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Nonspecific Effects of Ligands on the β-Adrenergic Receptors in Rabbit Abdominal Aorta *in vitro**

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The study was conducted on 30 New Zealand rabbits weighing 3-4 kg from which sample strips of the abdominal aorta were collected. The study investigated the *in vitro* reaction of rabbit aorta smooth muscle to ligands binding to β-adrenergic receptors. The response of aortic strips to β-adrenergic receptor agonists (dobutamine, isoproterenol, salbutamol) and the influence of β-adrenergic receptor antagonists (propranolol, betaxolol) on contractile activity was determined. All tested agonists induced contraction of the rabbit abdominal aorta muscle in a concentration-dependent manner (dobutamine >> isoproterenol > salbutamol). Enhanced reaction to low concentrations of agonists (dobutamine, isoproterenol) after administration of propranolol and inhibition of contractility in the presence of high concentrations thereof (dobutamine, salbutamol) was observed. Maximal reaction to agonists decreased after betaxolol pretreatment. The results indicate that all the substances with β-agonist activity also possess contracting properties (presumably by acting at α-adrenergic receptors), but are much weaker in the case of isoproterenol and salbutamol than for dobutamine. Propranolol and betaxolol reduce the contractile response of smooth muscle using probably other mechanisms than those associated with adrenergic receptors.

Key words: Aorta, contractility, β-adrenergic receptors, rabbit, propranolol.

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The aorta is innervated by the sympathetic nervous system that regulates its lumen and thereby affects the arterial blood pressure in the body. Norepinephrine released from the nerve endings binds mainly to α_1-adrenergic receptors resulting in aortic muscle contraction (DOCHERTY *et al.*1979; DREW *et al.* 1979; GNUS *et al.* 2012). In addition to the α-adrenergic receptors the blood vessel wall contains β-adrenergic receptors, stimulation of which initiates relaxation of vascular smooth muscle. The change in vascular reactivity after stimulation of β-adrenergic receptors may be associated with the development of hypertension (BORKOWSKI *et al.* 1992; OLIVER *et al.* 2009).

Originally vascular β-adrenergic receptors were classified as β_2 subtype (LANDS *et al.* 1967), then it was proven that the β_1 subtype also takes parts in vasorelaxation (O'DONNEL *et al.* 1985). Based on a number of functional *in vitro* studies carried out in subsequent years, the existence of atypical β-adrenergic receptors in the vascular wall was suggested (ORIOWO *et al.* 1994; BRAWLEY *et al.* 2000).

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Since the activation of β-adrenergic receptors is associated with relaxation response, the procedure used in *in vitro* experiments involves functional precontraction of the vessel strips. The substances usually used for this purpose are the α-agonists, particularly phenylephrine. However, we can not exclude the possibility that agonists and antagonists of β-adrenergic receptors are able to nonspecifically stimulate or block the α-adrenergic receptors or influence other pathways which play a role in vascular contractility.

This paper focuses on the demonstration of nonspecific activity of β-adrenergic ligands not related to the stimulation or blocking of the β-adrenergic receptors, which may be associated, among others, with effects on α-adrenergic receptors as well as with pathways not involving the adrenergic system.

Material and Methods

The study was conducted on 30 New Zealand rabbits weighing 3-4 kg from which specimens of the abdominal aorta were collected. The study was approved by the II Local Ethical Committee, approval no. 89/2010.

The experimental animals were euthanized by administering intravenous solution of pentobarbiturate (Morbital). Immediately after death, 4-5 cm long specimens of abdominal aorta were collected and cut into 0.5 cm wide rings. The diameter of the aorta in New Zealand breed rabbits weighing 3-4 kg was on average 4 to 6 mm. Strips were set up in an automatic water bath in four chambers of 20 ml capacity. The samples were placed transversely by threading the surgical thread safil 4.0 inside the aorta lumen and mounted so that the changes of the isolate transverse section were registered. The initial tonus of all strips was 9.8 mN (ZIYAL *et al.* 1997). This was the baseline value used as a reference for the obtained results. The time required to balance the record was determined experimentally at 60 minutes. Krebs-Henseleit buffer of the following composition was used as the incubation environment: 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. Incubation of the strips was carried out at 37°C in a gaseous mixture of oxygen and carbon dioxide used in the following proportion: 95% of O_2 and 5% of CO_2, in order to obtain a pH value of 7.3-7.5. Aortic contractions were registered with isotonic transducers (Letica Scientific Instruments) combined with bridge amplifiers (BridgeAmp, ADInstruments, Australia), and 4-channel data acquisition system (PowerLab/400, ADInstruments) connected to a Macintosh computer.

