**Introduction**

The activation of $\alpha_1$-adrenergic receptors ($\alpha_1$-ARs) and their downstream intracellular signal transduction pathways is a key event for the regulation of arterial contractility and control of blood pressure in situ but function inefficiently when studied in vitro. The present study examined the causes for these inconsistencies in native $\alpha_1$-adrenergic functional performance between the vascular smooth muscle and myocytes.

**Methods:** The $\alpha_1$-adrenergic mediated contraction, $Ca^{2+}$ signaling and the subcellular receptor distribution were evaluated using the Fluo-4, BODIPY-FL prazosin and subtype-specific antibodies.

**Results:** Rat aortic rings and freshly dissociated myocytes displayed contractile and increased intracellular $Ca^{2+}$ responses to stimulation with phenylephrine (PE, 10 µmol), respectively. However, the PE-induced responses disappeared completely in cultured aortic myocytes, whereas PE-enhanced $Ca^{2+}$ transients were seen in cultured rat cardiac myocytes. Further studies indicated that $\alpha_1D$-ARs, the major receptor subtype responsible for the $\alpha_1$-adrenergic regulation of aortic contraction, were distributed both intracellularly and at the cell membrane in freshly dispersed aortic myocytes, similar to the $\alpha_1A$-AR subcellular localization in the cultured cardiomyocytes. In the cultured aortic myocytes, however, in addition to a marked decrease in their protein expression relative to the aorta, most labeling signals for $\alpha_1D$-ARs were found in the cytoplasm. Importantly, treating the culture medium with charcoal/dextran caused the reappearance of $\alpha_1D$-ARs at the cell surface and a partial restoration of the $Ca^{2+}$ signal response to PE in approximately 30% of the cultured cells.

**Conclusion:** Reduction in $\alpha_1D$-AR total protein expression and disappearance from the cell surface contribute to the insensitivity of cultured vascular smooth muscle cells to $\alpha_1$-adrenergic receptor activation.

**Keywords:** $\alpha_1D$-adrenergic receptor; vascular smooth muscle cells; receptor sensitivity; receptor distribution; $Ca^{2+}$ signaling
are coupling, rendering a fine tuning for the α₁-adrenergic responsiveness to different biological signals and for adapting cells to changes in the internal milieu and to overall homeostasis. Therefore, exploring the receptor characteristics of and regulatory mechanisms for each subtype is important for understanding and explicating their functional roles in both physiological and pathophysiological processes. An increasing number of studies in vivo as well as in vitro have been performed to examine the cellular functions and regulatory mechanisms of α₁A-ARs and α₁D-ARs. Also, α₁D-ARs have been found to mediate contractile responses to catecholamines in several blood vessels with high potency. In addition, they are thought to be the critical mediator of noradrenaline in several blood vessels with high potency. In the current study, we sought to determine the causes for the inconsistencies in α₁AR regulatory effects on vascular contractility between the in vivo and in vitro studies by comparing α₁AR-mediated Ca²⁺ signaling and its subcellular distribution in native rat aortic and cardiac myocytes. α₁A-AR and α₁D-AR subtypes are known to contribute mainly to α₁ adrenergic mediation of constriction in the rat aorta and cardiac muscle, respectively.

**Materials and methods**

**Materials**

All reagents and drugs were used purchased from Sigma, except A61603 (N-[5-(+/-)-dihyro-1H-imidazol-2-yl)-2-hydroxy-5,6,7,8-tetrahydroquinoline-1-yl)methanesulfonamide hydrobromide) and Fluo-4/AM in DMEM at 37 °C for 30 min and washed three times with HBSS for 20 min. [Ca²⁺]i was then measured as described previously. Rat thoracic aortic myocytes were isolated as follows. Adipose and connective tissues, as well as endothelial cells, were removed, and aortas were digested in HBSS containing 1 mg/mL collagenase (type II, Worthington Biochem), 0.2 mg/mL elastase (type III, Sigma), and 1 mg/mL bovine serum albumin for 20 min at 37 °C. Following incubation, the aortas were massaged and flushed with HBSS. The dissociated VSMCs were collected by centrifugation and either seeded onto laminin-coated dishes for measurements of intracellular Ca²⁺ concentration ([Ca²⁺]i) in freshly isolated cells or plated into plastic tissue culture dishes and grown in Dulbecco’s modified Eagle’s medium (DMEM, Hyclone) containing 10% fetal bovine serum, 4 mmol L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin (Hyclone) at 37 °C under 5% CO₂–95% air. Cells were used from the third through the eighth passages.

