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Mechanism of Acitretin-Induced Relaxations in Isolated Rat Thoracic Aorta Preparations

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ABSTRACT

Acitretin is a member of vitamin A-derived retinoids and its effect on vascular smooth muscle had not been studied yet. The aim of this study is to investigate the effect of acitretin, a retinoid, on vascular smooth muscle contractility. Thoracic aorta preparations obtained from 34 male Sprague-Dawley rats (355 ± 15 g) were studied in isolated organ baths containing Krebs-Henseleit solution. The relaxation responses were obtained with acitretin (10^{-12}–10^{-4} M) in endothelium-preserved and endothelium-denuded aorta preparations precontracted with submaximal concentration of phenylephrine (10^{-6} M). The roles of retinoic acid receptor (RAR), nitric oxide, adenylyl and guanylyl cyclase enzymes, and potassium channels in these relaxation responses were investigated. Acitretin produced concentration-dependent relaxations, which were independent of its solvent dimethylsulfoxide, in endothelium-denuded phenylephrine-precontracted thoracic aorta preparations. While incubation with the RAR antagonist (AGN193109, 10^{-5} M) had no effect on these relaxations; nitric oxide synthase inhibitor (L-NAME, 10^{-4} M), adenylyl cyclase inhibitor (SQ2253, 10^{-5} M), guanylyl cyclase inhibitor (ODQ, 10^{-6} M), and potassium channel blocker (tetraethylammonium-TEA, 10^{-2} M) significantly eliminated the relaxation responses induced by acitretin. Acitretin induces relaxation in rat isolated thoracic aorta preparations without endothelium, which may be mediated by nitric oxide, cyclic adenosine monophosphate and cyclic guanosine monophosphate-dependent kinases and potassium channels.

Keywords: retinoids, acitretin, vascular smooth muscle, aorta, relaxation, dimethylsulfoxide

INTRODUCTION

Acitretin is a retinoid that contain vitamin A derivatives or chemically related compounds (Orfanos et al., 1997). Retinoids include the first (retinol, retinal, tretinoin [retinoic acid], isotretinoin), second (etretinate and acitretin), and third (adapalene, tazarotene) generation molecules (Orfanos et al., 1997, Mukherjee et al., 2006). Acitretin is the active metabolite of etretinate and belongs to the second generation retinoids. It has regulatory effects on epithelial cell proliferation and differentiation, and immunomodulatory and anti-inflammatory effects (Dogra and Yadav, 2014).

Retinoids act on nuclear receptors within the cell and regulate gene transcription. One of the retinoid receptors is the retinoic acid receptor (RAR) that belongs to the nuclear receptors family. This receptor family, which is important in the receptor-ligand interaction, is encoded by different genes and have three different subsets expressed as alpha, beta, gamma (RAR-α, -β, -γ) (Orfanos et al., 1997, Chandraratna, 1998). Acitretin has been shown to activate all three subtypes of RAR (Orfanos 1997, Saurat, 1999). RARs can form heterodimers with various nuclear receptors such as vitamin D3 and thyroid hormone T3 receptors. This heterodimeric structure plays a role in the crosstalk mechanism in signaling pathways (Orfanos et al., 1997, Chandraratna, 1998, Saurat, 1999).

Acitretin is mainly used in the treatment of psoriasis and has some advantages over etretinate such as less sequestration in adipose tissue and rapid elimination (Orfanos et al., 1997, Dogra and Yadav, 2014). Among the most common side effects of orally administered acitretin are cheilitis, skin peeling, alopecia, dry skin, rhinitis, and increased liver enzymes, lactate dehydrogenase, and triglyceride levels; the most serious unwanted effect being teratogenicity (Dogra and Yadav, 2014, Katz et al., 1999). In addition, there are case reports of erectile dysfunction in patients

treated with acitretin suggesting that acitretin may have an effect on smooth muscle (Coleman and Macdonald, 1994, Csaba and Geal, 1997, Reynolds, 1991, Rossi and Pellegrino, 2009). As a widely used retinoid in the treatment of psoriasis, the effect of acitretin on smooth muscle, which may be important in terms of efficacy and safety, has not been studied in detail.

Considering the potential effect of acitretin on smooth muscle, in this study we aimed to evaluate its effect on rat thoracic aorta *in vitro* and the mechanisms that may be associated with this effect.

MATERIAL AND METHODS

Animals

This study was approved by the Başkent University Animal Experiments Local Ethics Committee (ANNEX-1, project number: DA18 / 27) and was supported by the Başkent University Research Fund (Project Approval Date: 17.09.2018). All the study procedures were carried out according to the Guide for the Care and Use of Laboratory Animals (NIH, USA).