After the equilibration time the presence of functional endothelium was confirmed by the relaxation in response to acetylcholine ($3 \cdot 10^{-6}$ M). Following washout, aortae were then contracted with cumulative doses of agonist (phenylephrine, isoproterenol, dobutamine or salbutamol) until a maximal response was reached or incubated with an antagonist for 30 min (propranolol or betaxolol, $2 \cdot 10^{-5}$ M). Concentration-response curves to agonists were constructed after preincubation with antagonists. Neither of the antagonists at a concentration of $2 \cdot 10^{-5}$M caused inhibition of the reaction to increasing doses of the α_1-adrenergic agonist phenylephrine (data not shown).

The following chemical substances were added: phenylephrine- α_1-adrenergic receptor agonist (Sigma--Aldrich), isoproterenol – nonspecific β-adrenergic receptor agonist (Sigma-Aldrich), salbutamol – β_2-adrenergic receptor agonist (Sigma-Aldrich), dobutamine – β_1-adrenergic receptor agonist (Sigma--Aldrich), propranolol – β_1 and β_2 adrenergic receptor antagonist (Sigma-Aldrich), betaxolol – β_1 adrenergic receptor antagonist (Sigma-Aldrich).

We analysed the strength of contractions expressed in mN (contractility amplitude). The results of the tests expressed as mean and standard deviation (±SD) 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

Phenylephrine

Application of phenylephrine to the incubation chamber induced contraction of the rabbit aorta strips (n=9) in a concentration dependent manner. A minimal reaction was observed at a phenylephrine concentration of $3 \cdot 10^{-7}$ M, while the maximum (7.61±1.47 mN) at a concentration of $3 \cdot 10^{-5}$ M; further increase of concentration did not intensify the contractile response (Fig. 1). Log_{10} EC_{50} for phenylephrine was -5.85±0.22 (Fig. 2).

Dobutamine

Application of dobutamine to the incubation chamber induced contraction of the rabbit aorta strips in a concentration dependent manner (n=5). Minimal reaction (0.412±0.128 mN) was noted at a concentration of $3 \cdot 10^{-7}$ M, while the maximum (6.118±0.268 mN) at a concentration of $3 \cdot 10^{-5}$ M (Fig. 1A). Log_{10} EC_{50} for dobutamine was equal to -6.22±0.17 (Fig. 1B).

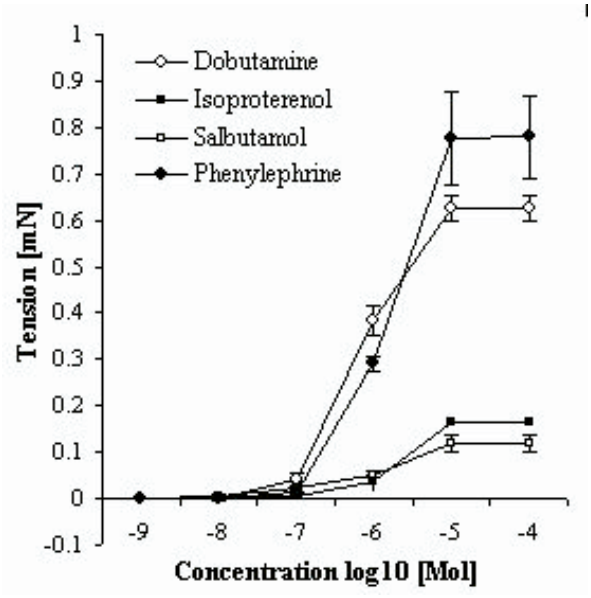

Fig. 1A. Response of rabbit abdominal aorta rings to phenylephrine and β-adrenoreceptor agonists (dobutamine, isoproterenol, salbutamol). Phenylephrine elicited the strongest maximum reaction (E_{max}=7.61±1.47 mN) followed by dobutamine (E_{max}=6.118±0.268 mN). Isoproterenol and salbutamol were less effective ($p \leq 0.0001$) in inducing contraction (E_{max}=1.625±0.094 mN and E_{max}=1.161±0.167 mN respectively).

