine, although this classification of potency does not apply to all vascular beds. Some studies show that β₁-adrenergic receptors also promote vasodilation, whereas other studies show that β₂-receptor subtypes participate in the vasodilator response of arteries of various species, such as human coronary arteries, rat aorta, and canine pulmonary arteries. In rat aorta, it was demonstrated that some relaxant responses appear to be mediated by a population of atypical receptors (presumably β₁), through the use of conventional agonists/antagonists that stimulate β₁, β₂, and β₁-β₂-receptor subtypes. On the other hand, other studies failed to confirm the participation of β₁-receptor subtypes and of this atypical receptor (β₁) in this preparation. In rat mesenteric arteries, β₁-adrenergic receptor also seems to be present, but these data were not confirmed in a subsequent study in these arteries.

Studies involving femoral and brachial arteries are scarce compared to more central and larger arteries, such as the aorta and mesenteric artery. In one of these few studies, the presence of β₁ and β₂-adrenergic receptors was observed through the use of selective agonists/antagonists in porcine femoral artery. On the other hand, in rabbit femoral arteries, only β₂-receptor subtypes were shown to mediate the vasodilator response.

Mechanism of action of β-adrenergic receptors

The multiple intracellular signaling pathways in response to the activation of β-adrenergic receptors in blood vessels modify according to the β-adrenoceptor subtype that is mediating relaxant responses and to the vascular bed studied. Although the activation of cyclic adenosine monophosphate (cAMP) is the classic pathway for the vasodilator response to β-adrenergic stimulation, dependent and independent mechanisms of formation of this second messenger contribute to the relaxant response induced by the activation of these receptors. For details, see Figure 1.

Signaling pathway: cAMP-protein kinase A

Adrenoceptors belong to a superfamily of membrane receptors closely related and coupled to G proteins. All these proteins share a common peptide structure, in which the amino-terminal portion (N), extracellularly, is connected to the carboxyl-terminal chain (C)
intracellularly by seven transmembrane domains. The relative size of N- and C-terminal chains and of the third intracellular loop varies considerably from receptor to receptor.55,66 The third intracellular loop of β-adrenoceptors is the site for coupling of these receptors to G protein. G proteins are heterotrimers, consisting of one hydrophilic α-subunit and two hydrophobic subunits, β and γ. In the absence of agonists, when G protein is inactive, a molecule of guanosine diphosphate (GDP) is bound to the α-subunit, forming a complex associated with β- and γ-subunits. In the presence of agonists, the activated receptor interacts with G protein and induces the conversion of GDP into guanosine triphosphate (GTP) in the α-subunit. After binding to GTP, the α-subunit dissociates from βγ-subunits and becomes active. The α-subunit remains free until GTP hydrolysis and formation of GDP occurs, leading to its reassociation with βγ-subunits. The α-subunit of Gs protein, when activated, leads to stimulation of adenylyl cyclase, which leads to the formation of cAMP second messenger from ATP breakdown. cAMP activates protein kinase A that will promote reduction in intracellular Ca\(^{2+}\) concentration in vascular smooth muscle cells, with consequent vasodilation.56,57 For details, see Figure 1.

**Signaling pathway by activation of calcium-dependent potassium channels**

Maintenance of relaxant activity of the aorta in response to isoprenaline, even in the presence of SQ 22,536 (an adenylyl cyclase inhibitor), supports the existence of cAMP-independent mechanism in certain vessels.51 In addition, relaxation is abolished in the presence of iiberotoxin, a K\(^{+}\) channel blocker, suggesting the involvement of large-conductance Ca\(^{2+}\)-activated K\(^{+}\) channels (MaxiK). These data are consistent with a previous study that demonstrated the importance of K\(^{+}\) channels in the relaxant response of the basilar artery of the guinea pig.66 Additionally, relaxation was shown to be dependent on MaxiK channels only for responses mediated by β\(_3\)- and β\(_2\)-adrenergic receptors whereas, for β\(_1\) receptors, K\(_{Ca}\) channels do not appear to be involved.51

The mechanism by which activation of β-adrenergic receptors promotes relaxation is carried out through the activation and opening of K\(^{+}\) channels, allowing their extracellular release, which, in turn, causes reduction in membrane potential, leading to cell hyperpolarization. This results in the closure of voltage-dependent Ca\(^{2+}\) channels. Ca\(^{2+}\) channel closure by membrane hyperpolarization causes a reduction in the Ca\(^{2+}\)-calmodulin complex and in the phosphorylation of the myosin light chain, leading to relaxation.10 For details, see Figure 1.

**Signaling pathway: nitric oxide-cGMP**

Another signaling pathway of β-adrenergic receptor-mediated, cAMP-independent relaxation is the endothelial pathway. Vasodilator response by stimulation of β-adrenergic receptors has been shown to be partially69,70 or completely14 inhibited by endothelium removal or in the presence of NO synthase inhibitors, such as L-NAME. Furthermore, inhibition of soluble guanylate cyclase in vessels without endothelium eliminates the vasodilator response, whereas addition of sodium nitroprusside restores vasorelaxation. Thus, these studies show that NO produced by endothelial cells is involved in β-adrenoceptor-induced relaxation.

