The study included 18 sections of the aneurysmally-changed abdominal aortas, obtained from patients of the Provincial Specialist Hospital in Wroclaw and 18 sections of normal abdominal aortas obtained from swine. The collected samples were placed horizontally in the incubation chamber. Changes in their transverse section area were registered. They were stretched to a tension of 5 mN. Krebs-Henseleit buffer was used as the incubatory environment. Incubation of the sections was performed at a temperature of 37°C, in the gaseous mixture of oxygen and carbon dioxide used in the following proportion: 95% of O₂ and 5% of CO₂. Contractions of the aorta were registered with isotonic transducers (Letica Scientific Instruments). In the studies, we examined the influence of α₁-adrenergic receptors (and their subtypes α₁A, α₁B, α₁D) on the contractility of the aortic muscle in humans and swine by their stimulation or inhibition with some selected agonists or antagonists. This time, it was shown that the stimulation of α₁-adrenergic receptors leads to contractions of the human and swine aortic muscle; the observed increase in the muscle tone may follow from the stimulation of all subtypes of alpha-1 receptor (α₁A, α₁B, α₁D). All three subtypes of 1-adrenergic receptor are engaged in vasoconstriction, especially of α₁A and α₁D subtypes; the α₁B subtype is less significant for aortic contractility. The contractile response of the aneurysmally-changed abdominal aorta in humans to agonists of α₁-adrenergic receptors was significantly less intense than that of the normal porcine aorta. It can be concluded that aneurysms influence the contractile response of the aorta.

**Key words:** α₁-adrenergic receptor, aneurysm, aorta, contractility, tone, adrenoceptors, phentolamine, hypertension

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**INTRODUCTION**

Abdominal aortic aneurysm (AAA) and aortoiliac occlusive disease (AIOD) constitute two most common diseases with an indication for elective surgery of the abdominal cavity. Aneurysm is a local widening of the arterial lumen by 50%, as compared to the normal, unchanged part of the artery, right above the aneurysm (1-4). This is a relatively common ailment, found in older individuals mostly. The majority of the cases are asymptomatic. The process of arterial widening follows gradually, over a few years, leading to the rupture of the aneurysm, which often results in patient's demise. The most common location of the aneurysm is the abdominal aorta, right below the renal arteries, but the pathological process may involve other arteries as well.

Mechanical properties of arteries, *i.e.* their stretching potential, are conditioned by the presence of two proteins: collagen and elastin. Collagen of the arteries is mostly of the I and III type and is constantly produced by arterial smooth muscles, throughout the whole life. Collagen deficits in the arterial wall may be effectively reversed if the mechanisms of synthesis are efficient and the cause of collagen damage is quickly eliminated. Collagen is a protein that is not susceptible to stretching forces. It is more like a scaffolding of a vessel. The second protein building the vessel is the elastin. This is a stretchable protein which provides vessel elasticity allowing for heart-related changes in the vascular diameter. Elastin is not synthesised in the aortic wall of adults. Its half-time is 70 years, so its amount in the aortic wall decreases with age, which leads to aortic weakening and aneurysm formation, as a consequence of coexistence of many other predisposing factors.

Normal contractility of the abdominal aorta is extremely important in the regulation of the lumen diameter and blood pressure. The influence of the adrenergic system, and especially of the α₁-adrenergic receptors, is also of significance in this process. The α₁-adrenergic receptors regulate many processes in the brain (all their subtypes), smooth muscles (α₁A and α₁D - adrenergic subtypes), liver (α₁A and α₁D - adrenergic subtypes), heart, and prostate (α₁D-adrenergic subtype).

The aim of this work was to show the effects of agonists and antagonists of α₁-adrenergic receptors on the contractility of aortic smooth muscles within the aneurysmally-changed aorta of humans and the normal aorta obtained from pigs, as well as to show the effects of the abdominal aorta aneurysm on the contractility.
MATERIAL AND METHODS

The experiments were conducted on 18 sections of normal abdominal aortas obtained from pigs weighing from 50 to 70 kg and on 18 sections of aneurysmally-changed abdominal aortas from humans. The human material for the study was collected during elective surgeries of aneurysm correction conducted at the Provincial Specialist Hospital in Wroclaw. Patients from whom the aortic samples were collected, were operated on in an elective or emergent setting, due to an extensive, life-threatening aneurysm of the abdominal aorta. The study was approved by the Ethical Review Board.