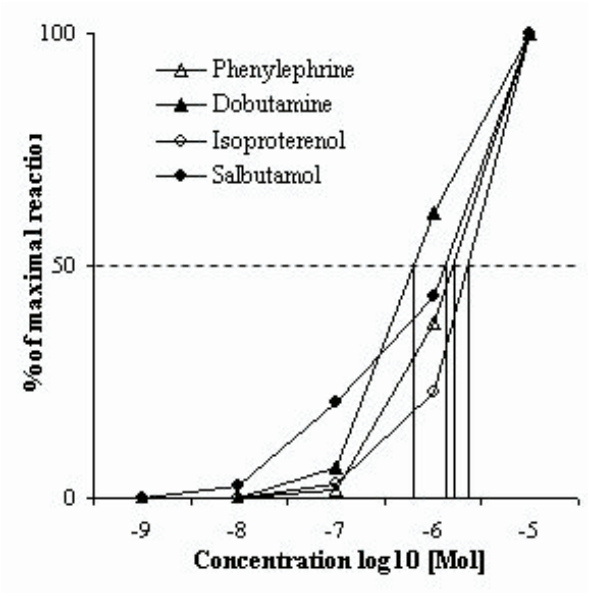

Fig. 1B. Relative potencies of phenylephrine (log_{10} EC_{50} = -5.85±0.22), isoproterenol (log_{10} EC_{50} = -5.66±0.2), dobutamine (log_{10} EC_{50} = -6.22±0.17) and salbutamol (log_{10} EC_{50} = -5.9±0.19) in inducing contraction in rabbit abdominal aortae rings.

Isoproterenol

The minimum concentration of isoproterenol causing contraction of the rabbit aorta strips (n=5) was $3 \cdot 10^{-7}$ mol. Tissue tonus increased by 0.051±0.054 mN. The strongest reaction occurred at isoprenaline concentration of $3 \cdot 10^{-5}$ M and amounted to 1.625±0.094 mN (Fig. 1A). Log_{10} EC_{50} for isoproterenol was equal to -5.66±0.2 (Fig. 1B).

Salbutamol

High concentrations of salbutamol caused a slight increase in tonus of rabbit abdominal aortic rings (n=7). The lowest concentration at which the tissue contraction was visible was $3 \cdot 10^{-8}$ M, and the tonus increased up to 0.028 mN. The strongest reaction was observed at a concentration of $3 \cdot 10^{-5}$ M, and it amounted to 1.161±0.167 mN (Fig. 1A). Log_{10} EC_{50} for salbutamol was -5.9±0.19 (Fig. 1B).

Propranolol and Dobutamine

Pre-incubation of the rabbit abdominal aortic rings (n=5) in propranolol ($2 \cdot 10^{-5}$ M) altered the tissue reaction to dobutamine. The contraction appeared at a dobutamine concentration of $3 \cdot 10^{-8}$ M, and the tone changed by 0.098 mN. Elevating the concentration of the agonist after preincubation in the antagonist induced a weaker reaction than while using the agonist alone. The maximum reaction was weaker and amounted to 1.161±0.167 mN, which accounted for 43.7% of the maximum response to dobutamine alone (Fig. 2A). The EC_{50} value was higher and amounted to -5.75±0.25.

Betaxolol and Dobutamine

Pre-incubation of the rabbit abdominal aortic rings (n=8) in betaxolol ($2 \cdot 10^{-5}$ M) had a similar effect as propranolol. Reaction to dobutamine appeared at a concentration of $3 \cdot 10^{-8}$ M and maximum contraction was weaker, 2.882±0.26 mN (47.11% of the maximum response elicited by an antagonist alone agonist alone).

Propranolol and Isoproterenol

Pre-incubation of the rabbit abdominal aortic rings (n=7) in propranolol ($2 \cdot 10^{-5}$ M) altered the tissue reaction to isoproterenol. The addition of the antagonist to the buffer resulted in contractile reaction to the agonist at a concentration of $3 \cdot 10^{-8}$ M, and the tissue tone changed by 0.07 mN. The contractile reaction to isoproterenol concentrations of $3 \cdot 10^{-7}$ and $3 \cdot 10^{-6}$ M were also escalated. However, the tissue response to high concentrations of isoproterenol ($3 \cdot 10^{-5}$ M) was slightly lower following the application of the antagonist and amounted to 1.596±0.139 mN (statistically insignificant). EC_{50} for isoproterenol after preincubation in propra-