**Measurement of intracellular Ca²⁺**

The rat VSMCs from rats or mice were loaded with 4 μmol Fluo-4/AM in DMEM at 37 °C for 30 min and washed three times with HBSS for 20 min. [Ca²⁺]i, was then measured as described previously.

**Immunocytochemistry**

VSMCs were fixed in 4% formaldehyde in phosphate-buffered saline (PBS) for 10 min, permeabized with 0.1% Triton X-100, and then blocked in PBS containing 5% bovine serum albumin, 5% horse serum and 0.05% Triton X-100 for 1 h. Antibodies specific for α₁A-ARs or α₁D-ARs were added and incubated overnight at 4 °C at a dilution of 1:100 (Santa Cruz). Secondary antibodies used were Alexa Fluor 594 goat anti-rabbit or Alexa Fluor 488 goat anti-goat (Invitrogen) at a dilution of 1:50. For the binding measurement of BODIPY FL prazosin, living myocytes were washed twice with HBSS, loaded with HBSS containing 2 μmol BODIPY FL prazosin, 1% bovine serum albumin and 0.1% F127 for 30 min at room temperature. Analysis of subcellular immunostaining was performed using a Leica SP 5 confocal microscope equipped with a 40x oil immersion objective lens (NA 1.25).

**Immunoblotting**

Rat aortic smooth muscle and cultured myocytes were lysed in RIPA buffer containing 1 mmol polymethlysulfonyl fluoride and 2 μg/mL protease inhibitor cocktail (Santa Cruz) for 1 h on ice. Homogenates were centrifuged for 15 min at 14000×g at 4 °C. Lysates of 30 μg in cells or 15 μg in tissue were heated for 5 min, resolved on a 10% SDS-PAGE gel and transferred to PVDF membrane. Membranes were blocked with 5% non-fat milk powder in Tris-buffered saline containing 0.1% (v/v) Tween 20 for 60 min at room temperature and then probed connected to a carrier amplifier and analyzed with AcqKnowledge software (MP150, Biopac Systems, Inc.)
with specific antibodies against α₁A- or α₁D-ARs at a dilution of 1:500 overnight at 4 °C. The immunoblotted membrane was then incubated with horseradish peroxidase-conjugated secondary antibody for 1 h, and immunoreactive bands were detected using an enhanced chemiluminescence kit (ECL-plus).

Statistical analysis and data presentation
Mechanical responses of the arteries were expressed as the increase in force (g). All results are reported as means±SEM (n=number of cells or arteries, as indicated). Statistical differences between means were analyzed using a two-way paired or unpaired Student’s t-test or χ² test, when appropriate. Probability levels <5% were considered significant.

Results
Contractile and internal Ca²⁺ signal responses of rat and mouse aortas to α₁AR activation

To investigate the native α₁AR function at the tissue level, the non-subtype selective α₁AR agonist phenylephrine (PE)-induced vasoconstriction was evaluated in rat aortic rings. Similar to the response induced by 80 mmol KCl depolarization, PE (10 µmol) evoked a strong tonic constriction in the vessels, which was completely abolished by prazosin, a specific antagonist for α₁ARs (Figure 1A). In a Ca²⁺-free medium, a transient contractile response to PE was followed by a relaxation to the baseline level, suggesting an internal Ca²⁺ release-related constriction. Upon the re-addition of extracellular CaCl₂ (2.0 mmol) in the presence of PE, further sustained force was induced (Figure 1B). This force could be decreased significantly by nifedipine (1 µmol, data not shown), demonstrating an extracellular Ca²⁺-dependent constriction of this part. Additionally, as a reference for vasoconstriction regulation among GPCR members, angiotensin II (Ang II), an agonist of the angiotensin receptor, was also examined in this study; Ang II produced similar responses to PE in the rings (Figure 1B).

