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In vitro Contractility of Normal and Aneurysmal Abdominal Aorta Muscle Coat Sections in Human and Animal Material*

Jan GNUS, Albert CZERSKI, Wojciech ZAWADZKI, Wojciech WITKIEWICZ, Willy HAUZER, Agnieszka RUSIECKA, and Stanisław FERENC

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The objective of the study was to demonstrate spontaneous contractile activity of the smooth muscle coat of the aorta in human and animal material. Spontaneous contractility of smooth muscle tissue, or tonus, is essential for the proper function of many internal organs as observed in the many types of muscle cells which make up the internal structures. The spontaneous contractile activity of the muscle tissue in blood vessels is particularly marked in resistance vessels, regulating circulation within organs or tissues. It can also be observed in large blood vessels such as arteries and veins. The contractile activity of muscular tissue isolated from arteries is the result of a number of factors, including endogenous paracrine substances, neurotransmitters released at postganglionic endings (mostly within the sympathetic system), cells capable of spontaneously generation of functional potentials (pacemaking cells) and the vascular endothelium. Pacemaking cells present in the aortic wall are an important factor in the development of the spontaneous contractility of the muscular coat of the aorta. They are capable of generating functional potentials, resulting in the constant tonus of the smooth muscular coat (comprising the aortic wall) due to tonic contraction. *In vitro* studies were carried out on abdominal aortic sections collected from 30 New Zealand rabbits with a body mass of 3-4 kilograms each and also on aneurysmal abdominal aortic sections collected during elective aneurysm repair procedures in humans (10 abdominal aortic sections). The 1.5 cm-long sections were mounted in chambers of an automated water bath. The sections were oriented in a transverse and longitudal fashion in order to compare contractility. The incubation medium consisted of Krebs-Henseleit buffer. Spontaneous contractile activity was observed during the study, characterized by rhythmic contractions of the muscular layer of the aorta.. The contractile tension within the sections was 0.15 mN in the case of rabbit sections and 0.8 mN in the case of human sections. The average duration of a single contraction was 38.3 ± 15.05 seconds. The average contraction frequency, i.e. the average number of contractions per minute, was 1.61 ± 0.54 contractions per minute. The spontaneous contraction is modulated by many factors like endogenous paracrine substances, neurotransmitters or vascular endothelium.

Key words: Aorta, contractility, tonus, rabbit, human.

Jan GNUS, Wojciech WITKIEWICZ, Willy HAUZER, Stanisław FERENC, Regional Specialist Hospital in Wrocław, Research and Development Center, Kamieńskiego 73a, 51-124 Wrocław, Poland.
E-mail:janus@tlen.pl
witkiewicz@wssk.wroc.pl
willyhauzer@gmail.pl
ferenc1@tlen.o2.pl
Albert CZERSKI, Wojciech ZAWADZKI, Agnieszka RUSIECKA, Department of Animal Physiology and Biostructure, Institute of Animal Physiology, Wrocław University of Environmental and Life Sciences, K. C. Norwida 31, 50-375 Wrocław, Poland.
E-mail: alb5@tlen.pl
wojciech.zawadzki@up.wroc.pl
agnieszka.balcerzak@up.pl

Blood vessels transport blood to all cells of the body, ensuring a constant supply of nutrients and the effluence of metabolites. The successful function of blood vessels is achieved owing to the ap-

