

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

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Received September 24, 2012

Accepted May 29, 2013

### 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  $4.75 \pm 0.47$  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<sub>1</sub>-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<sub>1</sub>-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  $\alpha_{1B}$ -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:  $\alpha_{1A} > \alpha_{1B} > \alpha_{1D}$ .

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Alpha<sub>1</sub>-adrenergic receptors are present in different tissues including the brain (all subtypes), smooth muscles (α<sub>1A</sub>- and α<sub>1D</sub>-adrenergic), liver (α<sub>1A</sub>- and α<sub>1D</sub>-adrenergic), heart, and prostate (α<sub>1A</sub>-adrenergic).

The aim of this experiment was to study the effect of α<sub>1</sub>-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, CaCl<sub>2</sub> – 2.5 mM, MgSO<sub>4</sub> – 1.6 mM, NaHCO<sub>3</sub> – 24.3 mM, KH<sub>2</sub>PO<sub>4</sub> – 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 O<sub>2</sub> and 5% of CO<sub>2</sub>, 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 α<sub>1</sub>-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 α<sub>1</sub>- adrenergic receptors)

Administration of phentolamine 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 α<sub>1</sub>-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  $1.16 \pm 0.19$  mN. A 10-fold lower concentration of phentolamine ( $0.002 \mu\text{M}$ ) had no influence on the aortic muscle response to phenylephrine.

**Prazosin (non-selective antagonist of  $\alpha_1$ -adrenergic receptors)**

Blockade of  $\alpha_1$ -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  $0.002 \mu\text{M}$  dose, there was a decrease in the muscle tonus of  $-0.09 \pm 0.02$  mN. An increase of prazosin concentration, up to  $0.02 \mu\text{M}$ , caused further dilatation of the aortic muscle of  $-0.58 \pm 0.05$  mN. Blockade of  $\alpha_1$ -adrenergic receptor by addition of prazosin to the incubation chamber at a concentration of  $0.02 \mu\text{M}$  caused a decrease in response to  $0.05 \mu\text{M}$  of phenylephrine by 62%.

**5-methylurapidil (selective antagonist of  $\alpha_{1A}$ -adrenergic receptors)**

Administration of 5-methylurapidil at a dose of  $0.02 \mu\text{M}$  caused a significant ( $P \leq 0.01$ ) decrease in the aortic muscle tonus in rabbits, that is of  $-1.17 \pm 0.09$  mN. After the blockade of the  $\alpha_{1A}$ -adrenergic receptor by addition of 5-methylurapidil to the incubation chamber, the administration of  $0.05 \mu\text{M}$  phenylephrine resulted in an increase of the aortic muscle tonus of  $0.79 \pm 0.19$  mN. Phenylephrine dose elevation up to  $0.5 \mu\text{M}$  caused further increase of muscle tonus by  $0.7 \pm 0.29$  mN.

**Cyclazosin (selective antagonist of  $\alpha_{1B}$ -adrenergic receptors)**

Administration of cyclazosin at a dose of  $0.002 \mu\text{M}$  caused a decrease in the muscle tonus of  $-0.49 \pm 0.10$  mN. After blockade of the  $\alpha_{1B}$ -adrenergic receptor by  $0.02 \mu\text{M}$  cyclazosin, the administration of  $0.05 \mu\text{M}$  phenylephrine resulted in an increase of the aortic muscle tonus of  $1.49 \pm 0.14$  mN. An increase in phenylephrine dose up to  $0.5 \mu\text{M}$  caused further increase in the muscle tonus of  $2.89 \pm 0.19$  mN.

**L-765.314 (selective antagonist of  $\alpha_{1B}$ -adrenergic receptors)**

Administration of L-765.314 at a dose of  $0.0002 \mu\text{M}$  to the incubation chamber caused a decrease of the aortic muscle tonus of  $-0.19 \pm 0.09$  mN. After the blockade of the  $\alpha_{1B}$ -adrenergic receptor by  $0.02 \mu\text{M}$  of L-765.314, the administration of  $0.005 \mu\text{M}$  phenylephrine resulted in an increase of the aortic muscle tonus of  $1.49 \pm 0.14$  mN. Phenylephrine dose elevation up to  $0.05 \mu\text{M}$  caused further increase of muscle tonus by  $2.98 \pm 0.16$  mN.

**BMY 7378 (selective antagonist of  $\alpha_{1D}$ -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  $\alpha_{1D}$ -adrenergic receptor by the administration of BMY 7378 at a dose of  $0.02 \mu\text{M}$ , the administration of  $0.05 \mu\text{M}$  phenylephrine resulted in an increase of tissue reaction of  $2.57 \pm 0.25$  mN.