The mechanisms by which β-adrenergic receptors promote NO release seem to involve several signaling pathways, such as mitogen-activated protein kinase (MEK), p42/p44 mitogen-activated protein kinase (MAPK) or ERK1/2, and phosphatidylinositol 3-kinase (PI3K), both in humans and laboratory animals.71-73 The activation of these enzymes by β-adrenergic receptors leads to activation of endothelial NO synthase (eNOS) present in endothelial cells, which,
in turn, will promote oxidation of a terminal nitrogen of the guanidine group of L-arginine, forming equimolar amounts of NO and L-citrulline. Once formed, NO diffuses rapidly from endothelial to smooth muscle cells, where it interacts with the heme group of soluble guanylate cyclase, stimulating its catalytic activity and leading to the formation of cyclic guanosine monophosphate (cGMP), which, in turn, reduces intracellular Ca\(^{2+}\) levels. For details, see Figure 1. The mechanisms by which NO/cGMP pathway induces vasodilation include inhibition of IP3 generation, increased sequestration of cytosolic Ca\(^{2+}\), dephosphorylation of the myosin light chain, inhibition of Ca\(^{2+}\) influx, protein kinase activation, stimulation of membrane Ca\(^{2+}\) ATPase, and opening of K\(^{+}\) channels.\(^4\)

### Phosphodiesterases and vascular disease

The importance of cAMP and cGMP as mediators of various cellular functions, including regulation of vascular tone, proliferation of smooth muscle cells, and inhibition of platelet adhesion and aggregation, has given rise to several studies for the development and synthesis of various compounds in order to increase or control intracellular levels as a treatment for several diseases, such as erectile dysfunction, cardiac and vascular diseases.\(^5\)

Intracellular cAMP and cGMP levels are controlled by enzymes called phosphodiesterases, which catalyze hydrolysis of these mediators, leading to the formation of 5’cAMP and 5’cGMP, respectively. There are at least 11 isoforms of phosphodiesterase, including phosphodiesterases type 3 and 5, which are highly selective for degradation of cAMP and cGMP, respectively.\(^5\) The compound cilostazol, a selective inhibitor of phosphodiesterase 3, has been widely used in clinical practice for the treatment of intermittent claudication in peripheral arterial occlusive disease, promoting increased intracellular cAMP levels. The administration of cilostazol promotes potent vasodilation and inhibition of platelet aggregation, improving pain and walking ability.\(^2,10\) Furthermore, β-adrenergic receptor activation leads to activation of two important second messengers, cAMP and cGMP, whose therapeutic target is the focus of major drug companies, highlighting the importance of investigating the role that these receptors play in vascular disease. Recently, German researchers have synthesized the compounds BAY 41-2272 and BAY 58-2667, direct soluble guanylate cyclase activators, which have proven to be potent vasodilators with great therapeutic potential for vascular diseases such as thrombosis and peripheral arterial occlusive disease.\(^76\)

Thus, drug therapy for vascular diseases still requires further advances, and its association with non-pharmacological therapy, such as physical exercise, deserves attention within the area of angiology, since physical exercise promotes important changes in the vascular system, especially in the endothelium.

### Physical exercise and endothelial activation

Physical exercise is characterized by skeletal muscle contraction, and during exercise performance significant cardiovascular alterations occur, such as: increased blood flow into the muscles in activity, reduction in peripheral vascular resistance proportional to the increase in cardiac output, and, consequently, increased systolic blood pressure. To adjust all cardiovascular alterations that physical exercise causes, there are mechanisms for neural and humoral regulation. Humoral factors that will cause reduction in peripheral vascular resistance and, consequently, in blood pressure are primarily dependent on the endothelium.\(^16\)

Increased pulsatile blood flow and pressure that blood exerts on the vascular wall produce the so-called shear stress, which acts on the intima of vessels where endothelial cells are found. Shear stress is a powerful stimulus for the generation of the vasodilator agent NO in the vascular system. Associated with this phenomenon, physical exercise is an important stimulus to increase blood flow and, consequently, promotes increased NO production that triggers beneficial effects, such as vasorelaxation and inhibition of platelet aggregation, preventing diseases such as hypertension and atherosclerosis.\(^6\) NO plays a protective role in the atherosclerosis process by two signaling pathways. First, NO prevents the formation of oxidized LDL-cholesterol molecules, through its antioxidant action (which is concentration-dependent), decreasing the formation of reactive species of oxygen, which are fundamental for the process of oxidation of LDL-cholesterol molecules; second, by its inhibitory action on platelet adhesion and aggregation, preventing thrombus formation and subsequent partial or total ischemia of the tissues involved.\(^6\) Studies evaluating hypercholesterolemic animals showed increased expression of the antioxidant enzyme superoxide dismutase (SOD) and better relaxant sensitivity through vascular β-adrenoceptor-activated NO/cGMP pathway in chronic response to exercise.\(^77,78\)

The mechanisms by which shear stress promotes increased NO production involves the activation of different membrane proteins called mechanosensitive channels. These mechanoreceptors may be Gs proteins, ion channels, caveolin, and integrins, which capture tension changes on...
the cell wall and convert mechanical stimuli into chemical stimuli for eNOS activation. The pathways involved in this process are related to the activation of PKC, cSrc, and Akt/PI3K, which phosphorylate eNOS activating it.

