

## Comparison of the Effect of $\alpha_1$ - and $\alpha_2$ -Adrenoceptor Agonists and Antagonists on Muscle Contractility of the Rabbit Abdominal Aorta *in vitro*

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The aim of the study was to demonstrate the effect of selected agonists and antagonists of  $\alpha$ -adrenergic receptors on muscle contractility of the rabbit abdominal aorta *in vitro* with particular emphasis on  $\alpha_2$ -adrenergic receptor subtypes. The study was conducted on 30 New Zealand breed rabbits from which specimens of the abdominal aorta were collected. The sections were set up in an automatic water bath in a Krebs-Henseleit buffer at 37°C. The experiments showed that  $\alpha_1$ -adrenergic receptors played the main role in the contractile response of the rabbit abdominal aorta. Stimulation of  $\alpha_1$ -adrenergic receptor by administration of phenylephrine resulted in an increase in smooth muscle tonus of the rabbit abdominal aorta by an average of 4.75 mN. The reaction after stimulation of  $\alpha_2$ -adrenergic receptors by similar doses of their agonists was much weaker. Prolonged tissue response time and time needed to reach maximum tonus for  $\alpha_2$ -adrenergic receptor agonists were observed. The obtained results confirm the thesis that the  $\alpha_1$ -adrenergic receptor is the most important factor controlling the contractility of the rabbit abdominal aorta, but the  $\alpha_2$ -adrenergic receptor is also involved in maintaining muscle tissue tonus.

Key words: Abdominal aorta, adrenergic receptors, contractility, *in vitro* studies, rabbit.

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Activation of the sympathetic system plays an important role in the regulation of vascular resistance (OSWALD *et al.* 1983). It is generally assumed that the postsynaptic cooperation of  $\alpha_1$ - and  $\alpha_2$ -adrenergic receptors in blood vessels is an important element of lumen regulation (DOCHERTY *et al.* 1979; DREW *et al.* 1979).

The  $\alpha_1$ -adrenergic receptors are stimulating receptors responsible for regulation of many biological processes; they are heterogeneous, i.e. they have different subtypes. There are 3 subtypes of the  $\alpha_1$ -adrenergic receptor:  $\alpha_{1A}$ ,  $\alpha_{1B}$ ,  $\alpha_{1D}$  (HIEBLE *et al.* 1995; HIEBLE & RUFFOLO 1996; DOCHERTY 1998; BRODDE & MICHEL 1999; LANGER 1999).

The  $\alpha_1$ -adrenergic receptor is connected with calcium signalling, which means that its stimula-

tion leads to an increased concentration of intracellular calcium ions (SMITH *et al.* 1997). Stimulation of the  $\alpha_1$ -adrenergic receptor causes an intracellular influx of  $\text{Ca}^{2+}$  ions by opening calcium channels sensitive to 1,4-dihydropyridine, while the  $\alpha_{1B}$ -adrenergic receptor releases intracellular  $\text{Ca}^{2+}$  (HAN *et al.* 1987; MINNEMAN 1988; SUZUKI *et al.* 1990). The  $\alpha_1$ -adrenergic receptors are present in the brain (all subtypes), smooth muscles ( $\alpha_{1A}$ - and  $\alpha_{1D}$ -adrenergic), liver ( $\alpha_{1A}$ - and  $\alpha_{1D}$ -adrenergic), heart and prostate ( $\alpha_{1A}$ -adrenergic).

The  $\alpha_2$ -adrenergic receptors are involved in the regulation of many physiological processes including the cardiovascular system. They belong to the family of G proteins that inhibit adenylyl cyclase and thus lower the intracellular concentration of cAMP.

According to the current classification adopted by the International Union of Pharmacology Subcommittee on Nomenclature for Adrenoceptors, there are 3 subtypes of the  $\alpha_2$ -adrenergic receptor:  $\alpha_{2A}$ ,  $\alpha_{2B}$  and  $\alpha_{2C}$ . This classification is the result of adopting pharmacological criteria and using molecular cloning techniques (BYLUND *et al.* 1988; LOMASNEY *et al.* 1991; HEIN *et al.* 1995; BYLUND 1998). An  $\alpha_{2D}$  subtype was also distinguished, but now it is assumed to be the same as the  $\alpha_{2A}$  receptor, though identified in other animal species.