## Discussion

Stimulation of the  $\alpha_1$  and  $\alpha_2$ -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 (Kochová et al. 2009). Substances that block  $\alpha$ -adrenergic receptors are used as additional therapy in the treatment of hypertension. Expression of  $\alpha_1$ -adrenergic receptor subtypes varies

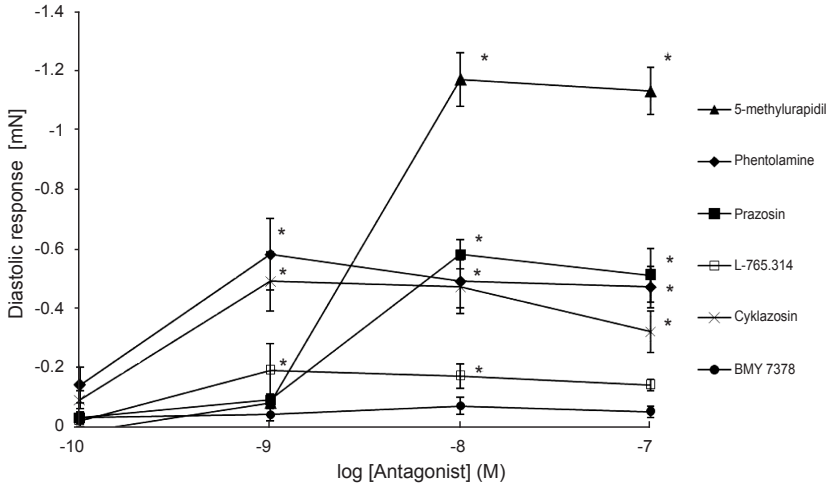


Fig 1. The relaxation responses of abdominal aortic strips to selected  $\alpha_1$ -adrenoreceptor antagonists ( $*P \leq 0.01$ ).

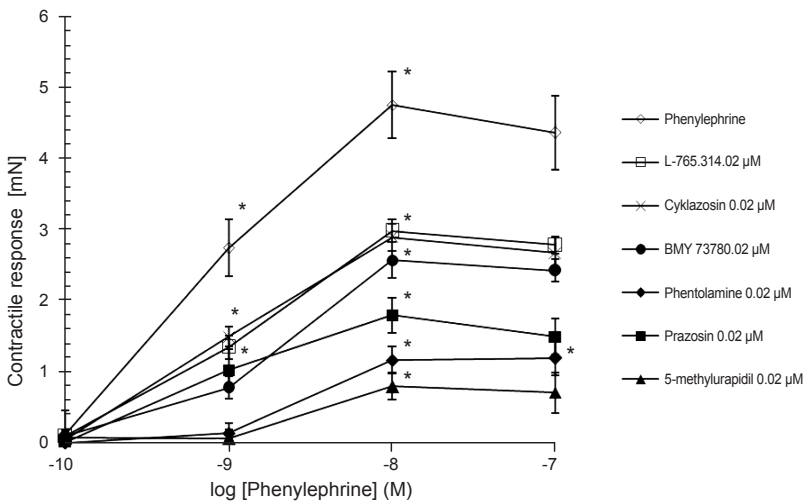


Fig 2. The effect of pretreatment with  $\alpha_1$ -adrenoreceptor antagonists on the response of abdominal aorta strips to following doses of phenylephrine ( $*P \leq 0.01$ ).

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  $\alpha_{1B}$ -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  $\alpha_{1A}$ -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  $\alpha_{1B}$ -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 <sup>3</sup>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 (Sato 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  $\alpha_1$ -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  $\alpha_1$ -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.

#### Acknowledgments

This publication is part of the project “Wrovasc – Integrated Cardiovascular Centre”, co-financed by the European Regional Development Fund within the Innovative Economy Operational Program, 2007–2013 realized in the Provincial Specialized Hospital, Research and Development Center in Wrocław; “European Funds – for the development of innovative economy”; Grant No. POIG.01.01.02-02-001/08-00.

