Impact of $\alpha_1$-adrenoceptor expression on contractile properties of vascular smooth muscle cells

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Abstract

Low-frequency blood pressure oscillations (Mayer waves) are discussed as a marker for sympathetic modulation of vascular tone. However, the factors that determine the frequency response of the vasculature to sympathetic stimuli are not fully understood. Possible mechanisms include functions related to α₁-adrenergic receptors (α₁-AR) and post-receptor processes involved in the vascular contractile response. The purpose of the present study was to examine the hypothesis that expression levels of α₁-AR and their subtype distribution determine velocity and magnitude of α₁-AR-mediated vascular smooth muscle cell (VSMC) contraction.

α₁A⁺, α₁B⁻ and α₁D⁻-AR subtypes were transfected into VSMCs from rat aorta and characterized immunocytochemically via confocal microscopy. Functional studies in isolated cells were performed using video microscopy. The α₁-AR agonist phenylephrine produced dose-dependent contractions of VSMCs. All transfected groups were more sensitive to phenylephrine compared with controls. Maximal contraction velocity almost doubled in transfected cells. However, no differences in observed parameters were found between the three transfected groups. Contractile properties in response to membrane depolarization with KCl were similar in all groups.

In conclusion, α₁-AR density determines velocity and sensitivity of α₁-AR-mediated contraction in VSMCs. α₁-AR subtype distribution does not appear to influence vasoconstriction to sympathetic stimuli.

Keywords: α₁-adrenoceptors, alpha1 nervous system, sympathetic Mayer waves vascular smooth muscle cells gene transfection
Introduction

It is under debate whether low-frequency blood pressure oscillations, also referred to as Mayer waves, are a useful parameter for the estimation of sympathetic modulation of vascular tone. The association of peripheral sympathetic outflow and low-frequency blood pressure variability has been demonstrated by microneurographic techniques (11; 32; 44). Moreover, frequency and amplitude of Mayer waves can be altered in a variety of pathological conditions, such as diabetes (17), congestive heart failure (37), acute brain injury (13), and hypertension (33). However, despite these findings, relatively little is known regarding the underlying physiology of these oscillations (24).

Short-term control of arterial pressure is achieved by a variety of regulatory systems that rely on sympathetic pathways, endothelial factors, angiotensin II, and others (34). The role of these factors in blood pressure regulation is often studied by the power spectral approach (5; 30; 36; 41). With this technique, the contribution of blood pressure fluctuations at specific frequencies to the overall blood pressure variability is assessed. The general idea is that different blood pressure regulatory systems can modulate arterial pressure only in very specific frequency ranges.

One of several sources causing blood pressure variability originates from sympathetic-mediated vasomotions. These vascular reactions are elicited by contractions and relaxations of vascular smooth muscle cells (VSMCs) in response to periodic stimulation of $\alpha_1$-adrenergic ($\alpha_1$-AR) (3) or other (e.g. purinergic) (10) receptors by sympathetic transmitters. The presence of the resulting blood pressure oscillations between 0.1 and 0.4 Hz requires that sympathetic transmission to the vasculature is rapid enough to mediate fluctuations in this frequency range.

Experiments in healthy rats using electrical stimulation of the paraventricular nucleus of the hypothalamus at multiple frequencies while recording splanchnic nerve activity and mesenteric blood flow suggest that the rate-limiting step of sympathetic-mediated blood pressure oscillations lies not at the central or sympathetic nervous system site but in the vascular...
1-adrenoceptors and contraction of VSMCs

reactivity (42). A more recent study using isolated rat aortic VSMCs suggests that the time course of sympathetic-mediated smooth muscle cell contraction may be limited by mechanisms related to $\alpha_1$-AR (25). However, the specific factors determining velocity and magnitude of sympathetic-mediated vasoconstriction and, therefore, underlying the different frequency response characteristics in various physiological and pathological conditions remain to be elucidated.