Figure 1. Differences in contractile and intracellular Ca²⁺ signal responses to α₁-adrenergic receptor activation between the rat thoracic aorta and cultured aortic smooth muscle cells. (A) Stimulating rat aortic rings with PE or high KCl concentration (80 mmol) (indicated with an arrow) induced a tonic force in Ca²⁺ containing HBSS. Pretreatment of the rings with 1 µmol prazosin for 10 min abolished the effect of PE, but not that of high KCl. (B) PE or Ang II caused a transient aortic contraction in Ca²⁺-free medium, followed by a tonic constriction upon Ca²⁺ addition to the medium. (C and D) A [Ca²⁺] increase upon stimulation with high KCl (C) or Ang II (D), but not with PE, in cultured aortic VSMCs loaded with fluo4. Numbers for each curve in all panels ranged from 6 to 12 separate experiments.
Therefore, these data demonstrate a common characteristic of Ca²⁺-dependent vasoconstriction by the activation of two different GPCR members, consistent with previous studies on arteries[25, 27, 28].

To investigate the native α₁AR function at the cellular level, α₁AR-mediated [Ca²⁺]i signaling was further measured in isolated aortic VSMCs by confocal microscopy. Unlike in rat aortic rings, the cultured VSMCs did not respond to PE (10 to 300 µmol), but exhibited a striking [Ca²⁺]i increase to the addition of 40 mmol KCl and stimulation with Ang II (Figures 1C and D). This observation was generally identical in the third to eighth cultured VSMCs passages and also in all passages of A7R5 cells (a cell line of rat aorta, data not shown), suggesting that a similar phenomenon occurs in different culture of cells.

Since the α₁D-AR subtype is tightly linked to blood pressure control in mice[15, 16], we also investigated the α₁AR-mediated Ca²⁺ signaling responses in vessels and VSMCs in mice. ATP, an activator of the purinic receptor (a member of GPCR superfamily)[26], was used for comparison, because Ang II was unable to cause observable vasoconstriction in the mouse thoracic aorta. Similar to the situation in rats, PE caused a remarkable aortic contraction in mouse aorta, but failed to induce any Ca²⁺ signal response in the cultured VSMCs, whereas ATP elicited a relatively weak tension in the vessel and a profound rise in [Ca²⁺], in cultured mouse VSMCs (Figures 2A and B). This much weaker aorta contraction induced by ATP than by the other GPCR receptor activators is likely due to ATP’s additional relaxing effect on vessels caused by activation of protein kinase A[29] and the delayed ATP-elicited potassium channel[30].

Intracellular Ca²⁺ signal responses to α₁AR activation in freshly isolated thoracic aortic myocytes

The above results indicate that among the three members of the GPCR family, only the α₁AR-coupled Ca²⁺ signaling pathway in the vessels was somehow masked in the culture condition, implying some alteration in the α₁AR themselves occurred during the cell dissociation or/and culturing procedures. Thus, we further examined the α₁AR-mediated Ca²⁺ signals in rat aortic myocytes of freshly isolated, and primarily cultured for <1 day, ≥2 days or beyond 3 days. VSMCs were identified morphologically and functionally by their ability to respond to 40 mmol KCl by contracting and [Ca²⁺]i increasing. Cells that did not exhibit a Ca²⁺ response to high K⁺ depolarization were not used. As shown in Figure 3 and Table 1, PE induced an obvious cell shortening and simultaneous [Ca²⁺], increase in 91.6±4.3% of freshly dispersed and 55.2±4.9% of cultured <1 day VSMCs (n=32 and 40, respectively), but completely failed to evoke any [Ca²⁺], response in the cells cultured for 2 days or beyond, all of which remained responsive ability to either high K⁺ or Ang II stimulation. Therefore, the results indicate that the α₁AR-coupled Ca²⁺ signaling pathway in VSMCs is gradually and ultimately blocked by cell culture, not cell isolation.