Shear stress-induced NO production occurs regardless of Ca²⁺ presence, since Akt protein reduces eNOS sensitivity to this ion. The ability of endothelial cells to perceive and respond to changes in blood flow is an essential factor in the regulation of vascular tone and involves the activation of cell growth factors, promoting the remodeling of the arterial wall and maintenance of endothelial integrity. Thus, one of the beneficial effects of regular physical activity is closely related to its ability to stimulate NO synthase by endothelial cells and, consequently, to control blood pressure. Increased NO production also promotes antithrombotic effects, preventing thromboembolic diseases and atherosclerosis, a phenomenon that is due to inhibition of platelet aggregation by NO.

**Effects of physical activity on vascular β-adrenergic sensitivity**

The effects of exercise on vasomotor function have been extensively studied using both vasoconstrictors, such as norepinephrine and phenylephrine, and vasodilator agents, such as acetylcholine and bradykinin. Norepinephrine induces vasoconstriction by activating α-adrenergic receptors present within vascular smooth muscle cells, whereas acetylcholine promotes vasodilation by activating muscarinic receptors present within endothelial cells. On the other hand, information about the effects of exercise on β-adrenergic receptor-mediated vasodilator responses is much more scarce, and these few conflicting data show reduced, increased or no effect of physical exercise on the relaxant response. Most existing studies associate relaxant responses of β-adrenergic receptors with the aging process or cardiovascular diseases.

The first studies analyzing the involvement of vascular β-adrenergic receptors in response to physical training date from the late 1970s and have investigated vascular reactivity in response to chronic use of β-blockers in exercise-trained and sedentary rats. It was observed that trained animals without β-blockade showed higher skin temperature when exposed to 5 °C room temperature, in addition to increased vasodilator response to isoprenaline, as judged from the increase in body temperature during a training period. The author suggested increased sensitivity of β₂-adrenoceptors or decreased sensitivity of α-adrenoceptors as an explanation for these phenomena. Subsequent studies used blood flow and coronary vascular resistance to access the effects of β-adrenergic receptor blockade. It was demonstrated that the β₂-adrenergic receptor selective antagonist ICI 118.551 significantly decreased coronary blood flow velocity and increased late diastolic coronary resistance in dogs during a running session. These data showed the important participation of β-adrenergic response in the relaxant response of coronary arteries during exercise. A later study confirmed these results and even showed that coronary resistance and diameter appeared to be also affected by α-adrenergic receptor activity, since the application of phentolamine (a non-selective α-adrenoceptor antagonist) with propranolol (a non-selective β-adrenoceptor antagonist) reduced the vasoconstrictor effect of β-adrenoceptor blockade during exercise.

More recently, studies have been conducted with older animals showing that exercise improved sensitivity of β-adrenergic receptors when the vasodilator response mediated by these receptors had been previously reduced by the aging process. Thus, the results showed that a 6-week training program of 5 days/week swimming exercise improved vasodilator response to the non-selective β-adrenoceptor agonist isoproterenol, in coronary arteries, compared with the sedentary group. Another study, conducted with old and young rats, showed that a 10- to 12-week treadmill program of 5 days/week running exercise, with 60-minute sessions, improved vasodilator response to isoproterenol in gastrocnemius muscle vessels from old rats, but not in young animals. Collectively, these studies show the beneficial effects of physical exercise on vascular sensitivity in the aging process. However, β-adrenoceptor responsiveness to exercise is not homogeneous, depending on several factors, such as the region of vascular bed to be studied (regions of different diameters in the same artery may respond differently to physical exercise). Another important variable is the type of artery studied; resistance vessels (forearm) or conductance vessels (brachial artery) showed different responses in relation to blood flow both to endothelium-dependent agonist (acetylcholine) and to endothelium-independent sodium nitroprusside. Likewise, there may be differences in responses according to the animal studied.

Thus, studies related to relaxant responses mediated by β-adrenergic receptors need to be better designed regarding the classification of receptors mediating vasodilator responses in different vessels, the role of physical exercise in this response, and the possible beneficial effects that these receptors may play in the prevention and treatment of vascular diseases.
Conclusions

The effects of exercise on the vasodilator response mediated by β-adrenergic receptors are conflicting. Overall, previous studies show improvement in vascular reactivity to β-adrenoceptor agonists in response to exercise in old animals, but studies involving vascular diseases are scarce and less conclusive. For a better understanding of the effects of physical exercise on vascular β-adrenergic sensitivity, signaling pathways, such as cAMP, cGMP, and ion channels, should be considered and investigated, since factors such as oxidant activity and functional status of the endothelium are involved in the activation of these receptors. Collectively, the existing data show that the area of vascular pathophysiology is an open field for the discovery of new compounds and advances in clinical practice.

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