Role of $\alpha_1$-adrenergic receptor subtypes in contractility of the rabbit abdominal aorta in vitro

Jan Gnus$^{1}$, Albert Czerski$^{1,2}$, Stanisław Ferenc$^1$, Wojciech Zawadzki$^{1,2}$, Wojciech Witkiewicz$^1$, Agnieszka Rusiecka$^{1,2}$, Jolanta Bujok$^{1,2}$, Willy Hauzer$^1$, Maciej Janecz$^2$, Aleksander Chrószcz$^2$

$^1$General and Vascular Surgery Ward of Regional Specialist Hospital, Research and Development Centre in Wroclaw, Wroclaw, Poland
$^2$Wrocław University of Environmental and Life Sciences, Faculty of Veterinary Medicine, Department of Animal Physiology and Biostructure, Wroclaw, Poland

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Abstract

Investigation of the effect of $\alpha_1$-adrenergic receptor subtypes on the contraction of the abdominal aorta will allow for more effective treatment of hypertension by use of selective antagonists. The aim of the study was to evaluate the participation of $\alpha_1$-adrenergic receptor subtypes in the contractility of the aortic smooth muscle cells in rabbits. The in vitro experiments were performed in isolated tissue preparations from 30 adult female New Zealand rabbits. The abdominal aortic sections were placed in organ bath chambers and contracted with increasing doses of non-selective $\alpha_1$-adrenergic receptor agonist phenylephrine without pre-incubation or after incubation in $\alpha_1$-adrenergic receptor subtype-selective or non-selective antagonists. Separate sections were incubated with increasing concentrations of antagonists. Phenylephrine caused maximal rise in arterial smooth muscle tone to 4.75 ± 0.47 mN. The most potent in blocking phenylephrine induced contraction was 5-metylurapidil ($\alpha_1A$-adrenergic receptor antagonist) followed by phentolamine and prazosin (non-selective $\alpha_1$-adrenergic receptor antagonists); BMY 7378 ($\alpha_1D$-adrenergic receptor antagonist), cyclazosin and L-765.314 ($\alpha_1B$-adrenergic receptor antagonists) were less effective. All antagonists, except BMY 7378 elicited relaxation of non-precontracted aorta in dose dependent manner. Our results indicate that postsynaptic $\alpha_1A$ receptors are the most potent in producing rabbit abdominal aorta contraction, while $\alpha_1B$ and $\alpha_1D$ subtypes are less effective.

Vascular smooth muscle cells, aortic section, contraction, 5-metylurapidil, cyclazosin, $\alpha_1$-adrenergic antagonist

Alpha$\_1$-adrenergic receptors belong to stimulating receptors which are responsible for the regulation of many biological processes. They regulate blood pressure by changing the tonus of the vascular muscles. According to the current classification developed by the International Union of Pharmacology Subcommittee on Nomenclature for Adrenoceptors, there are three subtypes of $\alpha_1$-adrenergic receptor: $\alpha_{1A}$, $\alpha_{1B}$, $\alpha_{1D}$ (Hieble and Ruffolo 1996; Docherty 1998; Langer 1999).

Alpha$\_1$-adrenergic receptors are connected with calcium signalling. Stimulation of $\alpha_1$-adrenergic receptors cause an intracellular influx of Ca$^{2+}$ ions by opening calcium channels sensitive to 1.4-dihydropyridine, while the $\alpha_{1D}$-adrenergic receptors release intracellular Ca$^{2+}$ (Han et al. 1987; Minneman 1988; Suzuki et al. 1990). Stimulation of $\alpha_1$-adrenergic receptors leads to the activation of phospholipase C (PLC) and its inositol-phosphate-hydrolysing activity. The second mechanism of signal transmission through the $\alpha_1$-adrenergic receptor is the influx of Ca$^{2+}$ ions to the cells by the activation of calcium channels of L type. In many studies it was shown that the effectiveness of $\alpha_1$-adrenergic receptors in activating inositol phosphate hydrolysis and increasing the intracellular concentrations of calcium ions is as follows: $\alpha_{1A} > \alpha_{1B} > \alpha_{1D}$.
Alpha₁-adrenergic receptors are present in different tissues including the brain (all subtypes), smooth muscles (α₁A- and α₁D-adrenergic), liver (α₁A- and α₁D-adrenergic), heart, and prostate (α₁A-adrenergic).