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propriate anatomical structure and biophysical properties of vascular walls. In general, the vascular wall is comprised of an innermost layer of endothelium cells, a medial layer containing numerous smooth muscle cells with collagen and elastin fibres and adventitia surrounding the vessel. The biophysical properties of blood vessels are determined by the quantitative ratio of these three constituents and by the ratio of vascular wall thickness to the inner radius of the vessel. The walls of major arteries are characterized by a low wall thickness to the inner radius ratio (average of 1:6). They are predominantly composed of elastin fibres which ensure their extensibility. The presence of smooth muscular tissue in the arteries provides control over the lumen diameter. Muscular contraction results in a reduction of a cross-sectional vessel area. The mechanism of aortic smooth muscle coat contraction is initiated by the release of Ca^{2+} ions. The cytoplasmic levels of the free Ca^{2+} ions rise up to 1 μmol/l which during relaxation is more than 10 times lower. Ca^{2+} ions may be released as a result of numerous factors including the stimulation of membrane receptors located in myocytes, a stretching of the vascular walls, or an increased intravascular pressure. The released Ca^{2+} ions cause the detachment of a regulatory protein, called caldesmon, in the case of smooth muscle tissue. When the Ca^{2+} levels are low, caldesmon remains actin-bound and prevents binding between actin and myosin. The detachment of caldesmon from the actin molecule (as a result of high Ca^{2+} levels) frees the anchor point for myosin side chains. A similar role is performed by another regulatory protein, calponin, which is also actin-bound. The role of calponin consists of inhibiting the activity of the enzyme myosin actin ATP-ase. The inhibition is suppressed upon phosphorylation of the light myosin chains. This process allows the formation of cross-bridges that bind myosin to actin. The process of actin-myosin bridge formation is initiated by Ca^{2+} ions attaching to the regulatory protein calmodulin. The Ca^{2+}-bound calmodulin activates myosin light chain kinase (MLCK) which transfers phosphate residues from ATP molecules onto four light chains of myosin. Contraction is a result of alternating formation and the breaking of cross-bridges between the contractile proteins actin and myosin (ECKERT *et al*. 1995; VAN BREEMEN *et al*. 1989; WALSH *et al*. 1985). The calcium that initiates the contraction originates from the sarcoplasmic reticulum and extracellular matrix (HILL 1999). It enters the sarcoplasm through the calcium channels. The smooth muscle coat of major blood vessels contains L-type (long lasting activation) and T-type (transient activation) calcium channels. L-type calcium channels are voltage-dependent. They open as the intracellular potential rises to ca. -50 mV and close very slowly and thus cause long-lasting depolarization. This type of calcium channel may be blocked by calcium antagonists – blockers such as nifepidin or dihydropyridin derivatives. The dominant role of voltage-dependent calcium channels in the spontaneous contractility of the vascular muscle coat is proved by the fact that blocking these channels results in a total suppression of spontaneous contractility. These observations have been made on portal veins in rats, arteries, mesenteric arteries, cardiac vessels and femoral arteries in rabbits, and auricular arteries (HAYASHIDA *et al*. 1986; CHEMTOB *et al*. 1992; GUSTAFSSON 1993; OMOTE & MIZUSAWA 1993; OMOTE *et al*. 1993; STORK & COCKS 1994).

The objective of the study was to demonstrate the spontaneous contractile activity of the smooth muscle coat of the aorta in human and animal material. This study is a preliminary work as part of research on the role of the aortic muscle coat in the mechanisms regulating blood pressure and aneurysm formation.

Material and Methods

The experiments were performed following approval from the local 2nd Bioethics Committee, approval no. 89/2010.

Studies were carried out on abdominal aortic sections collected from 30 New Zealand rabbits with a body mass of 3-4 kilograms each and also on aneurysmal abdominal aortic sections collected during elective aneurysm repair procedures in humans (10 abdominal aortic sections).

The experimental animals were euthanized by intravenous administration of a pentobarbiturate solution (Morbital). Immediately after death, 4-5 cm sections of the abdominal aorta were collected and subsequently cut into 1.5 cm-long sections. The average diameter of the aorta in New Zealand rabbits with a body mass of 3-4 kg each was 4-6 mm. The sections were mounted in 4 chambers of an automated water bath with 20 ml chamber size. Sections were oriented in two ways in order to compare contractility. First, the sections were mounted in a transverse fashion by pulling a Safil 4/0 surgical suture through the aortic lumen and installing the suture so as to observe changes in the cross-sectional area of the isolate. Next, the sections were mounted in a longitudinal fashion in order to demonstrate contractility along the aortic axis.