The size of aorta sections collected for examination from pigs and from humans, was the same, i.e. 0.5x1 cm. The human samples were transported in the Krebs-Henseleit buffer, at 4°C. Transport time did not exceed 1 hour. Any atherosclerotic plaque together with the endothelium were removed from the delivered aorta with the help of a scalpel. The sections were placed horizontally in 4 chambers of the automatic water bath, with a volume of 20 ml each. Every section was punctured at its edges with a needle, and a surgical thread safil 4.0 was inserted through the holes. All the samples were stretched to a tension of 5 mN. That tone was a baseline used for comparisons of the obtained results (5, 6). The time required to balance the record was determined experimentally at 20 minutes (7). The Krebs-Henseleit buffer was used as the incubation environment. It consisted of NaCl - 118 mM, KCl - 4.7 mM, CaCl2 - 2.5 mM, MgSO4 - 1.6 mM, NaHCO3 - 24.3 mM, KH2PO4 - 1.18 mM, and glucose 5.6 mM (5, 7). Incubation of the sections was carried out at a temperature of 37°C, in the gaseous mixture of oxygen and carbon dioxide used in the following proportion: 95% of O2 and 5% of CO2, in order to obtain the pH value of 7.3-7.5. Aortic contractions were registered with isotonic transducers Letica Scientific Instruments combined with bridge amplifiers (BridgeAmp, ADInstruments, Australia), a 4-channel data acquisition system (PowerLab/400, ADInstruments) connected with a Macintosh computer. Spontaneous contractile activity of the aortic muscle was recorded for 20 minutes (8). Afterwards, agonists and antagonists of adrenergic receptors were introduced to the incubation chambers with an isolated section material. The following chemical substances were added: adrenaline - i.e. agonist of α- and β-adrenergic receptors (Sigma-Aldrich), phenylephrine - agonist of α2-adrenergic receptors (Sigma-Aldrich), prazosine - antagonist of α1-adrenergic receptors (Sigma-Aldrich), cyclazocine - antagonist of α1B receptors (Sigma-Aldrich), L-765314 - antagonist of α1B receptors (Sigma-Aldrich), BMY 7378 - antagonist of α1B receptors (Sigma-Aldrich), 5-methylurapidil - antagonist of α1A receptors (Sigma-Aldrich).

Doses of the preparations were established experimentally by 10-fold dilutions. When defining the experimental dose, we introduced the preparation to the incubation chamber, starting with the highest dilution and not washing the chamber between the administration of subsequent doses (which led to the accumulation of the doses), until a visible effect was obtained on the chart. The experimental dose was defined as the lowest concentration of the preparation, leading to the required effect confirmed in a few subsequent experiments.

The following experimental designs were used in the study: 1. an agonist was introduced to the incubation chamber, with dose accumulation 2. an antagonist was introduced to the incubation chamber, with dose accumulation 3. an antagonist, followed by phenylephrine, was introduced to the incubation chamber, with dose accumulation

Between particular experiments, the incubation chambers were rinsed with Krebs-Henseleit buffer at a temperature of 37°C, three times, and the whole buffer was always removed from the chamber. Only one type of substance was always tested on one section. Before another substance was introduced, the chamber was washed with distilled water and the section was changed for a different one.

When evaluating the obtained results, we analysed the strength of contractions expressed in mN (contractility amplitude). The results of the tests were processed with the use of Microsoft Office Excel 2000 spreadsheets and analysed statistically with Student's t-test and a single-factor analysis of variance (ANOVA) for independent variables.

RESULTS

Sections of the aneurysmally-changed abdominal aorta, obtained from individuals during elective surgeries of aneurysm...
correction had a long life-span and could survive in vitro for 6-8 hours. The survival time was similar for aortic sections collected from pigs (no statistically significant differences). Proper handling of the section material was very important. After sampling, the material was washed in a cooled Krebs-Henseleit buffer several times, to get rid of blood and all impurities. The sections had to be transported to the in vitro laboratory as soon as possible. Not following the commonly accepted transportation procedures results in a slow death of the sampled material. After 20 minutes of control recording (during which the strength of the muscle tone stabilised and the metabolic activity of the tissue was rising due to temperature and oxygenation increase), agonists and antagonists of adrenergic receptors were added to the incubation chamber, in the system of accumulated doses, starting from the lowest concentration. Sections of porcine abdominal aortas were handled in the same manner i.e. stored in a cool buffer for 30 minutes. Both the aneurysmally-changed abdominal aorta from humans and the normal porcine aorta respond to the administration of selected agonists and antagonists with muscle tone change. An increase (vasoconstriction) or a decrease of muscle tone (dilatation) is observed. Figs. 1-5 show the obtained results, for a better visualisation of changes and differences in the contractility of aneurysmally-changed aortas and normal porcine aortas.