Intracellular Ca²⁺ signal responses to α₁AR activation in primary cultured cardiac ventricular myocytes

Unlike in the VSMCs, functional α₁AR-mediated Ca²⁺ signals, such as increasing [Ca²⁺], or spontaneous Ca²⁺ transient rate have been shown in primary cultured neonatal mouse or rat ventricular myocytes (NRVM)[21-24], implying unaltered responsibility for cardiomyocytes to α₁AR activation even in the culture state. To clarify this, we testified the PE effect on [Ca²⁺], in freshly isolated and cultured NRVM for <1 day and ≥2 day for compatibility with the aortic myocytes. As expected, all of the cardiomyocytes from either group

| Time of culture (d) | PE 10 (µmol) | KCl 40 (mmol) | Ang II 1 (µmol) |
|---------------------|--------------|---------------|-----------------|
| 0                   | 91.6±4.3     | 100           | 100             |
| <1                  | 55.2±4.9     | 100           | 100             |
| ≥2                  | 0            | 100           | 100             |
| Beyond 3            | 0            | 100           | 100             |
exhibited a robust increase in \([\text{Ca}^{2+}]_i\) or the spontaneous \(\text{Ca}^{2+}\) transient frequency due to 10 µmol PE stimulation (Figure 4).

**Different \(\alpha_2\)-AR subtypes in mediating \(\text{Ca}^{2+}\) signaling between vascular and cardiac myocytes**

The different results between cultured VSMCs and cardiac myocytes after \(\alpha_1\)-AR activation presumably suggest that distinctive receptor subtypes are responsible for the respective intracellular couplings. We thus investigated the functional \(\alpha_1\)-AR subtype in the rat aorta and compared our data with previous results obtained in cardiomyocytes\cite{20, 24, 31–33}. Similar to other studies\cite{9, 13, 14, 24, 32}, we combined selective antagonists for each subtype with selective agonists to distinguish among contributions of the different subtypes to vasoconstriction. BMY 7378, a selective antagonist of \(\alpha_1D\)-ARs, attenuated the PE-induced constriction in a dose-dependent manner and completely abolished the constriction at a concentration of 30 µmol. Selective inhibition of \(\alpha_{1A}\)-ARs with 5-Mu (30 nmol) did not affect the PE effect, and A61603 (1 µmol), a highly selective \(\alpha_{1A}\)-AR agonist, did not induce any tension above baseline (data not shown). The irreversible antagonist chloroethyl-clonidine (CEC) at 10 µmol, the only available antagonist of \(\alpha_1B\)-ARs at present, inhibited the PE-induced contraction by approximately 30% (Figure 5), implying an involvement of \(\alpha_1B\) -ARs to some extent; however, these data do not provide a definite identification of the responsible subtype because of the low selectivity (5- to 10-fold) of CEC for \(\alpha_1B\)-AR over the other \(\alpha_1\)-AR subtypes\cite{34}.

Taken together, these data demonstrated that \(\alpha_1\)-AR functional relevance in the rat aorta and cardiac myocytes, especially for intracellular \(\text{Ca}^{2+}\) regulation, can be attributed to the activation of \(\alpha_{1D}\)-AR and \(\alpha_{1A}\)-AR subtypes, respectively, in agreement with previous reports\cite{13–15, 24, 31–33}.
Differential distribution of α_{1D}-ARs between freshly dispersed and cultured aortic myocytes

This study, thus far, has demonstrated that, unlike the functional receptor subtype in cardiomyocytes, α_{1D}-ARs in VSMCs lost their sensitivity to activation after the cells were cultured. We then investigated the expression and subcellular distribution of α_{1D}-ARs between freshly dissociated VSMCs (obvious Ca^{2+} signal response in more than 90% cells) and cultured VSMCs (no Ca^{2+} signal response at all) and compared these data with the distribution pattern of α_{1A}-ARs in cultured cardiomyocytes.