The human material consisted of fragments of abdominal aorta resected during scheduled abdominal aortic aneurysm repair procedures in the Regional Specialist Hospital in Wrocław. The ma-

terial collected during the procedures was immediately transferred to the *in vitro* lab at 5°C in the Krebs-Henseleit buffer which was also used as the incubation medium during the experiments. The average size of the abdominal aortic wall sections isolated during the procedure was 3-4 cm. After delivery to the lab, sections were divided into smaller sections, sized 0.5 × 1.5 cm. Atherosclerotic plaque was removed from the sections and thus the prepared aortic isolates were mounted in the automated water bath according to the protocol described in the case of the rabbits.

All sections, both human and animal, were mounted on the apparatus with an initial tonus of 5 mN. This value defined the foundation to which the results were later compared (LOW *et al*. 1993; ECKERT 2000). A 20-minute time limit for record equilibration was determined experimentally (HILL 1999). The incubation medium consisted of the Krebs-Henseleit buffer with the following chemical composition: NaCl – 118 mM; KCl – 4.7 mM; $CaCl_2$ – 2.5 mM; $MgSO_4$ – 1.6 mM; $NaHCO_3$ – 24.3 mM; KH_2PO_4 – 1.18 mM; glucose 5.6 mM (ECKERT 2000; HILL *et al*. 1999). The sections were incubated at 37°C while administering a gaseous mixture of oxygen and carbon dioxide composed of 95% O_2 and 5% CO_2 so that the pH of the solution was maintained within the range of 7.3-7.5. The aortic contractions were recorded using isotonic transducers (Letica Scientific Instruments) connected to bridge amplifiers (BridgeAmp, ADInstruments, Australia) and a 4-channel data acquisition system (PowerLab/400, ADInstruments) which was connected to a Macintosh computer. The spontaneous contractile activity of the aortic muscle coat was recorded over 4 hours. The results were processed with a focus on the number of contractions per minute (contraction frequency), the contraction force in mN (contraction amplitude) and the contraction duration in seconds. The results were processed in MS Excel 2000 and submitted for statistical analysis using the *t*-Student test and single-factor variance analysis (ANOVA) for uncorrelated variables.

Results

The isolated sections of the abdominal aorta collected from rabbits are characterized by spontaneous contractile activity and also by contractions occurring at regular intervals. The sections of rabbit aorta were characterized by contraction amplitude of 0.19 ± 0.048 mN in the case of longitudinal orientation within the incubation chamber and 0.15 ± 0.048 mN in the case of transverse orientation. The difference between the average contraction forces in the sections oriented in longitudinal and transverse fashions was small and statistically insignificant. This proves that the aorta is capable of contracting both longitudinally and in when studying sections of that size. Figure 1 presents the spontaneous contractile activity of the rabbit abdominal aorta sections. The average duration of a single contraction was 38.3 ± 15.05 seconds in the case of longitudinal orientation and 36.05 ± 16.93 seconds in the case of longitudinal orientation. The difference between the average contraction duration in sections oriented in longitudinal and transverse fashions was small and statistically insignificant. The average contraction frequency, i.e. the average number of contractions per minute was 1.61 ± 0.54 contractions/minute in sections oriented in longitudinal a fashion and 1.69 ± 0.52 contractions/minute in sections oriented in a transverse fashion. The average difference between the average spontaneous contraction frequency in sections oriented in longitudinal and transverse fashions was small and statistically insignificant.