The goal of the present study was to test the hypothesis that $\alpha_1$-AR density and expression of the three subtypes $\alpha_{1A}$, $\alpha_{1B}$, and $\alpha_{1D}$ determine magnitude and velocity of $\alpha_1$-AR-mediated smooth muscle contraction. To examine the role of receptor-independent mechanisms, we induced contractions by membrane depolarization with KCl.
Materials and Methods

Animals

For experiments, six-week-old male Sprague-Dawley rats (Charles River Laboratories, Sulzfeld, Germany) were used. After shipping, rats were housed in the institutional animal care facility under standardized conditions for at least one week prior to experiments. All studies were approved by the federal animal rights committee and were performed in accordance with institutional guidelines for health and care of experimental animals.

Materials

Mammalian expression vectors containing the \( \alpha_1 \)-AR genes were kindly provided by Dr. Robert J. Lefkowitz (Duke University Medical Center, Durham, NC, USA): pBC12Bl\( \alpha_{1A/C} \) (\( \alpha_{1A} \)-AR, bovine) (40), pBC12Bl\( \alpha_{1B} \) (\( \alpha_{1B} \)-AR, hamster) (6), and pCMV5\( \alpha_{1A} \)RA42 (\( \alpha_{1D} \)-AR, rat) (29). The pEGFP-C1 expression vector was obtained from CLONTECH, Heidelberg, Germany.

Subtype-selective goat polyclonal \( \alpha_1 \)-AR antibodies raised against residues in the cytoplasmatic tail of the \( \alpha_1 \)-AR were obtained from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA. The rabbit pan-specific \( \alpha_1 \)-AR antibody raised against residues in the third intracellular loop of \( \alpha_1 \)-AR was purchased from Alexis Deutschland GmbH, Grünberg, Germany.

Other materials were obtained from the following sources: DMRIE-C Transfection Reagent, Alexa 594-labeled secondary antibody (goat anti-rabbit), Dulbecco’s modified Eagle’s medium (DMEM), penicillin, streptomycin, foetal bovine serum, horse serum, and phosphate buffered solution (PBS) from Invitrogen, Karlsruhe, Germany; monoclonal anti-\( \alpha \)-Sm-1 antibody (mouse IgG2a, clone 1A4), bovine serum albumin (BSA), gelatin, collagenase, Triton X-100, paraformaldehyde, phenylephrine, NaCl, KCl, CaCl\(_2\), MgSO\(_4\), KH\(_2\)PO\(_4\), NaHCO\(_3\), and glucose.
from Sigma, Taufkirchen, Germany; VECTASHIELD Mounting Medium with DAPI from Alexis Deutschland GmbH, Grünberg, Germany; and Texas Red-labeled secondary antibody (rabbit anti-goat) from Acris GmbH, Hiddenhausen, Germany.

**Cell Isolation and Transient Transfection**

Rats were anesthetized with pentobarbital sodium (150 mg/kg i.p.), and the thoracic aorta was quickly removed. Aortic cells were isolated by collagen dispersion and propagated in gelatin-coated Lab-Tek™ Chamber Slide™ Systems (Nunc, Wiesbaden, Germany) at 37°C in a humidified 5% CO₂ incubator in DMEM supplemented with 100 units/ml penicillin, 100 µg/ml streptomycin, 10% foetal bovine serum, and 5% horse serum. Cells were grown to approximately 50% confluence and used for experiments 5 days after being plated. Characterization of VSMCs was performed by α-smooth muscle actin immunostaining using the monoclonal anti-α-Sm-1 mouse IgG2a (clone 1A4; Sigma).