An interesting report in α_{1D}-AR transfected HEK293 cells has suggested that the treatment of culture medium with charcoal/dextran (C/D) increases the α_{1D}-AR distribution on cell membranes and increases receptor’s sensitivity to activation. Thus, we determined the subcellular localization of α_{1D}-AR subtypes with BODIPY-FL prazosin in live cells and using specific antibodies for individual subtypes in permeabilized cells. The tested cells were divided into four groups: freshly isolated VSMCs, VSMCs cultured for 2 days in DMEM in the presence of 2% charcoal/dextran (+C/D) or with the absence of 2% charcoal/dextran (-C/D), and NRVM cultured for 2 days.

As shown in Figures 6A and B, the binding signals for BODIPY-FL prazosin and anti-α_{1D}-AR antibody were located both intracellularly and on the cell surface in freshly isolated VSMCs as well as in aorta tissue (data not shown), but membrane labeling disappeared in VSMC cultured -C/D, and was instead uniformly distributed inside the cytoplasm. Interestingly, cell membrane labeling could be detected in part of cultured VSMCs +C/D (membrane binding was detected in 34.67±4.1%, n=84). Compared with α_{1D}-ARs, α_{1A}-AR binding appeared much less in VSMCs, but was apparent in most cultured NRVM, with a pattern of both cytosolic and cell membrane distributions.

Accordingly, a robust internal Ca^{2+} signal response to 10 µmol PE was observed in both freshly isolated VSMCs and cultured NRVM. Further, while VSMCs cultured -C/D showed no response to PE (Figure 6C), an obvious PE-induced rise in [Ca^{2+}] (but lower than that in the freshly isolated VSMCs) could be induced in 29.5±1.2% of tested VSMCs cultured +C/D (n=81), a response sensitive to the inhibitory effect of BMY 7378 (data not shown).

Finally, the protein expression of the α_{1A} AR subtypes in aorta tissue and cultured myocytes was analyzed by western-blotting. Compared with the labeling signal for α_{1A}-ARs, α_{1D}-ARs seemed to be the predominant receptor subtype in vascular muscle and were expressed at a higher level in the aorta tissue than in the cultured aortic myocytes, in accordance with the staining results. Additionally, the signal intensities for the α_{1D}-AR bands were almost equal for VSMCs cultured -C/D and VSMCs cultured +C/D, indicating no significant effect of the C/D treatment on the native VSMC α_{1D}-AR expression (Figure 7).

Discussion

Our data showed that in rat or mouse aortas, a marked Ca^{2+}-dependent contractile response was induced upon α_{1D} AR activation (Figures 1A and B, and 2A). In this response, the α_{1D}-AR subtype played the most important role (Figure 5 and refs 13–15). However, when VSMCs were isolated from the aorta and then cultured, the α_{1A}-AR-associated Ca^{2+} signal response completely disappeared (Figures 1C and D, and 2B), a phenomenon also observed in other studies. Under identical conditions, the cultured cardiac myocytes did exhibit a robust intracellular Ca^{2+} signal to α_{1A} AR activation (Figure 4) due to a different functional receptor subtype, the α_{1A}-ARs, responsible for this effect (see refs 24, and 31–33). Additionally, α_{1A}-ARs in the cultured cardiac myocytes were found both intracellularly and at the cell membrane distributions, whereas α_{1D}-ARs were most frequently concentrated intracellularly in cultured VSMCs (Figures 6A and B), consistent with existing research. Interestingly, similar α_{1D} AR distribution patterns, α_{1A} adrenergic-sensitive cell shortening and [Ca^{2+}] increases (as manifested in the cardiomyocytes) were clearly observable in most of the freshly isolated aortic myocytes, but disappeared completely in VSMCs cultured ≥ 2 days (Figures 3
Importantly, treating the culture medium with charcoal/dextran for 2 days caused the reappearance of α₁D-ARs on the cell surface and the partial recovery of the α₁AR-associated Ca²⁺ signal response in approximately 30% of the cultured VSMCs (Figure 6). Therefore, these results indicate a strong correlation between the cell membrane-expressed α₁D-ARs and functional intracellular Ca²⁺ coupling, which is somehow lost during cell culture.