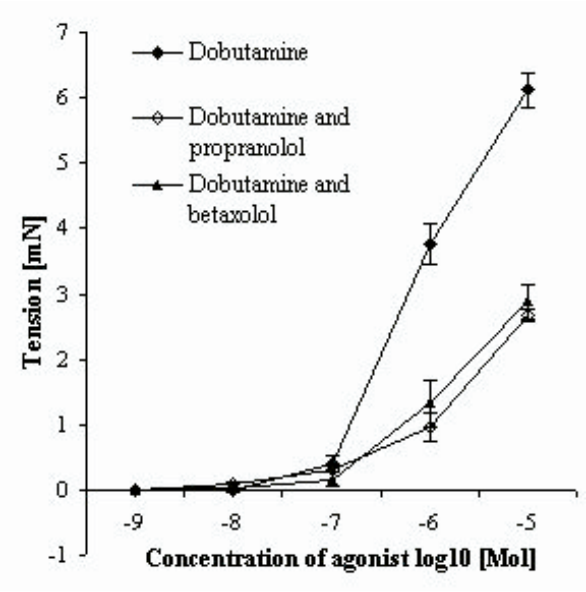

Fig. 2A. Contraction in response to dobutamine alone (n=5) and after preincubation in propranolol (n=5) or betaxolol (n=8) ($2 \cdot 10^{-5}$ M). The concentration of the agonist eliciting minimum reaction was lower ($3 \cdot 10^{-8}$ M) after preincubation in propranolol and betaxolol. Both antagonists inhibited the reaction to dobutamine in a noncompetitive manner, although they were not able to abolish the reaction. E_{max} after propranolol pretreatment amounted to 1.161±0.167 mN (43.7% of the maximum reaction to dobutamine alone, $p \leq 0.0001$), and with betaxolol was equal to 2.882±0.26 mN (47,11% of the maximum reaction to agonist alone, $p \leq 0.05$).

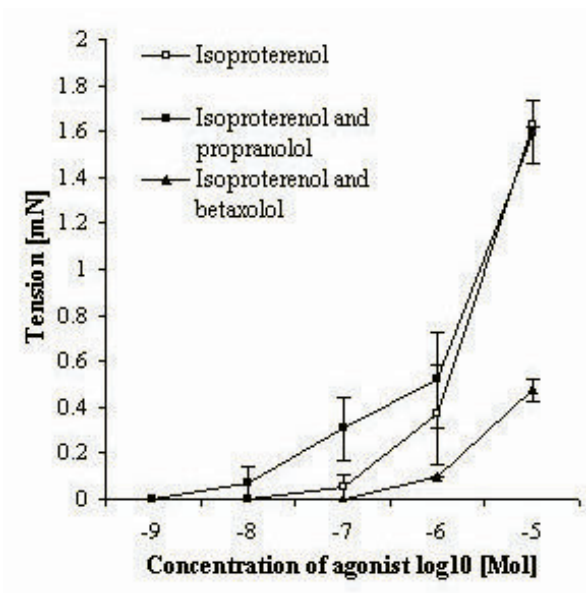

Fig. 2B. Concentration contraction curve of rabbit abdominal aorta to isoproterenol alone after preincubation in propranolol or betaxolol. Isoproterenol is characterized by low efficiency to induce contraction (E_{max}=1.625±0.094 mN). Propranolol pretreatment induced stronger contractile reaction to isoproterenol at lower concentrations ($10^{-8} - 10^{-6}$ M) without influence on maximum reaction. Betaxolol strongly inhibited the contractile response to isoproterenol (E_{max}=0.475±0.05 mN, 29.23% of the maximum response to agonist alone, $p \leq 0.05$).

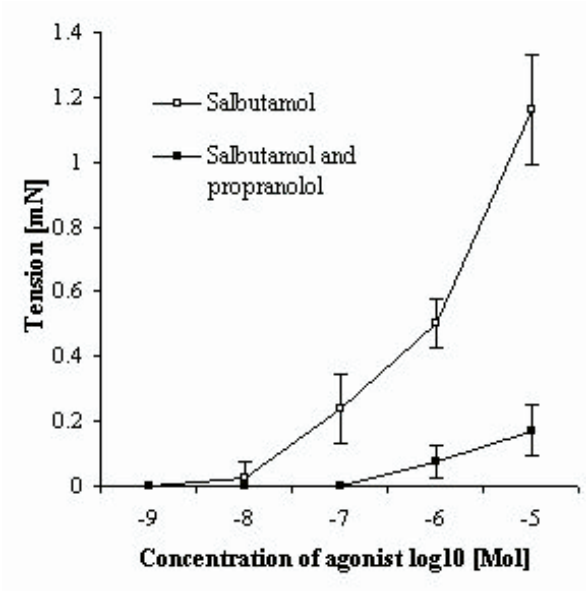

Fig. 2C. Contraction in response to increasing concentrations of salbutamol with and without preincubation in propranolol. Salbutamol (n=7) elicited weak contraction of rabbit abdominal aortae rings in a concentration dependent manner (E_{max}=1.161±0.167 mN). Maximal reaction to salbutamol ($3 \cdot 10^{-5}$ M) was weaker in the presence of propranolol (n=5; $2 \cdot 10^{-5}$ M) and was 0.172±0.08 mN ($p \leq 0.0001$). Betaxolol (n=4; $2 \cdot 10^{-5}$ M) abolished the contractile reaction to salbutamol (not shown).

nolol did not differ significantly from the EC_{50} for the agonist alone (Fig. 2B).