The  $\alpha_2$ -adrenergic receptor subtypes are involved in the regulation of distinct physiological functions. Existing pharmacological and genetic studies indicate that the  $\alpha_{2A}$  subtype is responsible for most of the reactions typical for  $\alpha_2$ -adrenergic receptors, including adjustment of anti-hypertensive mechanisms (ALTMAN 1999). In addition, it plays a central role in presynaptic noradrenaline release, increases the local blood flow in the prefrontal cortex and is responsible for working memory performance (MACMILLAN *et al.* 1996; ALTMAN 1999; AVERY *et al.* 2000).  $\alpha_{2A/D}$  and  $\alpha_{2C}$  receptors are also associated with Attention Deficit Hyperactivity Disorder (CHO *et al.* 2008). Moreover, activation of  $\alpha_2$ -adrenoceptors affects digestive system motility, especially activation of the  $\alpha_{2A}$  subtype, which is probably responsible for its functioning.  $\alpha_2$  receptors are also involved in the regulation of uterine contractile activity. A study conducted *in vivo* on rat uterus sections showed that  $\alpha_{2A}$  and  $\alpha_{2C}$  subtypes were responsible for reducing the contractile response after administration of norepinephrine in the final days of pregnancy (GASPAR *et al.* 2007).

Compared to the  $\alpha_{2A}$  subtype, the typical functions of the  $\alpha_{2B}$  and  $\alpha_{2C}$  subtypes are less known. In contrast to the presynaptic localisation of  $\alpha_{2A}$  and  $\alpha_{2C}$  subtypes,  $\alpha_{2B}$  receptors are postsynaptic (DREW *et al.* 1979; PHILIPP *et al.* 2002). In addition, the  $\alpha_{2B}$  subtype mediates the induction of uterine contractions in pregnant rats, unlike  $\alpha_{2A}$  and  $\alpha_{2C}$  subtypes, which inhibit uterine contractions.

The  $\alpha_{2C}$ -adrenoceptor subtype is localised mainly in the central nervous system. Its role in hemodynamic processes has not been fully understood yet; it seems to mediate vein contraction (GAVIN *et al.* 1997), but it was also found in the arterial smooth muscle (CHOTANI *et al.* 2000). It participates in the regulation of the cerebral cortex, and activation of these receptors in a depressive mood may be beneficial for the treatment of neuropsychiatric disorders and diseases (GYIRES *et al.* 2009).

Summing up, separate locations within the body may indicate that  $\alpha_2$ -adrenoceptor subtypes ( $\alpha_{2A}$ ,  $\alpha_{2B}$ ,  $\alpha_{2C}$ ) are involved in various biological processes and have different regulatory functions.

## Material and Methods

The study was approved by the Local II Ethical Review Board, approval no. 89/2010.

The study was conducted on 30 New Zealand breed rabbits, weighing 3–4 kg, from which specimens of the abdominal aorta were collected.

The experimental animals were euthanized by administering an intravenous solution of pentobarbital (prep. Morbital), and immediately after death, 4–5 cm long specimens of the abdominal aorta were collected and cut into 1.5 cm long sections. The sections were cleared from fat and connective tissue. The aorta diameter in a New Zealand breed rabbit weighing 3–4 kg was on average 4 to 6 mm. The sections were set up in an automatic water bath in four 20 ml chambers. The samples were placed horizontally by threading safil 4.0 surgical thread inside the aorta lumen and mounted so that changes in the area of their transverse sections were registered.

All the samples were stretched to a tension of 5 mN. The time required to balance the record was determined experimentally at 40 minutes. The Krebs-Henseleit buffer was used and the incubation environment contained: NaCl – 118 mM; KCl – 4.7 mM;  $\text{CaCl}_2$  – 2.5 mM;  $\text{MgSO}_4$  – 1.6 mM;  $\text{NaHCO}_3$  – 24.3 mM;  $\text{KH}_2\text{PO}_4$  – 1.18 mM; glucose – 5.6 mM (ECKERT 2000). Incubation of the sections was carried out at a temperature of 37°C in a gaseous mixture of oxygen and carbon dioxide used in the following proportions: 95% of  $\text{O}_2$  and 5% of  $\text{CO}_2$ , in order to obtain a pH value of 7.3–7.5. The aortic contractions were registered with isometric transducers (Letica Scientific Instruments) combined with bridge amplifiers (BridgeAmp, ADInstruments, Australia) and a 4-channel data acquisition system (PowerLab/400, ADInstruments) connected to a Macintosh computer. The spontaneous contractile activity of the aortic muscle was recorded for 30 minutes. Afterwards, the agonists and the antagonists of the adrenergic receptors were introduced into the incubation chambers with an isolated section. The agonists were always added to the chambers 40 minutes after the antagonists.