Vectors containing one of the three α₁-AR subtypes were transfected into VSMCs together with cDNA encoding a green fluorescent protein (EGFP), which served for detection of transfected cells. The ratio of α₁-AR and EGFP plasmid concentration was 10 µg/ml to 1.0 µg/ml in order to increase the probability of α₁-AR coexpression in EGFP-positive cells. Controls were transfected with cDNA for EGFP only. Transfection was accomplished by incubation of cells in serum-free DMEM with cDNA (11.0 µg/ml) and DMRIE-C (33 µl/ml) at 37°C for 6 hours. The process was terminated by incubation of cells in DMEM containing 10% foetal bovine serum and 5% horse serum for 1 hour. To maintain a contractile phenotype of cells, the medium was subsequently exchanged for serum-free DMEM and cells were incubated for 36-48 hours before they were used for immunocytochemical and functional studies.
Immunocytochemistry and Laser Scanning Confocal Microscopy

Membrane expression of $\alpha_1$-AR was determined immunocytochemically in individual cells via laser scanning confocal microscopy. The slides of the Lab-Tek$^{\text{TM}}$ Chamber Slide$^{\text{TM}}$ Systems with cells attached were washed in PBS and fixed in 3% paraformaldehyde for 10 min. All washes were in PBS containing 1% BSA and 0.1% Triton X-100 to permeabilize cells. After fixation, cells were washed again and 400 $\mu$l of blocking solution (10% serum and 1% BSA in PBS) was placed on the glass slides and incubated at room temperature for 1 hour. After washing, the primary antibody (anti $\alpha_1$-AR, subtype-selective $1:200$ or anti $\alpha_1$-AR pan-specific $1:1000$ in PBS with 1% BSA and 0.1% Triton X-100) was placed on the glass slides and incubated for 16 hours at 4°C. Subsequently, the glass slides were washed and the secondary antibody was applied for 1 hour at room temperature. After a wash in PBS, the glass slides were mounted with VECTASHIELD Mounting Medium, covered with coverslips, and taken for viewing. For immunostainings with subtype-selective $\alpha_1$-AR antibodies rabbit serum and a Texas Red-labeled secondary antibody (rabbit anti-goat, $1:500$ in PBS) were used. Stainings with the pan-specific $\alpha_1$-AR antibody were performed using goat serum and an Alexa 594-labeled secondary antibody (goat anti-rabbit, $1:200$ in PBS). For negative controls, primary antibodies were replaced with serum. No significant labelling was seen under these conditions.

Cells were imaged using the Leica TCS 4 D laser scanning confocal microscope, which was equipped with a PL FLUOTAR 40x oil immersion objective. For detecting Texas Red and Alexa 594 fluorescence an argon-krypton-laser at a wavelength of 568 nm was used. The setting on the laser was constant for all experiments. Only cells of the same rat that were located on one glass slide and which all underwent the same immunocytochemical protocol, were compared. Images were stored on an optical disk as eight-bit, 512-483 pixel tagged image file format files and transferred to a personal computer for off-line analysis.
Functional Studies

Ten rats were used for functional cell culture studies. From each rat, 1-3 cells per group were investigated. Spindle-shaped EGFP-positive cells were selected by fluorescence microscopy. During experiments, cells were monitored under brightfield conditions by a video camera mounted on a microscope (Zeiss, Axiovert S 100). The magnification resulted from a 40x objective lens and projection (1x) on a 0.3” chip digital camera. The spatial resolution was 0.2 µm. The video sequences were captured to a personal computer by a frame grabber card at 2 frames per second. The cell culture medium was replaced by oxygenated and carbonated Krebs’ buffer (118.3 mM NaCl / 4.7 mM KCl / 2.5 mM CaCl_2 / 1.2 mM MgSO_4 / 1.2 mM KH_2PO_4 / 25 mM NaHCO_3 / 11 mM glucose) and adjusted to 37°C by heating the stage of the microscope. First, contraction was elicited by bolus application of high KCl solution (100 mM). The concentration was selected to be high enough to ensure maximal contraction. Subsequently, non-cumulative concentration-response curves were generated for changes in cell length and contraction velocity in response to bolus application of increasing doses of the selective α_1-AR agonist phenylephrine (1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 100 µM). Cells that disconnected from the surface or showed significant morphological changes during successive applications of phenylephrine were excluded from data analysis. Application of Krebs’ buffer alone did not induce significant contractions.
Data Analysis

Immunocytochemistry

The fluorescent intensity of the cell boundary was measured in a constant-sized rectangular area adjacent to the cell surface using the image analysis software NIH Image J 1.32e. Values from ten randomly selected sections per cell were averaged. Mean fluorescence was calculated for each cell group and is presented as percentage of control ± the S.E.M. (n represents the number of cells).