DISCUSSION

The study material comprised of section samples of the abdominal aorta changed by aneurysms and of normal abdominal aortas collected from sows. The human material included sections collected from patients subjected to surgery of aneurismal correction. The age of those patients ranged from 60 to 92 years. Their mean age was 73 years, which proves that aortic aneurysms develop in individuals over 60, this is at least when surgery is needed. Very often, such patients have many comorbidities apart from the aneurysm. In the study group, there were 3 patients with diabetes, 13 with hypertension, 11 with heart diseases, and one with lung and CNS disease. Most of the patients (13 individuals) did not reveal any symptoms of disease connected with the presence of the aneurysm of the abdominal aorta. Three patients were operated on in acute emergency procedures due to aneurismal rupture, and two showed symptoms of pain of the sacrolumbar spine and abdomen. Clot in the aortic lumen was found intraoperatively in all cases; in 4 cases, there was wall thickening and atherosclerotic changes, and in 13 patients there was a thin-walled aneurysm.

The α₁-adrenergic receptor belongs to stimulating receptors, and it is heterogeneous, which means that it has different subtypes. According to the current classification developed by the
International Union of Pharmacology Subcommittee on Nomenclature for Adrenoceptors, there are 3 subtypes of $\alpha_1$-adrenergic receptor: $\alpha_{1A}$, $\alpha_{1B}$, and $\alpha_{1D}$ (9-15). The $\alpha_1$-adrenergic receptor is connected with calcium signaling, which means that its stimulation leads to an increased concentration of intracellular calcium ions (5, 16-18). The signal is transmitted from the receptor to the cell, by means of such enzymes as: phospholipase C (PLC), phospholipase D (PLD), and phospholipase A$_2$ (19, 20).

Studies have shown that the effectiveness of $\alpha_1$-adrenergic receptors in increasing the intracellular concentration of calcium ions is as follows: $\alpha_{1A} > \alpha_{1B} > \alpha_{1D}$. Apart from different subtypes of $\alpha_1$-adrenergic receptor in humans, there are also nine splice variants of the $\alpha_{1A}$-adrenergic receptor, i.e. $\alpha_{1A,1}$, $\alpha_{1A,2a}$, $\alpha_{1A,2b}$, $\alpha_{1A,3a}$, $\alpha_{1A,3b}$, $\alpha_{1A,4}$, $\alpha_{1A,5}$ (10, 21-24).

The experiments showed that the stimulation of the $\alpha$-adrenergic receptor causes a contraction of the aortic muscle,

Fig. 4. Influence of blocking the subtypes of $\alpha$-adrenergic receptors with particular agonists and antagonists on the contractile response of the aneurysmally-changed abdominal aorta in humans to phenylephrine administration in the system of dose accumulation.

Fig. 5. Influence of blocking the subtypes of $\alpha$-adrenergic receptors with particular agonists and antagonists on the contractile response of the porcine aorta to phenylephrine administration in the system of dose accumulation.
both the normal one (swine), as well as the pathologically changed one (humans). There was a correlation between tissue reaction and agonist concentration. With increasing concentration, the intensity of the contraction was increasing as well (Fig. 3). Aneurysmally-changed aortas showed a significantly less intense reaction to agonists of α-adrenergic receptors as compared to porcine aortas (Fig. 3). The strength of the contraction is lower. The size of the sections used for examinations was the same for humans and for swine. This shows that aneurysms inhibit the contractility and aortic muscle reaction to agonists of α-adrenergic receptors.

Aneurysms of the abdominal aorta reveal features of a chronic inflammation, destructive reconstruction of the extracellular matrix, decreased number of smooth muscle cells in the tunica media, and a significant reduction of its thickness, as well as neovascularisation (25, 26). As it may be noticed, aneurysms significantly change aortic biomechanics, by influencing the processes of lumen regulation.

The authors distinguished four phases of contraction after - adrenergic agonist administration: 1) phase of a rapid increase, lasting for about 1 minute, with contraction strength of up to 2 g; 2) phase of a slow increase, lasting for 1.5 minute, on average, with contraction strength of up to 3 g; 3) plateau phase lasting for about 4 minutes, with no change in the muscle tone; 4) phase of muscle relaxation, lasting for about 4.5 minutes, with a slow dilatation of the aortic muscle (27, 28). The important factor was the previous removal of the aortic endothelium which significantly reduces muscle contractility and muscle reaction to agonists, which was proved by many authors (7, 27-30). Differences in the contraction strength may also follow from the method of section placement in the incubation chamber (21).

Blockade of α-adrenergic receptors with phentolamine (nonselective antagonist of α-adrenergic receptor) results in the lack of contractions in response to adrenaline and phenylephrine (Fig. 4, 5). As shown in the study, these are the α1-adrenergic and not the β-adrenergic receptors that are engaged in the observed vasoconstriction of the aneurysmally-changed aortic muscle in humans and of the normal porcine aorta. Administration of phentolamine alone leads to aortic muscle dilatation (Figs. 1, 2).