The aim of this experiment was to study the effect of α₁-adrenergic receptor and its subtypes on the contractility of aortic smooth muscle in rabbits.

Materials and Methods

The study was conducted on 30 adult female New Zealand rabbits, weighing 3–4 kg, from which the specimens of the abdominal aorta were collected. The study was approved by a local II Ethical Review Board (approval No. 89/2010).

The experimental animals were euthanized by intravenous injection of sodium pentobarbital at the dose of 50 mg/kg weight. Immediately after death, 4–5 cm long specimens of the abdominal aorta were collected and cut into 1.5 cm long sections. The aortic diameter was from 4 to 6 mm on average. The sections were placed in 4 chambers (volume of 20 ml each) of an automatic water bath. The samples were placed horizontally by threading the Safil 4.0 surgical thread through the lumen of the aorta.

All the samples were stretched to an initial tension of 5 mN. That tonus was a baseline used for comparisons of the obtained results (Eckert 2000). The time required to balance the record was determined experimentally at 20 min (Caryl 1999). 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 (Eckert 2000; Fraňová et al. 2009). 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 40 min. Afterwards, agonists and antagonists of adrenergic receptors were introduced to the incubation chambers with an isolated section material.

The following chemical substances were added (Sigma-Aldrich, USA): phenylephrine, prazosin, phentolamine, cyclazosin, L-765.314, BMY 7378 and 5-methylurapidil.

The experimental protocol was as follows: agonists were added to organ baths in which aorta strips were mounted. The doses-cumulation system was used (0.0005–0.5 µM). Preparations were treated for 30 min with each concentration of agonist, to obtain the maximal tonus. Following concentrations of antagonists 0.0002–0.2 µM were added into the incubation chambers with aorta strips. Tissue sections were treated for 30 min with each doses of antagonist in order to obtain maximal parameters of reaction. Aorta strips were treated with 0.02 µM of antagonist for 30 min. Afterwards the agonist in doses-cumulation system was added.

When evaluating the obtained results, we analyzed the strength of contractions expressed in mN (contractility amplitude). 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 abdominal aorta, obtained from rabbits had a long life-span and could survive in vitro for 6-8 h. After 20 min of control recording, agonists and antagonists of adrenergic receptors were added to the incubation chamber. The results are summarized in the following sections.

Phenylephrine (non-selective agonist of α₁-adrenergic receptors)

Administration of phenylephrine at a concentration of 0.005 µM caused an increase in the aortic muscle tonus by 2.74 ± 0.4 mN. After 10-fold increase in phenylephrine concentration (up to 0.05 µM), the aortic muscle tonus increased by 4.75 ± 0.47 mN.

Phentolamine (non-selective antagonist of α₁-adrenergic receptors)

Administration of phenylephrine at a concentration of 0.002 µM to the incubation chamber caused a decrease in the muscle tonus (dilatation) by a mean of -0.58 ± 0.12 mN. A 10-fold increase in the phentolamine concentration (up to 0.02 µM) did not cause any further changes in the muscle tonus. Blockade of α₁-adrenergic receptors by addition of 0.02 µM phentolamine to the incubation chamber resulted in reduction of tissue reaction to
phenylephrine by 75%. The maximal tonus produced by an agonist was 1.16 ± 0.19 mN. A 10-fold lower concentration of phentolamine (0.002 μM) had no influence on the aortic muscle response to phenylephrine.

Prazosin (non-selective antagonist of α₁-adrenergic receptors)

Blockade of α₁-adrenergic receptors by addition of prazosin to the incubation chamber resulted in a gradual dilatation of the aortic muscle in rabbits. In case of a 0.002 μM dose, there was a decrease in the muscle tonus of -0.09 ± 0.02 mN. An increase of prazosin concentration, up to 0.02 μM, caused further dilatation of the aortic muscle of -0.58 ± 0.05 mN. Blockade of α₁-adrenergic receptor by addition of prazosin to the incubation chamber at a concentration of 0.02 μM caused a decrease in response to 0.05 μM of phenylephrine by 62%.