Betaxolol and Isoproterenol

Pretreatment of rabbit aortae rings (n=8) with betaxolol abolished the contractile reaction to isoproterenol at a concentration of $3 \cdot 10^{-7}$ M. The maximum reaction was weaker and amounted to 0.475±0.05 mN, which accounted for 29.23% of the maximum response to isoproterenol alone.

Propranolol and Salbutamol

Preincubation of rabbit abdominal aortic rings (n=5) in propranolol reduced the contractile reaction induced by salbutamol. Ring tonus changed by 0.076±0.049 mN only at a salbutamol concentration of $3 \cdot 10^{-6}$ M. The maximal reaction to salbutamol ($3 \cdot 10^{-5}$ M) was weaker in the presence of propranolol and was 0.172±0.08 mN. EC_{50} for salbutamol plus propranolol was -5.5±0.23 (Fig. 2C). Pretreatment of tissues (n=4) with betaxolol abolished the contraction in response to salbutamol (not shown).

Discussion

Administration of β-adrenergic receptor agonists induced the contractile response of the rabbit abdominal aorta strips, which most probably resulted from α-adrenergic receptor stimulation. The most pronounced reaction was observed after administration of dobutamine and the strength of the tissue response was similar to the case of phenylephrine administration (Fig. 1A). Phenylephrine is the α_1-agonist serving as a reference substance for aorta contractions (KENAKIN 1981) and was used as such in this study. The resulting increase in tonus after administration of dobutamine is consistent with the observations made by other researchers who proved that dobutamine acted as an α-agonist in the blood vessels (KENAKIN 1981). Its (-)isomer is particularly effective in binding to and stimulating the α_1 -adrenergic receptor. A mixture of isomers is half as effective as the pure (-)isomer, as dobutamine (+)isomer binds to the α_1-adrenergic receptor without stimulating it. Moreover, (+)isomer stimulates β_1-adrenergic receptors which may affect the contractile response (RUFFOLO *et al.* 1981).

The contractile reaction after administration of isoproterenol was significantly weaker than the contraction induced by phenylephrine (α-agonist). Maximal contraction after isoproterenol accounted for 21.35% of the response to administration of phenylephrine at its highest concentration. These results are consistent with the literature data claiming that an isoproterenol concentration of 10^{-7} to 10^{-6} M induced the contractile response (FLEISCH *et al.* 1970). Contraction following isoproterenol administration is blocked by prior incubation of the strips in phentolamine (α-antagonist), and the removal of endothelium, the main location of β-adrenergic receptors, intensifies the response (KAMATA *et al.* 1989; TURPAPATY *et al.* 1975). This demonstrates the ability of isoproterenol to stimulate α-adrenergic receptors at higher concentrations.

The contractile reaction of rabbit aortic strips to salbutamol was equal to 15.26% of the response to phenylephrine. Salbutamol is considered to be a selective agonist of the β_2-adrenergic receptor. Experimental results suggest that salbutamol may bind to α-adrenergic receptors at higher concentrations and slightly stimulate them. The possibility of salbutamol binding to the binding sites of the α-adrenergic receptors is also indicated by its ability to relax the strips precontracted with phenylephrine, and the lack of the relaxing effect on strips precontracted with PGF2α, thus indicating binding to α-adrenergic receptors (BRAHMADEVARA *et al.* 2003; KOZŁOWSKA *et al.* 2005; SHAFIEI *and* MAHMOUDIAN 1999). Antagonistic action towards strong agonists, such as phenylephrine, is typical of partial agonists. Additionally, studies with a radioligand demonstrated that salbutamol displaced ^{3}H-prazosin from its binding sites (BRAHMADEVARA *et al.* 2004). However, the concentrations at which salbutamol induced the contraction of the aorta strips were much higher than clinical doses of the drug.

Similar results were obtained after administration of dobutamine which relaxed the aortic strips precontracted with phenylephrine, but did not affect the contraction induced by KCl or $PGF_{2\alpha,}$ and preincubation with dobutaminie affected the contractile response to phenylephrine without reducing the maximal response (AIKAWA *et al*. 1996).