Charcoal/dextran treatment in the medium has been indicated to be capable of reducing steroid levels by absorbing free hormones, free fatty acids and various serum factors in fetal bovine serum[39]. Previous studies have shown that charcoal/dextran increases chloride channel expression in cultured epithelial cells[40], increases α₁D-AR expression on the cell membrane and also increases cells’ responsiveness to recep-
tor stimulation in α1D-AR recombinant HEK293 cells[33]. The current study further confirms the charcoal/dextran effect in native α1D-ARs in VSMCs. As there was no significant change in VSMC receptor-protein expression, except for the cellular localization owing to charcoal/dextran treatment, the mechanism responsible for α1D-AR insensitivity after cell culture may be partially due to some elements in the culture medium that either desensitize the α1D-ARs by abrogating cell membrane insertion or facilitating internalization of the receptors in the native cells. Recently, several lines of evidence have implicated some critical factors for modulating α1D-AR functional expression at the cell membrane. For example, the truncation of 79 amino acids from the receptors’ N-termini results in translocation of the α1D-ARs from intracellular compartments onto the plasma membrane, and a three- to four-fold increase in IP3 formation due to norepinephrine stimulation[41]. Additionally, dystrophin proteins, a type of intracellular anchor protein, have been identified as essential elements for α1D-AR but not for α1A- or α1B-AR functional expression for both in vitro and in vivo situations[42]. Therefore, all the previous studies describe a clear picture indicating the complexity of regulating α1D-AR expression at the cell membrane, a critical process for the receptor efficient functional performance.

In addition to the disappearance of cell membrane α1D-ARs, a marked reduction in receptor protein expression also occurred in the cultured VSMCs relative to the tissues studied (Figure 7). It is difficult to determine whether the reduction is secondary to the internalization of the receptors from the cell membrane or vice versa. Nevertheless, compared with the response in the freshly dispersed aortic myocytes, the significantly lower amplitude and duration of the PE-induced [Ca2+]i increase in the charcoal/dextran-treated cultured VSMCs (Figure 6C) suggest only a partial restoration of the native receptor activity by the treatment of culture medium with 2% charcoal/dextran.

In summary, the parallel investigations between vascular and cardiac myocytes indicate that first cell-surface expressed α1-ARs are critical in triggering intracellular signal transduction cascades, especially for intracellular Ca2+ signaling. Second, α1D-ARs in the vessels primarily distribute intracellularly and on the cell membrane in situ, but are somehow reduced and abrogated from the membrane localization after the VSMCs are cultured. Third, unlike the α1A-ARs of vascular myocytes, the α1B-ARs, actually likely α1A-ARs, in cardiac myocytes are not dramatically altered during the procedures of either cell isolation or culturing. These findings may help provide answers for the inconsistencies reported in α1D-AR function in vascular myocytes between in situ and in vitro studies. Treatment of the culture medium with charcoal/dextran can partially prevent native α1D-AR losses from the cell membrane and coupling intracellular signal machinery that occur in the cell culture process.

Acknowledgements

This study was supported by grants from the National Natural Science Foundation (No 30772574), the Beijing Municipal Project for Developing Advanced Human Resources for Higher Education, the Scientific Research Common Program of the Beijing Municipal Commission of Education (DL), and the Beijing Natural Science Foundation (N_{2008}2018).

Author contribution

Prof Da-li LUO designed the research; Lin-lin FAN, Shuang REN, Hong ZHOU, Ying WANG, and Ping-xiang XU executed the research methods; Profs Jun-qi HE and Da-li LUO provided new reagents and analyzed data; and Lin-lin FAN and Shuang REN prepared the manuscript.