Functional Studies

The video sequences were analysed using imaging software (Khoros, Khoral Research, Inc., Albuquerque, NM) that generated time series of the cell length for each video frame (sampling rate 2 Hz). The cell length time series ($\delta(t)$) were fitted to the following four-parameter logistic response function:

$$\delta(t) = B + \frac{C - B}{1 + e^{-\frac{t-t_{50}}{\tau}}}$$

In this function, the parameter B represents the basal length, C equals the length in the constricted state, $t_{50}$ represents the time point at which 50 % of the maximum change in length is achieved, and $\tau$ is the time constant that determines contraction velocity. For KCl responses, changes in cell length are expressed as absolute changes in µm and as percentage of basal length. The maximum of the first derivative of the logistic response function was used as the
velocity of contraction. For KCl responses absolute (µm/s) and relative (%/s) velocity of contraction is presented.

In concentration-response curves, phenylephrine-induced responses are expressed as percentage of KCl-induced responses. Concentration-response curves were fitted by nonlinear regression to evaluate EC₅₀ and maximal contraction response (Eₘₐₓ). The EC₅₀ values were expressed as the negative logarithm of the drug concentration (pEC₅₀) that produces 50% of the maximal response.

**Statistical Analysis**

Differences between groups were determined by one-way ANOVA followed by the Bonferroni test for multiple comparisons. Since comparisons were made between 4 groups, critical α was reduced to 0.0125.
Results

\(\alpha_1\)-Adrenoceptor Expression in Vascular Smooth Muscle Cells

Subtype-selective antibodies were used to examine the membrane expression of individual \(\alpha_1\)-AR. Using laser scanning confocal microscopy, intense immunofluorescence was obtained with each antibody when assessed in VSMCs transfected with plasmids containing the corresponding receptor. Immunoreactivity was detected along the boundary of the cell, indicating receptor localization in the cell membrane. In line with previously reported observations in Rat1 fibroblasts and VSMCs (22) there was also intracellular immunostaining suggesting that receptors are localized in intracellular compartments as well (Fig. 1). Significantly less immunofluorescence was obtained in controls and in cells transfected with plasmids encoding \(\alpha_1\)-AR subtypes that did not correspond to the applied antibody. For example, using the anti-\(\alpha_{1A}\)-AR antibody fluorescent intensity of the cell boundary was 209±21%, 136±14%, and 124±13% of the control group for \(\alpha_{1A}\)-, \(\alpha_{1B}\)-, and \(\alpha_{1D}\)-AR-transfected cells (\(\alpha_{1A}\) vs. control, respectively, \(p<0.001\); \(\alpha_{1A}\) vs. \(\alpha_{1B}\) and \(\alpha_{1D}\), respectively, \(p<0.01\); \(n=15-17\) in each group; Fig. 2). Immunofluorescence produced by the anti-\(\alpha_{1B}\)-AR antibody was 257±28% of control in cells transfected with the corresponding \(\alpha_{1B}\)-AR subtype, while it was only 110±10% for \(\alpha_{1A}\)- and 149±22% for \(\alpha_{1D}\)-AR-transfected cells (\(\alpha_{1B}\) vs. \(\alpha_{1A}\) and control, respectively, \(p<0.001\); \(\alpha_{1B}\) vs. \(\alpha_{1D}\), respectively, \(p<0.01\); \(n=12-14\) in each group; Fig. 2). Fluorescent intensity produced by the anti-\(\alpha_{1D}\)-AR antibody was 167±16% of control for the \(\alpha_{1D}\)-AR-transfected group, but only 107±10% and 111±9% of control for \(\alpha_{1A}\)- and \(\alpha_{1B}\)-AR-transfected groups (\(\alpha_{1D}\) vs. \(\alpha_{1A}\), \(\alpha_{1B}\), and control, respectively, \(p<0.01\); \(n=15-16\) in each group; Fig. 2). Controls and cells transfected with plasmids encoding \(\alpha_1\)-AR subtypes that did not correspond to the applied antibody did not differ in their fluorescent intensity suggesting that overexpression of one transfected receptor subtype was not associated with up- or downregulation of other subtypes.
To determine overall \( \alpha_1 \)-AR expression including the endogenously expressed receptor fraction, immunostainings with a pan-specific \( \alpha_1 \)-AR antibody were performed. Compared with the control group, fluorescent intensity of the cell boundary was increased to 189±17\%, 224±27\%, and 201±15\% for the \( \alpha_{1A} \)-AR, \( \alpha_{1B} \)-AR, and \( \alpha_{1D} \)-AR (\( \alpha_{1A} \) and \( \alpha_{1D} \) vs. control, respectively, \( p<0.01 \); \( \alpha_{1B} \) vs. control, respectively, \( p<0.001 \); \( n=16-19 \) in each group; Fig. 3) suggesting increased overall \( \alpha_1 \)-AR expression levels in membranes of transfected cells. In contrast, no differences were found between the three transfected groups (Fig. 3).
Contractile Responses of Vascular Smooth Muscle Cells