Preincubation of rabbit aorta strips with propranolol or betaxolol ($2 \cdot 10^{-5}$ M) resulted in a reduction of the maximal response to both dobutamine and salbutamol. In the case of isoproterenol the maximal response was not statistically different for the strips incubated and not incubated with propranolol, whereas maximum reaction to isoproterenol after incubation with betaxolol was reduced. However, neither of the β-adrenergic receptor antagonists abolished the contractile reaction. Moreover, they did not influence the contraction of rabbit abdominal aorta in response to phenylephrine in our preliminary studies. In the case of isoproterenol and dobutamine propranolol and betaxolol induced the contractile response at lower concentration of agonists (10^{-8} M). Propranolol has properties of a nonspecific β-adrenergic receptor antagonist. It inhibits vessel relaxation caused by isoproterenol and norepinephrine (AL JEBOORY & MARSHALL 1977; FUJIMOTO & ITOH 1996). β_1-antagonistic properties of betaxolol are also well established. Blocking β-adrenergic receptors may enhance the contractile response following the stimulation of α-adrenergic receptors, if an agonist showing affinity to both types of receptors is used. This situation occurs for contractions induced by norepinephrine. Propranolol, by blocking β-adrenergic receptors, increases the contractile response to norepinephrine (FUJIMOTO & ITOH 1996). A similar phenomenon is observed for dobutamine and isoproterenol. The use of a β-adrenergic receptor blocker accelerated the occurrence of the contractile reaction; the contraction appears at lower concentrations and after a shorter period of time. However, a different tissue response occurred in the case of salbutamol. This might be related to a much weaker relaxation of the aortic strips precontracted with phenylephrine by salbutamol than isoproterenol (SHAFIEI & MAHMOUDIAN 1999). The desensitization of β-adrenergic receptors in the case of omitting the antagonist is rather unlikely, because aortic β_2 receptors have been shown to be insensitive to it (MARTIN & BROADLEY 1999).

Propranolol and betaxolol inhibited the contractile response of tissues to higher concentrations of agonists. The response of the aortic strips to the highest concentration of isoproterenol was slightly lower after preincubation in propranolol, but this was not statistically significant. In contrast, betaxolol significantly inhibited the maximum response to isoproterenol. Both β-adrenergic receptor antagonists blunted the contractile reaction to high concentrations of dobutamine and salbutamol. EC_{50} values were similar for both groups, so the competitive antagonism is rather unlikely the competitive antagonism. Maximum response to dobutamine was significantly reduced and amounted to less than 50% of the maximal response to dobutamine without antagonist preincubation, and it almost disappeared (14.77% of the maximal response to salbutamol without propranolol preincubation) or was entirely abolished (betaxolol) for salbutamol. This indicates a non-competitive antagonism. High concentrations of propranolol may limit the contractile response by binding to α_1-adrenergic receptors without stimulating them. It was shown that propranolol displaced ^{3}H-prazosin from its binding site on α_1-adrenergic receptor (BRAHMADEVARA *et al*. 2004). However, this mechanism of inhibition is contradicted by the chart showing the course of the contractile reaction and indicating the non-competitive antagonism. In addition, in the case of strong binding of propranolol to α_1-adrenergic receptors, the relaxation of strips precontracted with phenylephrine and then treated with dobutamine should be intensified, whereas a reverse phenomenon was observed (AIKAWA *et al.* 1996). Moreover, a lack of influence on the phenylephrine contraction opposes antagonism of propranolol and betaxolol against α-adrenergic receptors. Still, it is certain that propranolol and betaxolol relax the blood vessels, irrespective of their effect on β-adrenergic receptors, as both substances similarly inhibited contraction elicited by β_1-adrenergic and β_2-adrenergic agonists (dobutamine and salbutamol, respectively). Moreover, inhibition of the contractile response to dobutamine and salbutamol by propranolol and betaxolol was comparable, despite differing selectivity towards β-adrenergic receptors. In experiments with phenylephrine used as a contracting agent, relaxation of rat aorta was observed following administration of high concentrations of propranolol. The role of α-adrenoreceptors in the relaxation response was excluded after administration of endothelin-1 and U-4619 as contracting substances. This was not entirely correct, because our team has shown the ability of α-antagonistic substances to relax human artery sections *in vitro*, without prior application of the α-adrenergic receptor agonist (GNUS *et al*. 2012; PRIVIERO *et al.* 2006). Nevertheless, in the experiments of PRIVIERO *et al.* higher concentrations of propranolol were used. Other possible mechanisms of propranolol action may involve membrane stabilizing activity, effect on calcium channels, inhibition of thromboxane A_2 production, and antagonistic activity at 5-HT_1 receptors (MARANO *et al*. 2002; SAKANASHI & TAKEO 1983; ORIJI & SCHANZ 2001; SAXENA & VILLALÓN 1990; CEKIC *et al*. 2013). Propranolol and betaxolol were also equipotent in relaxing the bovine retinal microartery via a Ca^{2+} channel blockade. Betaxolol was shown to relax the rat aorta and rabbit ciliary artery similar to calcium antagonists as well (HOSTE & SYS 1998; HAYASHI-MORIMOTO *et al*. 1999). Many of the nonspecific characteristics of β-adrenergic receptor antagonists were evident only at high concentrations. In addition, experiments often require precontraction of the vessels. Phenylephrine, endothelin-1 and tromboxane A_2 analogues, which have high potency and efficacy, are typically used. It might be possible that the nonspecific properties of propranolol and betaxolol are more evident when applying a constrictor of low efficacy, which needs further investigation.