References

1. Wu D, Katz A, Lee CH, Simon ML. Activation of phospholipase C by alpha1-adrenergic receptors is mediated by the subunits of Gq family. J Biol Chem 1992; 267: 25798–802.
2. Graham RM, Perez DM, Hwa J, Piascik MT. Alpha1-adrenergic receptor subtypes. Molecular structure, function and signaling. Circ Res 1996; 78: 737–49.
3. Michelotti GA, Price DT, Schwinn DA. Alpha1-adrenergic receptor regulation: basic science and clinical implications. Pharmacol Ther 2000; 88: 281–309.
4. Albert AF, Large WA. Signal transduction pathways and gating mechanisms of native TRP-like cation channels in vascular myocytes. J Physiol 2006; 570 (Pt 1): 45–51.
5. Zhong H, Minneman KP. Alpha1-adrenoceptor subtypes. Eur J Pharmacol 1999; 375: 261–76.
6. Piascik MT, Perez DM. Alpha1-adrenergic receptors: new insights and directions. J Pharmacol Exp Ther 2001; 298: 403–10.
7. Milligan G. Exploring the dynamics of regulation of G protein-coupled receptors using green fluorescent protein. Br J Pharmacol 1999; 128: 501–10.
8. Chalothorn D, McCune DF, Edelmann SE, Garcia-Cazarin ML, Tsujimoto G, Piascik MT. Differences in the cellular localization and agonist-mediated internalization properties of the alpha1-adrenoceptor subtypes. Mol Pharmacol 2002; 61: 1008–16.
9. Théroux TL, Ebenshade TA, Peavy RD, Minneman KP. Coupling efficiencies of human alpha1-adrenergic receptor subtypes: titration of receptor density and responsiveness with inducible and repressible expression vectors. J Pharmacol Exp Ther 1996; 50: 1376–87.
10. Uberti MA, Hall RA, Minneman KP. Subtype specific dimerization of alpha1-adrenoceptors: effects on receptor expression and pharmacological properties. Mol Pharmacol 2003; 64: 1379–90.
11. Hein P, Michel MC. Signal transduction and regulation: Are all alpha1-adrenergic receptor subtypes created equal? Biochem Pharmacol 2007; 73: 1097–106.
12. Perez DM. Structure–function of alpha1-adrenergic receptors. Biochem Pharmacol 2007; 73: 1051–62.
13. Kenny BA, Chalmers DH, Philpott PC, Naylor AM. Characterization of an alpha2AR-adrenoceptor mediating the contractile response of rat aorta to noradrenaline. Br J Pharmacol 1995; 115: 981–6.
14. Gisbert R, Pérez-Vizcaino F, Cogolludo AL, Noguera MA, Ivorra MD, Tamargo J, et al. Cytosolic Ca2+ and phosphoinositide hydrolysis linked to constitutively active alpha2AR-adrenoceptors in vascular smooth muscle. J Pharmacol Exp Ther 2003; 305: 1006–14.
15. Tanoue A, Nasa Y, Koshimizu T, Shinoura H, Oshikawa S, Kawai T, et al. The alpha1D-adrenergic receptor directly regulates arterial blood pressure via vasoconstriction. J Clin Invest 2002; 109: 765–75.
16. Hosoda C, Koshimizu T, Tanoue A, Nasa Y, Oikawa R, Tomabechi T, et al. Two alpha1-adrenergic receptor subtypes regulating the
vascular smooth muscle phenotype. Focus on “Dual role of PKA in phenotype modulation of vascular smooth muscle cells by extracellular ATP”. Am J Physiol Cell Physiol 2004, 287: C260–2.

Serr K, Hayoz S, Fanachaouy M, Beny JL, Bychkov R. A delayed ATP-elicted K+ current in freshly isolated smooth muscle cells from mouse aorta. Br J Pharmacol 2006; 147: 45–54.

Woodcock EA, Du XJ, Reichelt ME, Graham RM. Cardiac alpha1-adrenergic drive in pathological remodeling. Cardiovasc Res 2008; 77: 452–62.