Basal cell length was 21.5±1.2 µm, 21.4±0.9 µm, 21.1±1.1 µm, and 22.0±1.2 µm for \( \alpha_{1A}\)-AR \((n=16)\), \( \alpha_{1B}\)-AR \((n=16)\), \( \alpha_{1D}\)-AR \((n=17)\), and control \((n=15)\) and did not differ between groups \((P>0.05)\). Cell length reduction in response to KCl (100 mM) was 2.37±0.23 µm (11.0±0.8%), 2.25±0.20 µm (10.5±0.8%), 2.26±0.22 µm (10.7±0.9%), and 2.30±0.27 µm (10.4±0.7%) for \( \alpha_{1A}\)-AR, \( \alpha_{1B}\)-AR, \( \alpha_{1D}\)-AR, and control. There was no significant difference between the four groups neither in absolute nor in relative \((P>0.05)\) changes in cell length. Velocity of contraction was 0.247±0.017 µm/s (1.20±0.08%/s), 0.259±0.021 µm/s (1.24±0.08%/s), 0.230±0.015 µm/s (1.14±0.06%/s), and 0.248±0.015 µm/s (1.17±0.06%/s) for \( \alpha_{1A}\)-AR, \( \alpha_{1B}\)-AR, \( \alpha_{1D}\)-AR, and control. Absolute and relative \((P>0.05)\) velocity of contraction was also similar in all groups suggesting that receptor-independent contractile characteristics were not altered in cells overexpressing \( \alpha_{1A}\)-AR.

The selective \( \alpha_{1A}\)-AR agonist phenylephrine produced concentration-dependent contractions in all groups. The control and cells transfected with one of the three cloned \( \alpha_{1A}\)-AR subtypes did not differ in their maximal length reduction, which was almost 100% of the KCl-induced response. However, sensitivity was increased in all three transfected groups compared with the control. pEC\(_{50}\) was 8.24±0.06, 8.22±0.10, 8.08±0.07, and 7.02±0.05 for \( \alpha_{1A}\)-AR, \( \alpha_{1B}\)-AR, \( \alpha_{1D}\)-AR, and control. Absolute and relative \((P<0.01)\) velocity of contraction was also similar in all groups \((p>0.05)\) suggesting that receptor-independent contractile characteristics were not altered in cells overexpressing \( \alpha_{1A}\)-AR.