The following chemical substances were added: phenylephrine-  $\alpha_1$ -adrenergic receptor agonist (Sigma-Aldrich), xylazine-  $\alpha_2$ -adrenergic receptor agonist (Sigma-Aldrich), oxymetazoline-  $\alpha_{2A}$ -adrenergic receptor agonist (Sigma-Aldrich), B-HT 933- selective agonist of the  $\alpha_2$  receptor (Sigma-Aldrich), phentolamine-  $\alpha_1$ -adrenergic receptor antagonist (Sigma-Aldrich), yohimbine-  $\alpha_2$  receptor antagonist (Sigma-Aldrich) and RX821002-selective antagonist of the  $\alpha_2$  receptor (Sigma-Aldrich).

Doses of the preparations were established experimentally by 10-fold dilutions. When defining the experimental dose, we introduced the preparation into 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 preparation triggering the required effect and confirmed in a few subsequent experiments.

We evaluated the results by analysing the strength of contractions expressed in mN (contractility amplitude), time to tissue reaction (from administration to a visible effect) and time to maximal tonus. The results of the tests were processed with the use of Microsoft Office Excel 2000 and analysed statistically with Student's t-test and a single-factor analysis of variance (ANOVA) for independent variables.

## Results

The *in vitro* lifetime of rabbit abdominal aorta sections amounted to 6-8 hours with no statistically significant difference in contractile activity. After 30 minutes of control recording (during which the strength of the muscle tone stabilised and the metabolic activity of the tissue rose due to temperature and oxygenation increase), agonists and antagonists of the adrenergic receptors were added to the incubation chamber, in a system of accumulated doses, starting from the lowest concentration.

The results were processed with the strength of contractions expressed in mN (contractility amplitude), time to tissue reaction (from administration to visible effect) and time to maximal tonus. Detailed reactions of the rabbit abdominal aorta are presented in the graphs: contractility amplitude (Figs 1, 2, 3), time to tissue reaction (Figs 4, 5, 6) and maximal tonus (Figs 7, 8).

Phenylephrine increased the smooth muscle tonus in the rabbit abdominal aorta by an average 4.75 mN at a concentration of 100  $\mu$ M. In the case of xylazine, a maximum increase in the muscle tonus was observed at a concentration of 100  $\mu$ M, and this amounted to 1.18 mN. In relation to the maximum response induced by the administration of phenylephrine at a concentration of 100  $\mu$ M, the maximum muscle tonus resulting from the administration of xylazine at the same concentration was almost four times smaller and equalled 24.8% of the initial tonus induced by phenylephrine. The administration of B-HT 933 at a concentration of 100  $\mu$ M caused a tonus increase constituting only 12% of the tonus recorded after the administration of

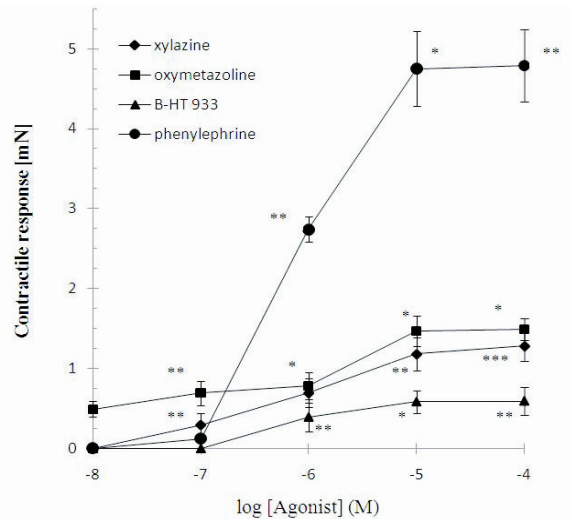


Fig. 1. Relationship between the increase of rabbit abdominal aorta muscle tonus stimulated by selected  $\alpha$ -adrenoceptor agonists and the agonist concentration, (\* $P \leq 0.001$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.05$ ).