Spontaneous contractile activity was also observed in abdominal aorta isolates which were collected from human subjects during elective procedures of surgical abdominal aortic aneurysm repair with straight or bifurcated grafts. The material collected during the procedure was cleaned of atherosclerotic plaque and cut into sections mounted into the incubation chamber in a manner similar to that in the case of the rabbit sections. The isolated sections of the human abdominal aorta showed spontaneous contractile activity lasting several hours. Muscle tissue contractions had an average amplitude of 0.8 ± 0.14 in the case of sections mounted in a longitudinal fashion and 0.76 ± 0.16 mN in the case of sections mounted in a transverse fashion. No statistical differences in contractile force (contraction amplitude) were observed between longitudinal and transverse sections. The average duration of aortic smooth muscle coat contraction in the case of spontaneous contractile activity recorded *in vitro* was 38.55 ± 8.59 seconds in isolates oriented in a longitudinal fashion and 39.46 ± 7.82 seconds in isolates oriented in a transverse fashion. No statistical differences in average

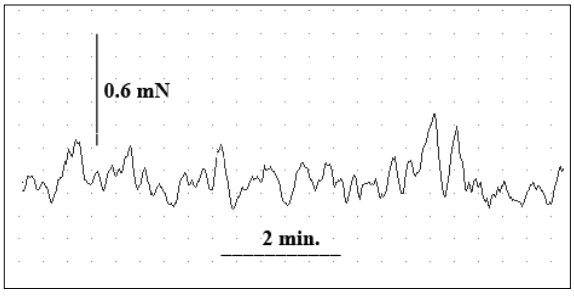

Fig. 1. Spontaneous contractile activity of aortic muscle coat isolated from rabbit. The section was oriented transversely to the instrument axis.

contraction duration were observed between longitudinal and transverse sections. The average contraction frequency was 0.54 ± 0.12 contractions/minute in the sections oriented in a longitudinal fashion and 0.48 ± 0.15 contractions/minute in the sections oriented in a transverse fashion. No statistical differences in the average contraction frequency were observed between longitudinal and transverse sections. Figure 2 presents the spontaneous contractile activity of human abdominal aorta fragments. In one experiment, a very high contractile force was observed in an abdominal aortic section, collected from a 65-year-old female subject who had undergone an elective abdominal aortic aneurysm repair procedure. The sample was delivered to the *in vitro* lab in a very short time. The average contractile force was 1.3 mN. The average duration of aortic smooth muscle coat contraction in this experiment was 41.55 ± 8.69 seconds and the average contraction frequency was 0.51 ± 0.12 contractions/minute.

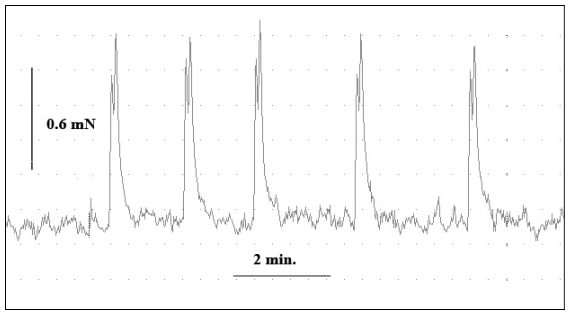

Fig. 2. Spontaneous contractile activity of aortic muscle coat isolated from human. The section was oriented transversely to the long instrument axis.

Discussion

Spontaneous contractility of smooth muscle tissue, or tonus, is essential for the proper function of many internal organs; it is observed in many types of muscle cells which constitute the internal structures. It is most pronounced within the gastrointestinal tract, but can also be observed within the ureters, urinary bladder, uterus and lymph node blood vessels, arteries and veins (TOMITA 1981; VAN HELDEN 1993; HASHITANI *et al*. 1996). Spontaneous contractile activity of the muscle tissue in blood vessels is particularly marked in resistance vessels which regulate circulation within organs or tissues. It is also observed in large blood vessels such as arteries and veins (SHIMAMURA *et al*. 1999; NILSSON & AALKJAER 2003; FUNK *et al*. 1983; MEYER *et al*. 2002). The occurrence of contractility is synchronous and sometimes encompasses longer segments of the blood vessels, thus affecting blood flow and vascular resistance (FUNK *et al*. 1983; MEYER *et al*. 2002; GRATTON *et al*. 1998). *In vitro*, contractility of the muscle coat of the aorta may be both spontaneous and induced by stimuli such as tissue compression, tissue stretching, drug application, or increased potassium levels within the extracellular matrix (DULING *et al.* 1981; HAYASHIDA *et al.* 1993; KATUSIC *et al.* 1988; CHEMTOB *et al*. 1992; PORRET *et al.* 1995; HILL *et al.* 1999). A stretching of dog-isolated abdominal aorta results in increased muscle tonus (KATUSIC 1987). Similar behaviour was observed in the aortic fragments isolated from rats (GHOSH 2004; WANG 1999; FRÈDÈRIC 2007).