In conclusion, even highly selective agonists and antagonists of adrenergic receptors used in high doses can non-specifically bind to other groups of receptors. This should be kept in mind, especially during designing the experimental model. The use of α-agonists as a contracting agent may result in an inadequate tissue reaction to β-adrenergic substances.

References

AIKAWA J., FUKAZAWA M., ISHIKAWA M., MOROI M., NAMIKI A., YAMAGUCHI T. 1996. Vascular smooth muscle relaxation by alpha-adrenoceptor blocking action of dobutamine in isolated rabbit aorta. J. Cardiovasc. Pharmacol. **7**: 33-36.

AL-JEBOORY A., MARSHALL R.J. 1977. The effects of dopamine and dobutamine on isolated coronary vascular smooth muscle of the pig [proceedings]. Br. J. Pharmacol. **59**: 514-516.

BORKOWSKI K.R., GROS R., SCHNEIDER H. 1992. Vascular beta-adrenoceptor-mediated responses in hypertension and ageing in rats. J. Auton. Pharmacol. **12**: 389-401.

BRAHMADEVARA N., SHAW A.M., MACDONALD A. 2003. Evidence against beta 3-adrenoceptors or low affinity state of beta 1-adrenoceptors mediating relaxation in rat isolated aorta. Br. J. Pharmacol. **138**: 99-106.

BRAHMADEVARA N., SHAW A.M., MACDONALD A. 2004. Alpha1-adrenoceptor antagonist properties of CGP 12177A and other beta-adrenoceptor ligands: evidence against beta(3)- or atypical beta-adrenoceptors in rat aorta. Br. J. Pharmacol. **142**: 781-787.

BRAWLEY L., SHAW A.M., MACDONALD A. 2000. Beta 1-, beta 2- and atypical beta-adrenoceptor-mediated relaxation in rat isolated aorta. Br. J. Pharmacol. **129**: 637-644.

CEKIC E.G., SOYDAN G., GULER S., BABAOGLU M.O., TUNCER M. 2013. Propranolol-induced relaxation in the rat basilar artery. Vascul. Pharmacol. [In press].

DOCHERTY J.R, MACDONALD A., MCGRATH J.C. 1979. Further sub-classification of alpha-adrenoceptors in the cardiovascular system, vas deferens and anococcygeus of the rat [proceedings]. Br. J. Pharmacol. **67**: 421-422.

DREW G.M., WHITING S.B. 1979. Evidence for two distinct types of postsynaptic alpha-adrenoceptor in vascular smooth muscle *in vivo*. Br. J. Pharmacol. **67**: 207-215.

FLEISCH J.H., MALING H.M., BRODIE B.B. 1970. Beta-Receptor Activity in Aorta: Variations with Age and Species. Circ. Res. **26**: 151-162.

FUJIMOTO S., ITOH T. 1996. Contraction and relaxation responses of femoral artery and aorta to norepinephrine in young rats. Gen. Pharmacol. **27**: 1355-1359.

GNUS J., CZERSKI A., FERENC S., ZAWADZKI W., WITKIEWICZ W., HAUZER W., RUSIECKA A. *et al*. 2012. *In vitro* study on the effects of some selected agonists and antagonists of α1-adrenergic receptors on the contractility of the aneurysmally-changed aortic smooth muscle in humans. J. Physiol. Pharmacol. **63**: 29-34.

HAYASHI-MORIMOTO R., YOSHITOMI T., ISHIKAWA H., HAYASHI E., SATO Y. 1999. Effects of beta antagonists on mechanical properties in rabbit ciliary artery. Graefes Arch. Clin. Exp. Ophthalmol. **237**: 661-7.