Blockade of that receptor with prazosin (nonselective antagonist of α1-adrenergic receptor) leads to the absence of tissue reaction to phenylephrine. Administration of prazosin to the incubation chamber resulted in a gradual dilatation of the muscle tissue, which confirms the role of α1-adrenergic receptor in the regulation of aortic vasoconstriction (Figs. 1, 2, 4, 5). Maybe the α1-adrenergic receptor is stimulated in vitro by agonists - endogenous noradrenaline secreted in the aortic wall, by postganglionic fibres of the sympathetic system (31). Similar results were obtained in other animals, when the influence of phenylephrine on arterial contractility was studied. In vitro studies showed that stimulation of α1-adrenergic receptor by intravenous phenylephrine caused constriction of the arterial vessels in pigs (32) and dogs (26, 33). The researchers showed a significant increase in the muscle tone of the arterial vessels, leading to an increased arterial blood pressure of the examined animals.

Blockade of α1-adrenergic receptor with 5-methylurapidil resulted in a decrease of the aortic muscle tone in humans and swine (Figs. 1, 2) and in a decrease of muscle reaction to phenylephrine (Figs. 4, 5).

The results confirmed the presence of α1-adrenergic receptors in the aortic muscle and its effects on contractility, and suggested a key role of this receptor subtype in aortic vasoconstriction caused by the stimulation of α1-adrenergic receptor. The α1A-adrenergic receptor is not the only receptor influencing the aortic vasoconstriction. Blockade of α1B-adrenergic receptors by administration of cyclazocine to the incubation chamber resulted in a statistically significant decrease in the muscle tone (Figs. 1, 2). Blockade of that receptor on the other hand caused a decrease in aortic muscle reaction to phenylephrine (Figs. 4, 5). However, the observed changes are much less pronounced than those registered for the α1A-adrenergic subtype. Analogous results were obtained with a blockade of α1A-adrenergic receptor by addition of L765314-selective antagonist of α1A receptor and blockade of α1B-adrenergic receptor by administration of BMY 7378 to the incubation chamber. (Figs. 1, 2, 4, 5).

The literature showed the influence of subtypes of α1-adrenergic receptor on blood vessels in animals. According to Willems et al. (32), phenylephrine influences the contractility of the carotid aorta in dogs, especially by means of α1A and α1B adrenergic receptors; the α1B subtype is less significant for aortic contractility. The authors showed that blocking the α1A-adrenergic receptor in dogs by administering 5-methylurapidil and BMY 7378 at a dose of 100 µg/kg intravenously, reduced the response to phenylephrine by approx. -20±8%, and a dose of 300 µg/kg completely blocked the response. Abound et al. (21) showed that the administration of 5-methylurapidil, benzoanithion and WB 4101 to the incubation chamber decreased the aorta contraction strength in rats in response to noradrenaline. Blockade of α1A-adrenergic receptor by addition of L-765314 at a dose of 1000 µg/kg did not have any influence on tissue reaction to phenylephrine (26, 34, 35). The role of α1B-adrenergic receptor in aortic vasoconstriction in dogs seems controversial. On the other hand, this receptor seems to have a crucial role in contractions of the spleen and splenic vessels, leading to blood expel from the spleen to the peripheral vessels (16). Administration of BMY 7378 blocked the α1B-adrenergic receptor in the aorta of the rats, leading to the dilatation (30, 36). However, its influence on vasa deferentia contractions was not that significant (37). There are different theories on the mutual functional coexistence of α1A and α1B adrenergic receptors in the regulation of vasoconstriction of the renal arteries in rats (30). Subtypes α1A and α1B, on the other hand, did not show any effects on the regulation of contractility of the arterial vessels in dogs (32) but they took part in regulating the contractions of the resistance arteries of the skin in rabbits (α1B) (38) and renal arteries in rats (α1A) (11).

The presented results as well as literature data confirm the influence of α1-adrenergic receptors on the aortic contractility and show a considerably lower contractile ability of the aneurysmally-changed tissue due to stimulation of α1-adrenergic receptor, as compared to a normal abdominal aorta. This phenomenon seems to be connected with histological changes in the aortic wall, accompanying aneurysm formation (20, 33). The observed loss of elastin and collagen, apoptosis of myocytes of smooth muscles and degradation of the tunica media in the aneurysmally-changed vessel not only inhibit the contractile and elastic function of the abdominal aorta in humans, but also change the reaction of that tissue to stimulation of α1-adrenergic receptors.

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