5-methylurapidil (selective antagonist of α₁A-adrenergic receptors)

Administration of 5-methylurapidil at a dose of 0.02 μM caused a significant (P ≤ 0.01) decrease in the aortic muscle tonus in rabbits, that is of -1.17 ± 0.09 mN. After the blockade of the α₁A-adrenergic receptor by addition of 5-methylurapidil to the incubation chamber, the administration of 0.05 μM phenylephrine resulted in an increase of the aortic muscle tonus of 0.79 ± 0.19 mN. Phenylephrine dose elevation up to 0.5 μM caused further increase of muscle tonus by 0.7 ± 0.29 mN.

Cyclazosin (selective antagonist of α₁B-adrenergic receptors)

Administration of cyclazosin at a dose of 0.002 μM caused a decrease in the muscle tonus of -0.49 ± 0.10 mN. After blockade of the α₁B-adrenergic receptor by 0.02 μM cyclazosin, the administration of 0.05 μM phenylephrine resulted in an increase of the aortic muscle tonus of 1.49 ± 0.14 mN. An increase in phenylephrine dose up to 0.5 μM caused further increase in the muscle tonus by 2.89 ± 0.19 mN.

L-765.314 (selective antagonist of α₁B-adrenergic receptors)

Administration of L-765.314 at a dose of 0.0002 μM to the incubation chamber caused a decrease of the aortic muscle tonus of -0.19 ± 0.09 mN. After the blockade of the α₁B-adrenergic receptor by 0.02 μM of L-765.314, the administration of 0.005 μM phenylephrine resulted in an increase of the aortic muscle tonus of 1.49 ± 0.14 mN. Phenylephrine dose elevation up to 0.05 μM caused further increase of muscle tonus by 2.98 ± 0.16 mN.

BMY 7378 (selective antagonist of α₁D-adrenergic receptors)

Introduction of BMY 7378 to the incubation chamber did not cause any visible changes in the aortic muscle tonus. After the blockade of α₁D-adrenergic receptor by the administration of BMY 7378 at a dose of 0.02 μM, the administration of 0.05 μM phenylephrine resulted in an increase of tissue reaction of 2.57 ± 0.25 mN.

Discussion

Stimulation of the α₁ and α₂-adrenergic receptor by in vitro addition of adrenaline or noradrenaline, as well as by a natural secretion of adrenaline by adrenal glands or noradrenaline at postganglionic adrenergic nerves causes a contraction of the arteries in animals. This is one of the mechanisms allowing for blood pressure regulation (Stephenson and Summers 1987; Willems et al. 2001). Sympathetic nervous system overactivity is one of the mechanisms playing role in the development of hypertension, which disrupts the function of many organs and leads to remodelling of the arterial wall (Kochoňová et al. 2009). Substances that block α₁-adrenergic receptors are used as additional therapy in the treatment of hypertension. Expression of α₁-adrenergic receptor subtypes varies
in different tissues and side-effects of the therapy can be minimized by greater receptor subtype selectivity.

We found that phenylephrine stimulates all subtypes of $\alpha_1$-adrenergic receptor in the aorta. Blockade of that receptor with prazosin led to the decrease of tissue reaction to phenylephrine in rabbit abdominal aorta. This showed that the $\alpha_1$-adrenergic receptor mediates the observed contraction in response to phenylephrine, i.e. aortic smooth muscle contractility. It is also interesting that the blockade of the $\alpha_1$-adrenergic receptor...
alone by adding prazosin led to a decrease in the aortic muscle tonus in vitro. Perhaps the \( \alpha_1 \)-adrenergic receptor is constitutively stimulated in vitro by endogenous agonist noradrenaline secreted in the aortic wall by postganglionic fibres of the sympathetic system (Hoffman and Lefkowitz 1996).

In vivo studies showed that stimulation of the \( \alpha_1 \)-adrenergic receptor by intravenous phenylephrine caused constriction of the arterial vessels in pigs and dogs (Willems et al. 2001). The researchers showed a significant increase in the muscle tonus of the arterial vessels, leading to an increased arterial blood pressure of the examined animals.