HOSTE A.M., SYS S.U. 1998. Ca2+ channel-blocking activity of propranolol and betaxolol in isolated bovine retinal microartery. J. Cardiovasc. Pharmacol. **32**: 390-6.

KAMATA K., MIYATA N., KASUYA Y. 1989. Involvement of endothelial cells in relaxation and contraction responses of the aorta to isoproterenol in naive and streptozotocin-induced diabetic rats. J. Pharmacol. Exp. Ther. **249**: 890-894.

KENAKIN T.P. 1981. An *in vitro* quantitative analysis of the alpha adrenoceptor partial agonist activity of dobutamine and its relevance to inotropic selectivity. J. Pharmacol. Exp. Ther. **216**: 210-219.

KOZŁOWSKA H., SCHLICKER E., KOZŁOWSKI M., SIEDLECKA U., LAUDAŃSKI J., MALINOWSKA B. 2005. Ligands at beta2-, beta3-, and the low-affinity state of beta1-adrenoceptors block the alpha1-adrenoceptor-mediated constriction in human pulmonary and rat mesenteric arteries. J. Cardiovasc. Pharmacol. **46**: 76-82.

LANDS A.M., LUDUENA F.P., BUZZO H.J. 1967. Differentiation of receptors responsive to isoproterenol. Life Sci. **6**: 2241-2249.

MARANO G., PALAZZESI S., FADDA A., VERGARI A., FERRARI A.U. 2002. Attenuation of aortic banding-induced cardiac hypertrophy by propranolol is independent of beta-adrenoceptor blockade. J. Hypertens. **20**: 763-769.

MARTIN S.W., BROADLEY K.J. 1999. Relative resistance of functional beta2-adrenoceptor-mediated smooth muscle responses to *in vitro* desensitization. Can. J. Physiol. Pharmacol. **77**: 156-165.

O'DONNELL S.R., WANSTALL J.C. 1985. Responses to the beta 2-selective agonist procaterol of vascular and atrial preparations with different functional beta-adrenoceptor populations. Br. J. Pharmacol. **84**: 227-235.

OLIVER E., MARTÍ D., MONTO´ F., FLACCO N., MORENO L., BARETTINO D., IVORRA M.D. *et al*. 2009. The Impact of α1-Adrenoceptors Up-Regulation Accompanied by the Impairment of β-Adrenergic Vasodilatation in Hypertension J. Pharm. Exp. Ther. **328**: 982-990.

ORIJI G.K., SCHANZ N. 2001. Nitric oxide in CsA-induced hypertension: role of beta-adrenoceptor antagonist and thromboxane A2. Prostaglandins Leukot. Essent. Fatty Acids **65**: 259-63.

ORIOWO M.A. 1994. Atypical beta-adrenoceptors in the rat isolated common carotid artery. Br. J. Pharmacol. **113**: 699-702.

PRIVIERO F.B., TEIXEIRA C.E., TOQUE H.A., CLAUDINO M.A., WEBB R.C., DE NUCCI G., ZANESCO A. *et al*. 2006. Vasorelaxing effects of propranolol in rat aorta and mesenteric artery: a role for nitric oxide and calcium entry blockade. Clin. Exp. Pharmacol. Physiol. **33**: 448-455.

RUFFOLO R.R. Jr, SPRADLIN T.A., POLLOCK G.D., WADDELL J.E., MURPHY P.J. 1981. Alpha and beta adrenergic effects of the stereoisomers of dobutamine. J. Pharmacol. Exp. Ther. **219**: 447-452.

SAKANASHI M., TAKEO S. 1983. Characterization of propranolol-induced relaxation of coronary artery. Jpn. J. Pharmacol: **33**: 603-610.

SAXENA P.R., VILLALÓN C.M. 1990. Cardiovascular effects of serotonin agonists and antagonists. J. Cardiovasc. Pharmacol. **15** Suppl **7**: S17-34.

SHAFIEI M., MAHMOUDIAN M. 1999. Atypical beta-adrenoceptors of rat thoracic aorta. Gen. Pharmacol. **32**: 557-562.

TURPAPATY P.D.M.V., CARRIER O. Jr, JUREVICS H. 1975. Effect of magnesium on isoproterenol-induced alpha and beta receptor responses of vascular smooth muscle. J. Pharmacol. Exp. Ther. **192**: 372-380.

ZIYAL R., ZIGANSHIN A.U., NICKEL P., ARDANUY U., MUTSCHLER E., LAMBRECHT G., BURNSTOCK G. 1997. Vasoconstrictor responses via P2X-receptors are selectively antagonized by NF023 in rabbit isolated aorta and saphenous artery. Br. J. Pharmacol. **120**: 954-60.