The contractile activity of muscular tissue isolated from arteries is the result of a number of factors. They include endogenous paracrine substances, neurotransmitters released at postganglionic endings (mostly within the sympathetic system), cells capable of spontaneous generation of functional potentials (pacemaking cells) and the vascular endothelium.

Endogenous paracrine substances affecting spontaneous arterial contractility include endothelium-derived contracting factors (EDCF) and arachidonic acid metabolites (FRÈDÈRIC 2007). The strongest effect is exerted by substances belonging to the $EDCF_3$ class, including endothelins. By activating the receptor and the regulatory protein G_q, endothelin activates phospholipase C and initiates the phosphatidylinositol cascade. The opposite reaction is achieved following the activation of ET_B. The activation of this receptor leads to activation of a secondary transmitter G_i which results in the inhibition of cGMP synthesis. Potassium channels are activated, myocytes undergo hyperpolarization and NO is released. The process leads to vasodilation. The reactions mobilize calcium from calcium reservoirs and initiate the contraction of the aortic muscle coat. The factors increasing the vascular tonus also include the arachidonic acid metabolites. They participate in the development of spontaneous aortic muscle tissue tonus *in vitro* (IMIG 1996; KATUSIC 1987), and include such substances as 20-HETE, thromboxane, and prostaglandins (PGH_2 and PGF_2) (SEKIGUCHI 1998).

The second factor affecting spontaneous contractility is sympathetic innervation. In living organisms, blood vessels, excluding capillaries and placental vessels, are innervated by postganglionic sympathetic fibres narrowing the blood vessels. The sympathetic nervous system is in a state of constant rest, leading to the constant resting tonus of the muscular layer comprising the blood vessels. Neurogenic tonus narrowing the blood vessels is characterized by the generation of functional potentials in postganglionic sympathetic fibres with a frequency of 1 Hz and up to 8 Hz in increased sympathetic tension conditions. Sympa-

thetic nerve endings release neurotransmitters, e.g. noradrenaline, neuropeptide Y (NPY) and the noradrenaline precursor – dopamine. Spontaneous contractility is also suppressed by the administration of a sympathetic nerve ending blocker – guanethidine (HILL 1999). Neurotransmitter diffusion occurs over a large area (volume diffusion), affecting numerous myocytes. When released, noradrenaline activates the α_1 postsynaptic adrenergic receptors. Notable is the fact that sparse postganglionic neurons may generate functional potentials while at rest, accounting for the so-called tonic- spontaneous sympathetic activity.

Pacemaking cells present in the aortic wall are another important factor in the development of spontaneous contractility of the muscular coat of the aorta. They are capable of generating functional potentials, resulting in a constant tonus of the smooth muscular coat which comprises the aortic wall due to tonic contraction. Pacemaking myocytes generate functional potentials. Many researches believe that voltage-dependent calcium channels ($VDCC_s$) play an important role in the process and the contraction is preceded by fluctuations of membrane potentials similar to those observed in pacemaking cells within myocardium (COLANTUONI *et al.* 1984; HAYASHIDA *et al.* 1986; HUNDLEY *et al.* 1988; BARLETT *et al.* 2000; HADDOCK & HILL 2002; OISHI *et al.* 2002; BOUSKELA & GRAMPP 1992; LEE *et al.* 1994). On the other hand, according to HILL (1999), the spontaneous rhythmic contractile activity of vascular muscle tissue is of myogenic origin and the contractions are induced by cyclic secretion of Ca^{2+} ions from intracellular reservoirs without any contribution from the voltage-dependent calcium channels. Gap junctions play an important role in synchronizing the contractions (HILL 1999).