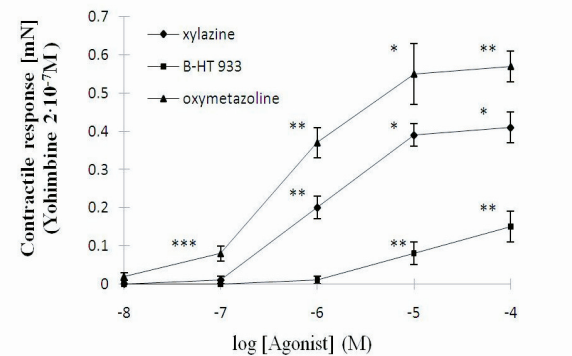


Fig. 2. Relationship between the increase of rabbit abdominal aorta muscle tonus stimulated by selected  $\alpha_2$ -adrenoceptor agonists and the agonist concentration.  $\alpha_2$  receptors were previously blocked by  $2 \cdot 10^{-7}$  M yohimbine administered to the incubation chamber, (\* $P \leq 0.001$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.05$ ).

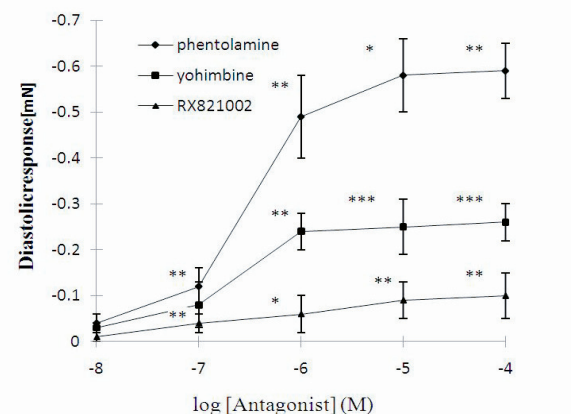


Fig. 3. Relationship between tonus decrease (relaxation of muscle) of the rabbit abdominal aorta stimulated by selected  $\alpha_1$ - and  $\alpha_2$ -adrenoceptor antagonists and the antagonist concentration (\* $P \leq 0.001$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.05$ ).

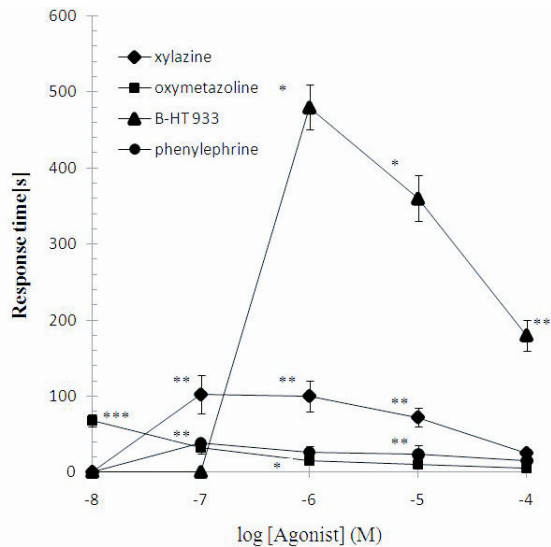


Fig. 4. Relationship between the response time of tissue stimulated by selected  $\alpha_2$ -adrenoceptor agonists and the agonist concentration, (\* $P \leq 0.001$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.05$ ).

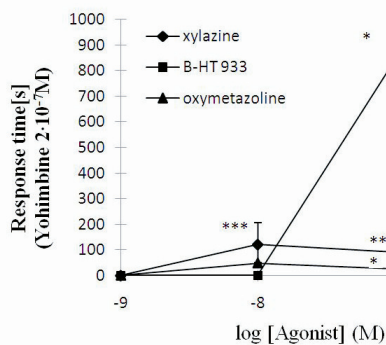


Fig. 5. Relationship between the response time of tissue stimulated by selected  $\alpha_2$ -adrenoceptor agonists and the agonist concentration.  $\alpha_2$  receptors were previously blocked by  $2 \cdot 10^{-7}$  M yohimbine administered to the incubation chamber, (\* $P \leq 0.001$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.05$ ).