In our study, we found that blockade of \( \alpha_{1A} \)-adrenergic receptor with 5-methylurapidil resulted in a decrease of the aortic muscle tonus in rabbits which proved that this receptor takes part in contractility. Similarly, blockade of \( \alpha_{1A} \)-adrenergic receptor with 5-methylurapidil significantly influenced the tissue response to phenylephrine. However, 5-methylurapidil was not able to completely block the phenylephrine contraction. This suggests that the \( \alpha_{1A} \)-adrenergic receptor is the most potent but not the only one receptor subtype influencing the aortic vasoconstriction in rabbits. Similar results were also obtained for \( \alpha_{1B} \)-adrenergic receptors. Blockade of \( \alpha_{1B} \)-adrenergic receptors by administration of cyclazosin to the incubation chamber resulted in a significant decrease in the muscle tonus. However, blockade of that receptor did not diminish tissue response to phenylephrine. This suggests that \( \alpha_{1A} \)-adrenergic receptor is influencing the aortic vasoconstriction in response to phenylephrine, but its role in the contractility of the rabbit abdominal aorta is not as important as \( \alpha_{1B} \)-adrenergic receptor.

We detected blockade of \( \alpha_{1D} \)-adrenergic receptors by administration of BMY 7378 to the incubation chamber that resulted in a non-significant decrease in the muscle tonus in rabbits. The administration of BMY 7378 influenced the contractile response to phenylephrine similarly to \( \alpha_{1D} \)-adrenergic receptor antagonists. This suggests that \( \alpha_{1D} \)-adrenergic receptor might be partially involved in the vasoconstriction of the abdominal aorta in rabbits. However, direct binding studies showed that BMY 7378 inhibits \( ^3 \)H-prazosin binding in the rabbit aorta with low affinity, presumably by binding to \( \alpha_{1A} \)- and \( \alpha_{1B} \)-adrenergic receptors. Moreover, relative level of expression of \( \alpha_{1D} \)-adrenergic receptor mRNA in rabbit aorta is approximately 50 \( \times \) lower than that of the \( \alpha_{1A} \)-adrenergic receptor mRNA (Satoh et al. 1998; Piao et al. 2000). Negligible \( \alpha_{1D} \)-adrenergic receptor function in aortic tonus regulation seems to be typical of rabbits, because administration of BMY 7378 blocked the \( \alpha_{1D} \)-adrenergic receptor in the aorta of rats, leading to its dilatation (Goetz et al. 1995).

According to Willems et al. (2001), phenylephrine influences the contractility of the carotid arteries and aorta in dogs, especially by means of \( \alpha_{1A} \)- and \( \alpha_{1D} \)-adrenergic receptors; the \( \alpha_{1B} \) subtype is less significant for aortic contractility. Abound et al. (1993) showed that the administration of 5-methylurapidil, benoxanthian, and WB 4101 to the incubation chamber decreased the aorta contraction strength in rats in response to noradrenaline. Blockade of the \( \alpha_{1B} \)-adrenergic receptor by addition of L-765.314 did not have any influence on tissue reaction to phenylephrine (Willems et al. 2001) which is not in agreement with our results. The role of \( \alpha_{1B} \)-adrenergic receptor in arteries vasoconstriction in dogs seems controversial. On the other hand, response to noradrenaline was decreased in \( \alpha_{1B} \)-adrenergic receptor knockout mice in vivo, thus indicating species differences in the participation of \( \alpha_i \)-adrenergic receptor subtypes in the vasoconstriction (Vecchione et al. 2002). Our results, which showed that \( \alpha_{1B} \)-adrenergic receptors are important in rabbit aorta contraction, are consistent with data obtained by Marucci et al. (2005).

The obtained results confirm that \( \alpha_i \)-adrenergic receptors play a key role in adrenergic regulation of the rabbit abdominal aorta contractility. It seems that postsynaptic \( \alpha_{1A} \)-receptors are the most potent in producing abdominal aorta contraction, \( \alpha_{1B} \) and \( \alpha_{1D} \) subtypes are less effective. There are differences between species in the presence of functional \( \alpha_1 \)-adrenergic receptor subtypes, which should be taken into account when
administering drugs affecting the sympathetic nervous system as well as when selecting experimental animal models.

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