Factors affecting the aortic muscle coat tonus also include substances secreted by the endothelium. The endothelium is responsible for local production of nitric oxide (NO). Nitric oxide is a strong vasodilator, which is a factor in charge of local blood vessel tonus-regulation. Blood flow and shear stress are the stimuli inducing the release of paracrine substances from the vascular endothelium. Shear stress deforms the endothelium, particularly proteoglycans and acidic glycoproteins. This deformation activates the store-operated calcium channels (SOC). This results in an intracellular influx of Ca^{2+} ions, accompanied by a difference in electrical potentials (I_{CRAC} – Ca^{2+} release-activated Ca^{2+} current). Calcium influx is additionally enhanced by open potassium channels and non-selective cation channels (NSCs). This results in the depolarization of vascular endothelial cells. As a result of depolarization, the endothelium releases nitric oxide, prostacyclin PGI_2, tissue plasminogen activator (tPa) and an endothelium-derived hyperpolarizing factor (EDHF). These factors stimulate the hyperpolarization of myocytes and inhibit the myogenic activity of the vascular wall. Blocking the voltage-dependent calcium channels by the administration of felodipine does not affect spontaneous contractility, while the administration of cadmium chloride – a selective calcium channel blocker – completely neutralizes the tonus of the vascular muscle coat (HILL 1999).

Experiments showed that the resting tonus of rat abdominal aortic muscle tissue *in vitro* was much higher in endothelium-deprived sections (FRÈDÈRIC 2007). The administration of N^{ω}-nitro-L-arginine methyl ester (L-NAME, an inhibitor of NO synthases) into the incubation chamber increased the spontaneous tonus of aortic smooth muscle coat in rats (FRÈDÈRIC 2007). A statistically significant increase was also observed in the resting tonus of sections collected from adult rats compared to young rats. On the other hand, administration of N^{ω}-nitro-D-arginine methyl ester (D-NAME) does not affect spontaneous contractility (HILL 1999). The vascular endothelium is not the only site where NO synthesis takes place. It is also produced outside the endothelium, within the aortic wall (FRÈDÈRIC 2007).

Sections from rabbit arteries were also used for the examination of the spontaneous contractile activity, in addition to the human material. It was not possible to compare the contractile activity of aneurismal and normal human aorta. Rabbits are easy and inexpensive animals for breeding. At the same time the size of a rabbit artery is suitable for *in vitro* studies and the availability of animal material permits many repetitions in order to reach reliable results.

Spontaneous contractile activity, characterized by rhythmic contractions of the muscular layer of the aorta, was observed during the study. The contractile tension within the sections was 0.15 mN in the case of the rabbit sections and 0.8 mN in the case of the human sections. HILL *et al*. (1999) demonstrated that the spontaneous contractile activity of arterioles isolated from rat iris was characterized by contraction amplitude of up to 4 μm and an average contraction frequency of 4 min^{-1}. Each contraction wave was preceded by the spontaneous depolarization of myocytes. The researchers demonstrated that the spontaneous contractile activity of sections equilibrated in terms of amplitude and frequency was over 15-20 minutes. The average time the spontaneous tonus was maintained was about 1.5 hours; a slow suppression of contractility was observed afterwards (HILL *et al*. 1999). However, tissue response to the electrical pulse stimulation was maintained for a much longer time (GOULD & HILL 1994).

In summary, the aneurismal human and rabbit aorta can develop spontaneous tone. The spontaneous contraction is modulated by many factors such as endogenous paracrine substances, neurotransmitters or vascular endothelium.

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