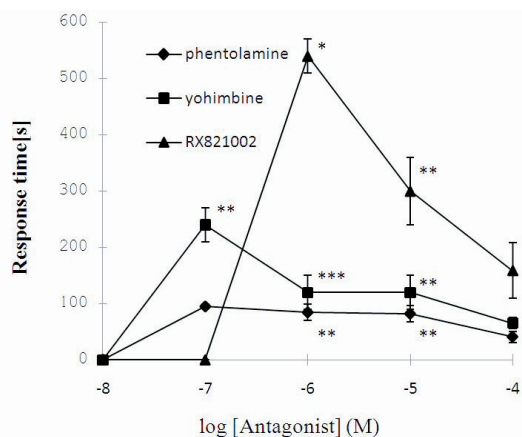


Fig. 6. Relationship between the response time of tissue stimulated by selected  $\alpha_1$ - and  $\alpha_2$ -adrenoceptor antagonists and the antagonist concentration, (\* $P \leq 0.001$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.05$ ).

phenylephrine. From among the studied agonists of the  $\alpha_{2A}$ -adrenergic receptor, the highest increase in the rabbit abdominal aorta smooth muscle tonus was observed after the administration of oxymetazoline. At a concentration of  $100 \mu\text{M}$ , it boosted the muscle tonus by 1.47 mN.

The results described above indicate that among the tested  $\alpha_2$ -adrenoceptor agonists, the highest stimulation of smooth muscle in the rabbit abdominal aorta can be induced by using oxymetazoline, the agonist of the  $\alpha_{2A}$ -adrenergic receptor subtype (Figs 1, 4).

At the same time, blocking the  $\alpha_2$ -adrenoceptors by  $2 \cdot 10^{-7}$  M yohimbine caused a decrease in the maximum response elicited by oxymetazoline by about 64% compared to the reaction without the antagonist (Fig. 2). In the case of xylazine, the observed reduction in response was 67%, and for B-HT 933, it exceeded 74% (Figs 2, 5, 8).

The experiments also examined the impact of  $\alpha_1$ - and  $\alpha_2$ -adrenoceptor antagonists on the contractile activity of the rabbit abdominal aorta. The greatest reduction in the vessel muscle tonus was induced by phentolamine - the  $\alpha_1$  receptor antagonist. It caused a tonus decrease by -0.58 mN. Yohimbine administered to the chamber at a concentration of  $2 \cdot 10^{-7}$  M reduced the tonus by -0.25 mN. RX 821002 had the weakest effect on contractility, causing a decrease in the tonus by -0.09 mN (Figs 3, 6, 8).

## Discussion

The  $\alpha_1$ - and  $\alpha_2$ -adrenergic receptors regulate the blood vessel lumen and affect the blood pressure (DOCHERTY *et al.* 1979; DREW *et al.* 1979). The  $\alpha_1$ -adrenergic receptors are localised postsynaptically. The main effect of  $\alpha_1$ -adrenergic receptor stimulation is narrowing of the blood vessel lumen, resulting in increased blood pressure in the body.  $\alpha_1$ -adrenoceptors play a major role in the contractile response of arteries, but there is little evidence for the direct involvement of the  $\alpha_2$ -adrenoceptors in blood vessel contractility (DOCHERTY 1998; GUIMARÃES & MOURA 2001). The aim of this study was to investigate the effect of  $\alpha_2$ -adrenoceptors on the smooth muscle contractility of the rabbit aorta *in vitro*. The second objective was to compare the effects of  $\alpha_1$ - and  $\alpha_2$ -adrenergic receptors stimulated by selected agonists inoculated to the incubation chambers on the contractility of isolated aortic muscle sections.

The results obtained show that rabbit abdominal aorta muscle contraction mediated by  $\alpha$ -adrenergic receptors stimulated by the administration of agonists depends mainly on the  $\alpha_1$ -adrenoceptor

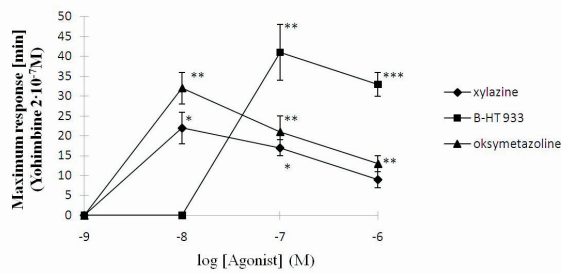


Fig. 7. Relationship between the time to reach maximum response of the tissue stimulated by selected  $\alpha_2$ -adrenoceptor agonists and the agonist concentration.  $\alpha_2$ -receptors were previously blocked by  $2 \cdot 10^{-7}$  M yohimbine administered to the incubation chamber, (\* $P \leq 0.001$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.05$ ).