There was also a dose-response relationship for contraction velocity to phenylephrine. Although sensitivity for this parameter did not differ between groups maximal velocity of contraction was almost 2-fold higher in transfected cells compared with the control group. E\(_{max}\) was 86.5±3.2%, 99.0±4.4%, 89.5±7.1%, and 53.5±6.0% of KCl for \( \alpha_{1A}\)-AR, \( \alpha_{1B}\)-AR, \( \alpha_{1D}\)-AR, and control \((\alpha_{1A}, \alpha_{1B}, \alpha_{1D} \text{ vs. control, respectively; } p<0.01; \text{ Fig. 5, left})\).
Discussion

Contractile velocity and magnitude of $\alpha_1$-AR-transfected aortic VSMCs in response to phenylephrine, was investigated. We found that elevated $\alpha_1$-AR expression density, irrespective of the expressed subtype, markedly enhanced velocity of contraction suggesting that regulation of receptor expression may be a possible mechanism determining the time course of sympathetic-mediated vasoconstriction. In contrast, a high $\alpha_1$-AR expression level was associated with increased sensitivity but had no influence on maximal magnitude of contraction in VSMCs overexpressing each of the three cloned $\alpha_1$-AR subtypes.

Recent experiments, in which isolated rat aortic VSMCs were periodically stimulated with either phenylephrine or KCl suggest that $\alpha_1$-AR-mediated velocity of smooth muscle contraction may be rate-limiting for sympathetic-mediated vasomotions (25).

The present study confirms the previously reported observation that VSMCs endogenously expressing $\alpha_1$-AR contract slower in response to phenylephrine than to KCl. Moreover, our results extend the previous findings (25) by suggesting that an elevation of the receptor expression level is associated with an increased velocity and sensitivity of cell contraction in response to phenylephrine. Thus, it is reasonable to assume that $\alpha_1$-AR density of the vasculature determines its sympathetic responsiveness, which in turn may have an impact on the frequency and the amplitude of sympathetic-mediated blood pressure oscillations.

Chronic exposure to high levels of humoral catecholamines has been reported to depress vascular $\alpha_1$-adrenergic receptor expression and responsiveness (27) suggesting a downregulation of these receptors by a negative autofeedback mechanism. In line with these findings other studies showed that vascular $\alpha_1$-adrenergic responsiveness is reduced in a variety of physiological and pathophysiological conditions associated with increased sympathetic tone, e.g. advanced age (7; 20), and congestive heart failure (1).
Interestingly, recent investigations in various cell models revealed that autofeedback regulation of $\alpha_1$-AR differs considerably between subtypes. While in these experiments the $\alpha_{1A}$- and $\alpha_{1B}$-AR were downregulated after chronic exposure to phenylephrine, the expression level of the $\alpha_{1D}$-AR increased or remained unchanged following stimulation with this agonist (4; 47).

Since the subtypes have been reported to couple with different efficiencies to inositol phosphate formation and calcium release (14; 35), it is reasonable to assume, that their modulation in cardiovascular tissues may, irrespective of the overall $\alpha_1$-AR expression level, also account for the altered vascular adrenergic responsiveness in age and various disease states. This hypothesis is supported by findings in aortas from rats, where overall $\alpha_1$-AR density remained unchanged with age, while subtype modulation occurred concomitantly with changes in contractile responses (18; 19).

Besides prolonged agonist exposure, various other pathogenetic factors have been associated with changes of $\alpha_1$-AR composition in vascular tissues. For example insulin and insulin-like growth factor I induce $\alpha_{1D}$-AR expression in cultured rat VSMCs (23). Other studies suggest that prolonged hypoxia selectively increases the expression of the $\alpha_{1B}$-AR in aortic smooth muscle cells (9) as well as in liver and in lung (8), a mechanism, which has been proposed to be involved in the pathogenesis of pulmonary hypertension (39).