Knowlton KU, Michel MC, Itani M, Shubeita HE, Ishihara K, Brown JH. The alpha_{2A}-adrenergic receptor subtype mediates biochemical, molecular, and morphologic features of cultured myocardial cell hypertrophy. J Biol Chem 1993; 268: 15374–80.

Luo D, Gao J, Fan L, Tang Y, Zhang Y, Han Q. Receptor subtype involved in alpha1-adrenergic receptor-mediated Ca2+ signaling in cardiomyocytes. Acta Pharmacol Sin 2007; 28: 921–1086.

Hanft G, Gross G. Subclassification of alpha1-adrenergic recognition sites by urapidil derivatives and other selective antagonists. Br J Pharmacol 1989; 97: 691–700.

Khattar SK, Bora RS, Priyadarsiny P, Gautam A, Gupta D, Tiwari A, et al. Molecular cloning, stable expression and cellular localization of human alpha1-adrenergic receptor subtypes: effect of charcoal/dextran treated serum on expression and localization of alpha1D-adrenergic receptor. Biotechnol Lett 2006; 28: 1731–9.

Mackenzie JF, Daly CJ, Pedian JI, Mcgrath JC. Quantitative imaging in live human cells reveals Intracellular alpha1-adrenoceptor ligand-binding sites. J Pharmacol Exp Ther 2000; 294: 434–43.

Woodsome TP, Polzin A, Kitazawa K, Eto M. Kitazawa T. Agonist- and depolarization-induced signals for myosin light chain phosphorylation and force generation of cultured vascular smooth muscle cells. J Cell Sci 2006; 119: 1769–80.

Koshimizu T, Tsujimoto G, Hirasawa A, Kitagawa Y, Tanoue A. T. Agonist- and depolarization-induced signals for myosin light chain phosphorylation and force generation of cultured vascular smooth muscle cells. J Cell Sci 2006; 119: 1769–80.

Koshimizu T, Tsujimoto G, Hirasawa A, Kitagawa Y, Tanoue A. T. Agonist- and depolarization-induced signals for myosin light chain phosphorylation and force generation of cultured vascular smooth muscle cells. J Cell Sci 2006; 119: 1769–80.

Koshimizu T, Tsujimoto G, Hirasawa A, Kitagawa Y, Tanoue A. T. Agonist- and depolarization-induced signals for myosin light chain phosphorylation and force generation of cultured vascular smooth muscle cells. J Cell Sci 2006; 119: 1769–80.

Koshimizu T, Tsujimoto G, Hirasawa A, Kitagawa Y, Tanoue A. T. Agonist- and depolarization-induced signals for myosin light chain phosphorylation and force generation of cultured vascular smooth muscle cells. J Cell Sci 2006; 119: 1769–80.

Koshimizu T, Tsujimoto G, Hirasawa A, Kitagawa Y, Tanoue A. T. Agonist- and depolarization-induced signals for myosin light chain phosphorylation and force generation of cultured vascular smooth muscle cells. J Cell Sci 2006; 119: 1769–80.

Koshimizu T, Tsujimoto G, Hirasawa A, Kitagawa Y, Tanoue A. T. Agonist- and depolarization-induced signals for myosin light chain phosphorylation and force generation of cultured vascular smooth muscle cells. J Cell Sci 2006; 119: 1769–80.

Koshimizu T, Tsujimoto G, Hirasawa A, Kitagawa Y, Tanoue A. T. Agonist- and depolarization-induced signals for myosin light chain phosphorylation and force generation of cultured vascular smooth muscle cells. J Cell Sci 2006; 119: 1769–80.

Koshimizu T, Tsujimoto G, Hirasawa A, Kitagawa Y, Tanoue A. T. Agonist- and depolarization-induced signals for myosin light chain phosphorylation and force generation of cultured vascular smooth muscle cells. J Cell Sci 2006; 119: 1769–80.

Koshimizu T, Tsujimoto G, Hirasawa A, Kitagawa Y, Tanoue A. T. Agonist- and depolarization-induced signals for myosin light chain phosphorylation and force generation of cultured vascular smooth muscle cells. J Cell Sci 2006; 119: 1769–80.