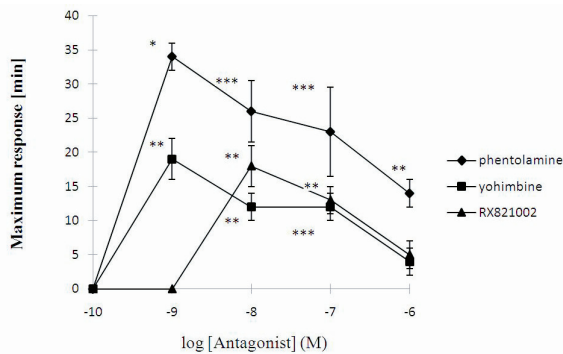


Fig. 8. Relationship between the time to reach maximum response of the tissue stimulated by selected  $\alpha_1$ - and  $\alpha_2$ -adrenoceptor antagonists and the antagonist concentration, (\* $P \leq 0.001$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.05$ ).

(Figs 1, 4). The administration of phenylephrine to the incubation chamber evoked a much stronger muscle response than the administration of a similar concentration of  $\alpha_2$ -adrenergic receptor agonists (Figs 1, 4). The response observed after the stimulation of the  $\alpha_2$ -adrenoceptors was much weaker. Compared to the maximum response induced by phenylephrine, the maximum contractile response induced by oxymetazoline represented approximately 31%, and by B-HT 933, 12% of the activity resulting from the stimulation of the  $\alpha_1$ -adrenoceptors by phenylephrine (Fig. 1).

The largest increase in contractile activity induced by oxymetazoline may be due to the fact that among three  $\alpha_2$ -adrenoceptor subtypes, the  $\alpha_{2A}$  receptors are the most involved in the norepinephrine-dependent contraction of blood vessels. At the same time, the concentration of  $\alpha_{2A}$  receptors in the blood vessels, both veins and arteries, is much higher than the concentration of other subtypes of adrenergic receptors. The dominant role of the  $\alpha_{2A}$  subtype in the regulation of the cardiovascular system has also been demonstrated in ex-

periments based on the deletion of genes encoding the  $\alpha_{2A}$ -adrenoceptor subtype (BREDE *et al.* 2002). The deletion led to an increase in blood pressure, increased heart rate and susceptibility to heart failure (BREDE *et al.* 2002). However, in tissues expressing the  $\alpha_{2A}$  subtype, the presence of the  $\alpha_2$ -adrenoceptor agonists induced the narrowing of blood vessels (MACMILLAN *et al.* 1996). Oxymetazoline is not fully selective towards  $\alpha_{2A}$ -adrenoceptors, and it is also a partial agonist of  $\alpha_{1A}$ -adrenoceptors (MINNEMAN & ESBENSHADE 1994) and 5-HT $_{2A}$  receptors (LACHNIT *et al.* 1997). Therefore, the aortic reaction to oxymetazoline should be compared to that induced by UK14304, a full agonist of the  $\alpha_2$ -adrenergic receptors.

Stimulation of the receptors by xylazine induced a slightly weaker reaction than using oxymetazoline. The weakest contractility was evoked by B-HT 933. At the same time, after blocking the adrenergic receptors, the greatest reduction in contraction response was observed for B-HT 933.

An interesting reaction was observed after the administration of yohimbine into the chamber (Figs 1, 2). With the exception of BHT-933, there is no rightward shift of the curves in the presence of yohimbine, but only a depression of the maximal response. In numerous studies performed in other groups, yohimbine behaved as a competitive antagonist of  $\alpha_2$ -adrenergic mediated contractile responses. Our antagonism experiments demonstrated that the constrictor response to oxymetazoline and xylazine was inhibited by yohimbine in a non-competitive fashion, although the non-competitive antagonism by yohimbine is quite unlike the response of the rabbit artery to those drugs. The reason for this discrepancy is unclear and further studies will be needed in order to resolve this phenomenon.