Although much progress in identifying the interactions of $\alpha_1$-AR with second messenger molecules has been made, it has proven extremely complex to discern the specific role of each $\alpha_1$-AR subtype in arterial pressure regulation. Due to the lack of highly selective agonists and antagonists for these receptors and due to their different vascular distribution, studies of the regulatory activities of $\alpha_1$-AR in vascular tissues are difficult to interpret. The advantage of our study is that we were able to compare the effects of individual $\alpha_1$-AR subtypes on the process of smooth muscle contraction in cells from one vascular region. Remarkably, these cells did not show any subtype-dependent differences in their contractile characteristics. This finding is in seeming contrast to experiments in other cells systems, where the three $\alpha_1$-AR subtypes have been shown to differ in their ability to accumulate intracellular IP and calcium (15; 35).
However, a simple extrapolation of the molecular biological findings may not be sufficient to explain the effects of these receptors on vascular contractility, since there may exist a nonlinear relationship between these factors. Recently, this issue has been addressed in a series of experiments on intact blood vessels. This study reported a non-parallelism between $\alpha_1$-AR-mediated IP formation and smooth muscle contraction in the rat aorta (12), which is possibly a result of sensitization mediated by intracellular enzymes, such as Rho kinase and protein kinase C (31; 48). Moreover, there is convincing evidence for activation of other signaling pathways by $\alpha_1$-AR. These include activation of phospholipase A2 and mitogen activated kinases (26; 45), production of reactive oxygen species (28), and activation of NADPH oxidase (46). Since these signal transduction cascades interact with each other as a network, the activation of one signal pathway can initiate, potentiate or inhibit other signaling systems, leading to a different functional response than expected from the increase of individual second messenger molecules. Recent studies employing oligonucleotide microarrays demonstrated a number of $\alpha_1$-AR subtype-specific changes in gene expression suggesting a link to independent signaling pathways for each subtype (14). Since the expression pattern of $\alpha_1$-AR varies between vascular beds, the distribution of signaling molecules involved in the $\alpha_1$-AR-mediated contractile response is also likely to differ. Thus, other vascular tissues need to be tested to determine, whether our findings in aortic smooth muscle cells are global or vessel-specific.

Since cultured smooth muscle cells lack a variety of conditions determining cell differentiation and function in intact blood vessels, e.g. endothelial and extracellular matrix influences, gap junctions, and permanent transmitter stimulation, the impact of $\alpha_1$-AR expression on Mayer waves needs to be assessed in whole arteries of different vascular beds including resistance vessels as well as in vivo. Correlations between $\alpha_1$-AR density and contractile responses in different vascular tissues may provide an answer to the question of whether differences in $\alpha_1$-AR expression account for the different frequency response characteristics of various vascular beds to sympathetic stimulation (2; 16). Another intriguing question is whether there exist species differences in vascular $\alpha_1$-AR expression and responsiveness. Although the frequency
of Mayer waves has been reported to differ among species (24), it is unknown whether kinetics of vascular contraction contribute to these differences. Further application of gene transfer approaches, comparisons of different vascular regions within one species and between species, as well as the use of genetically modified animals (21; 38; 43) would be attractive methods to resolve these questions.

In summary, the data of the present study suggest that $\alpha_1$-AR density determines velocity and sensitivity of sympathetic-mediated contraction in VSMCs. In contrast, the various $\alpha_1$-AR subtypes tested do not appear to influence vasoconstriction to sympathetic stimuli differently. An altered vascular $\alpha_1$-AR expression level may account for tissue-dependent differences in vascular sympathetic responsiveness as well as for frequency and amplitude changes of Mayer waves found in various pathological conditions.
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α<sub>1</sub>-adrenoceptors and contraction of VSMCs


α1-adrenoceptors and contraction of VSMCs


Figure Legends

Figure 1
Representative confocal fluorescent sections of VSMCs incubated with the α\textsubscript{1A}-AR-, α\textsubscript{1B}-AR-, or α\textsubscript{1D}-AR antibody. Cells transfected with plasmids containing the α\textsubscript{1}-AR subtype corresponding to the used antibody (left) and controls (right). Transfected cells were characterized by an increased fluorescent intensity of the cell boundary. Immunocytochemistry was carried out as described in Materials and Methods. Scale Bar=10 µm

Figure 2
Fluorescent intensity of the boundary of cells stained with subtype-selective anti-α\textsubscript{1}-AR antibodies was used as a relative measure of α\textsubscript{1}-AR subtype expression. For each antibody, fluorescence was determined in VSMCs transfected with each of the three the α\textsubscript{1}-AR subtypes and the respective control. Data are expressed as % of control (means ± S.E.M.; n=12-17 in each group).