#### References

- About R, Shafii M, Docherty JR 1993: Investigation of the subtypes of  $\alpha 1$ -adrenoceptor mediating contractions of rat aorta, vas deferens and spleen. *Br J Pharmacol* **109**: 80-87
- Caryl EH, Joyce E, Shaun LS 1999: Mechanisms underlying spontaneous rhythmical contractions in irideal arterioles of the rat. *J Physiol* **521**: 507-516
- Deng XF, Chemtob S, Varma DR 1996: Characterization of alpha 1 D-adrenoceptor subtype in rat myocardium, aorta and other tissues. *Br J Pharmacol* **119**: 269-276
- Docherty JR 1998: Subtypes of functional  $\alpha 1$ - and  $\alpha 2$ -adrenoceptors. *Eur J Pharmacol* **361**: 1-15
- Eckert RE, Karsten AJ, Útz J, Ziegler M 2000: Regulation of renal artery smooth muscle tone by  $\alpha 1$ -adrenoceptors: role of voltage-gated calcium channels and intracellular calcium stores. *Urol Res* **28**: 122-127
- Fraňová S, Janíček F, Nosáľová G, Šutovská M, Višňovský J 2009: In vitro contractile response of rabbit myometrium to  $BK_{Ca}$  and  $K_{ATP}$  potassium channel openers. *Acta Vet Brno* **78**: 13-18
- Goetz AS, King HK, Ward SD, True TA, Rimele TJ, Saussy DL 1995: BMY 7378 is a selective antagonist of the D subtype of alpha 1-adrenoceptors. *Eur J Pharmacol* **16**: 2-3
- Han C, Abel PW, Minneman KP 1987:  $\alpha 1$ -Adrenoceptor subtypes linked to different mechanisms for increasing intracellular  $Ca^{2+}$  in smooth muscle. *Nature* **329**: 333-335
- Hieble JP, Ruffolo JR 1996: Subclassification and nomenclature of  $\alpha 1$ - and  $\alpha 2$ -adrenoceptors. *Prog Drug Res* **47**: 81-130
- Hoffman BB, Lefkowitz RJ 1996: Catecholamines, sympathomimetic drugs, and adrenergic receptor antagonists. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Goodman A. The pharmacological basis of therapeutics. Goodman & Gilman's, New York, USA, 199-248
- Kochová P, Tonar Z, Matějka VM, Švíglerová J, Štengl M, Kuncová J 2009: Aorta remodelling associated with calcitonin gene related peptide concentration in rats with arterial hypertension. *Acta Vet Brno* **78**: 595-602
- Langer SZ 1999: History and nomenclature of  $\alpha 1$ -adrenoceptors. *Eur Urol* **36**: 2-6
- Marucci G, Angeli P, Buccioni M, Gulini U, Melchiorre C, Sagratini G, Testa R, Giardinà D 2005: (+)-Cyclazosin, a selective alpha1B-adrenoceptor antagonist: functional evaluation in rat and rabbit tissues. *Eur J Pharmacol* **522**: 100-107
- Minneman K 1988: Alpha 1- adrenergic receptor subtypes. Inositol phosphates and sources of cell calcium. *Pharmacol Rev* **40**: 87-93
- Piao H, Taniguchi T, Nakamura S, Zhu J, Suzuki F, Mikami D, Muramatsu I 2000: Cloning of rabbit alpha(1b)-adrenoceptor and pharmacological comparison of alpha(1a)-, alpha(1b)- and alpha(1d)-adrenoceptors in rabbit. *Eur J Pharmacol* **396**: 9-17
- Satoh M, Enomoto K, Niwano H, Fujimura H, Toyama Y, Takayanagi I, Koike K 1998: Regional differences in  $\alpha 1$ -adrenoceptor subtypes and mechanisms in rabbit arteries. *Eur J Pharmacol* **350**: 67-73
- Stephenson JA, Summers RJ 1987: Autoradiographic analysis of receptors on vascular endothelium. *Eur J Pharmacol* **134**: 35-41
- Suzuki E, Tsujimoto G, Tamura K, Hashimoto K 1990: Two pharmacologically distinct alpha 1-adrenoreceptor subtypes in the contraction of rabbit aorta: each subtype couples with a different  $Ca^{2+}$  signaling mechanism and plays a different physiological role. *Mol Pharmacol* **38**: 725-730
- Vecchione C, Fratta L, Rizzoni D, Notte A, Poulet R, Porteri E, Frati G, Guelfi D, Trimarco V 2002: Cardiovascular influences of alpha b-adrenergic receptor defect in mice. *Circulation* **105**: 1700-1707
- Willems EW, Valdivia LF, Saxena PR, Villalon CM 2001: The role of several  $\alpha 1$ - and  $\alpha 2$ -adrenoceptor subtypes mediating vasoconstriction in the canine external carotid circulation *Br J Pharmacol* **132**: 1292-1298