The second issue is that phentolamine, yohimbine and RX82002 seem to be inverse agonists in the rabbit abdominal aorta. This is explained by the constitutive mobilisation of intracellular  $Ca^{2+}$  and the inhibition of cAMP production. This could determine the increase in the number of receptors that are spontaneously active in the absence of an agonist (LEFKOWITZ *et al.* 1993; BLACK & SHANKLEY 1995; BOND *et al.* 1995; MILLIGAN *et al.* 1995). The antagonists bind to the receptor and change it from an activated state to an inactivated one or bind to inactive receptors and disrupt the equilibrium between the two states (active and inactive) in favour of the inactive state (SCHUTZ & FREISSMUTH 1992). New evidence suggests a two-state model, in which the receptors are in equilibrium between the inactive conformation and a spontaneously active conformation that couples to the G protein in the absence of the ligand. The first state can be fixed by inverse agonists

(BOND *et al.* 1995). The present work shows that phentolamine, yohimbine and RX82002 do not act as antagonists but as inverse agonists; they inhibit the increase in the resting tone in the absence of the agonist. In the case of yohimbine and RX82002, similar results were obtained by other authors (WADE *et al.* 2001). They concluded that several factors, such as the type of assay, receptor subtypes, cell type and local cellular G protein concentrations, may affect constitutive receptor activity.

The effects of the agonists and antagonists of the adrenergic receptors on the time to tissue reaction and the time necessary to reach the peak response of the rabbit aorta were assessed in the same study. The quickest reaction was noticed after the administration of oxymetazoline and phenylephrine to the incubation chamber, whereas the slowest tissue response was induced by 933rd B-HT. A similar relationship can be observed after blocking the aortic sections with yohimbine. The quickest aortic contractile response was recorded after the administration of oxymetazoline, and the slowest was in the case of 933rd B-HT. Phenylephrine and oxymetazoline are similarly effective in triggering the contractile response of the investigated sections. This may be due to a non-specific binding of oxymetazoline to  $\alpha_1$ -adrenergic receptors, particularly at higher concentrations of the agonist. Moreover, the reaction time after the administration of B-HT 933, a specific agonist of the  $\alpha_2$ -adrenergic receptors, was approximately 10 times higher than for oxymetazoline. A similar situation was found for antagonists; the time to tissue reaction after the administration of phentolamine was about 7 times shorter than for RX821002, a selective antagonist of  $\alpha_2$ -adrenergic receptors. This data indicates that the quickness of the rabbit abdominal aorta contractile reaction depends mainly on the  $\alpha_1$ -adrenergic receptors, but later on, it is further enhanced by the activation of the  $\alpha_2$ -adrenergic receptors.

Previous *in vitro* studies suggest that  $\alpha_1$  receptors constitute the main factors involved in norepinephrine-dependent contractions in the rat and mouse mesenteric artery (HUSSAIN *et al.* 2000). Other studies concerning the effect of  $\alpha_1$ -adrenoceptor subtypes on the contractility of the rabbit abdominal aorta show that the contractile response is mainly conditioned by postsynaptic  $\alpha_{1A}/\alpha_{1D}$  subtypes (ABOUD *et al.* 1993; GUIMARÃES & MOURA *et al.* 2001; GNUS *et al.* 2012). The available literature contains little evidence on the direct effect of  $\alpha_2$ -adrenoceptors on the arterial contractile response. It is currently assumed that a clear influence of the  $\alpha_2$ -adrenoceptors on vasoconstriction occurs in veins but not in arteries (GÖRNEMANN *et al.* 2007). Originally, this phe-

nomenon was described in pulmonary circulation in dogs (OHLSTEIN *et al.* 1989). Further studies revealed that the murine mesenteric vein was more sensitive to the vasoconstriction activity of the  $\alpha$ -adrenoceptor agonists than the mesenteric artery. It was also discovered that  $\alpha$ -adrenergic receptors in the veins were more resistant to agonist-induced desensitisation. The greater reactivity and resistance of  $\alpha$ -adrenergic receptors to desensitisation may be important for blood pressure regulation. Studies conducted on the arteries and veins in mice suggest the existence of different contraction mechanisms associated with the activation of  $\alpha$ -adrenoreceptors. The  $\alpha_1$  receptors mediate the contraction response in both veins and arteries, whereas the  $\alpha_2$  receptors operate in the veins. Such differences in the function and pharmacology of the adrenoreceptors account for the differences in reactivity between arteries and veins.

Some articles show that the role of the  $\alpha_2$ -adrenoceptors in the blood vessel contractile response is based on co-activation of both  $\alpha_1$ - and  $\alpha_2$ -adrenoceptors (BYLUND *et al.* 1988).

In summary, the  $\alpha_1$ -adrenergic receptors play a major role in the regulation of rabbit aorta contractility, but the  $\alpha_2$ -adrenergic receptors also affect the tonus of the blood vessel muscular layer.

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