Anti-α\textsubscript{1A}: α\textsubscript{1A} vs. control, respectively, #P<0.001; α\textsubscript{1A} vs. α\textsubscript{1B} and α\textsubscript{1D}, respectively, *P<0.01.

Anti-α\textsubscript{1B}: α\textsubscript{1B} vs. α\textsubscript{1A} and control, respectively, #P<0.001; α\textsubscript{1B} vs. α\textsubscript{1D}, respectively, *P<0.01.

Anti-α\textsubscript{1D}: α\textsubscript{1D} vs. α\textsubscript{1A}, α\textsubscript{1B}, and control, respectively, *P<0.01.
**Figure 3**

Fluorescent intensity of the cell boundary as a relative measure of overall $\alpha_1$-AR expression in the membrane of VSMCs transfected with each of the three the $\alpha_1$-AR subtypes and control (expressed as % of control; means ± S.E.M.; n=15-19 in each group; *P<0.01; #P<0.001; transfected cells vs. control, respectively). Cells were stained with a pan-specific $\alpha_1$-AR antibody as described in *Materials and Methods*.

**Figure 4**

Contractile responses of aortic VSMCs transfected with the $\alpha_{1A}$- (n=16), $\alpha_{1B}$- (n=16), or the $\alpha_{1D}$-AR subtype (n=17) and the control group (n=15) to membrane depolarization with KCl (100 mM). Reduction in cell length to KCl was similar in all groups (left). Values are expressed as percentage of the basal length and are presented as means ± S.E.M. Velocity of contraction did also not differ between groups (right). Values are expressed as percent changes per second (%/s) and are presented as means ± S.E.M.

**Figure 5**

Contractile effects of the selective $\alpha_1$-adrenergic receptor agonist phenylephrine in aortic VSMCs transfected with the $\alpha_{1A}$- (n=16), $\alpha_{1B}$- (n=16) or the $\alpha_{1D}$-AR subtype (n=17), and control (n=15). Concentration-response curves for reduction in cell length (left) were significantly left-shifted in transfected cells compared with the control group ($\alpha_{1A}$, $\alpha_{1B}$, $\alpha_{1D}$ vs. control, respectively; P<0.01). Maximum response was similar in all groups. Reduction in cell length is expressed as percentage of the KCl-induced response and is presented as means ± S.E.M. Maximal contraction velocity (right) was increased in transfected groups compared with
\(\alpha_1\)-adrenoceptors and contraction of VSMCs

the control group (\(\alpha_{1A}, \alpha_{1B}, \alpha_{1D}\) vs. control, respectively; \(P<0.01\)). Contraction velocity is expressed as percentage of the KCl-induced response and is presented as means ± S.E.M.
Transfected Cells | Control Group

anti-α\textsubscript{1A}

anti-α\textsubscript{1B}

anti-α\textsubscript{1D}
Fluorescent Intensity
(% of Control)

0 100 200 300 400

Control

\( \alpha_{1A} \)

\( \alpha_{1B} \)

\( \alpha_{1D} \)
Reduction in Cell Length (%) in Response to KCl (100 mM)

Velocity of Contraction (%/s) in Response to KCl (100 mM)
Phenylephrine (M)

Reduction in Cell Length (% KCl)

- Control
- $\alpha_{1A}$
- $\alpha_{1B}$
- $\alpha_{1D}$

Phenylephrine (M)

Velocity of Contraction (% KCl)