Thirty-four male Sprague Dawley rats (355 ± 15 g) were used. The acclimatization of rats was provided for 7 days in a ventilated room with constant temperature (25 ± 2°C) and relative humidity (32% ± 7%) under 12-hour light and 12-hour dark cycle. Standard rat chow and tap water were given to all animals *ad libitum.*

Surgery

Thoracic aorta of rats was removed through a midline thoraco-abdominal incision under general anethesia (60 mg/kg, ketamine and 7 mg/kg, xylazine; IM), and rats were sacrificed after collecting 6–8 ml of blood from the left ventricle. Approximately 1 cm proximal thoracic aorta was taken into a petri dish containing ice-cold physiological solution. Surrounding fat and connective tissues were carefully removed. Two ring-shaped aorta preparations of ~3 mm in

length were prepared. The endothelium was removed by gently turning a glass rod within its lumen 20 times in both directions around its axis in one of the preparations.

Experimental protocol

The isolated thoracic aorta ring preparations were mounted in 10 ml double jacketed glass organ baths containing Krebs-Henseleit solution ([mM]: NaCl, 118.2; KCl, 4.7; $MgSO_4$, 1.2; $CaCl_2$, 1.25; KH_2PO_4, 1.2; NaHCO, 25; glucose, 11.1). One end of tissue preparation was connected to isometric force-displacement transducer (FDT05, Commat Ltd., Turkey), the other end was fixed vertically to the organ sling. The solution was gassed continuously with 95% O_2 and 5% CO_2 mixture throughout the experiment and was kept at a constant temperature of 37°C with the help of a thermostatic controlled water circulator (WBC3044, Commat Ltd.). Isometric tension changes were recorded by a computerized physiological data acquisition and recording system (BIOPAC MP100, Commat Ltd.). All preparations were placed in isolated organ baths and maintained under 1 g resting tension for 60 min by washing with fresh solution every 15 min. Endothelium was accepted to be removed when the relaxation in response to acetylcholine (10^{-5} M) is less than 20% of the phenylephrine precontraction.

Concentration-contraction responses to the increasing phenylephrine concentrations (10^{-12}–10^{-4} M) were obtained for each preparation, and the concentration producing 60–80% of the maximum contraction response was accepted as the submaximal phenylephrine concentration. For all preparations, this concentration was determined to be 10^{-6} M. We also performed a time-control study for the response to phenylepherine, and found that the contractile response obtained with phenylephrine (10^{-6} M) persisted in the absence of acitretin (data not shown). The relaxation responses were obtained with increasing concentrations of acitretin in endothelium (+) and endothelium (-) preparations precontracted with submaximal concentration of phenylephrine

(10^{-6} M). Integrity of smooth muscle was confirmed by obtaining relaxation response to sodium nitroprusside (10^{-5} M) at the end of each experimental protocol.

In order to evaluate the role of retinoic acid receptors, nitric oxide, adenylyl cyclase and guanylyl cyclase, and potassium channels in the effect of acitretin on rat aorta preparations, the changes in relaxation responses obtained with acitretin were examined after 120-min incubation with RAR antagonist (AGN193109, 10^{-5} M), 30-min incubation with nitric oxide synthase (NOS) inhibitor (L-N^G-Nitro arginine methyl ester, L-NAME; 10^{-4} M)], 30-min incubation with adenylyl cyclase inhibitor (SQ22536; 10^{-5} M), 30-min incubation with guanylyl cyclase inhibitor (oxadiazolo [4,3-a] quinoxalin-1-one, ODQ; 10^{-6} M), and 30-min incubation with potassium channel blocker (tetraethylammonium, TEA; 10^{-2} M). Each antagonist was tested in separate tissues after obtaining initial responses to phenylephrine and acitretin. In order to evaluate whatever acitretin has an α_1-adrenergic receptor antogonistic effect or not, concentration-contraction response curves to the increasing concentrations of phenylephrine (10^{-8}–10^{-4} M) in endothelium (+) and (-) aorta preparations were obtained in the absence of acitretin and after 20-min incubation with three different concentrations of acitretin (10^{-8}, 10^{-7} and 10^{-6} M) (n = 6), and the difference between curves was investigated. All experimental protocols were repeated with the solvent of acitretin, dimethylsulfoxide (DMSO), in a concentration range that was used to dissolve 10^{-12}–10^{-4} M acitretin. The concentration of DMSO used to prepare 10^{-4} M acitretin stock solution was 1%,which was then serially diluted in water such that 10^{-8} M aciretin contains 0.0001% DMSO. Since acitretin caused significantly more relaxation than DMSO in endothelium (-) aorta preparations, the mechanism of vasorelaxant effect of acitretin was explored in endothelium (-) aorta preparations.

Chemicals

All of the chemicals and drugs used in the study were obtained from Sigma-Aldrich (Merck, Darmstadt, Germany). Acitretin (10^{-4} M), AGN193109 (10^{-5} M), SQ22536 (10^{-5} M) and ODQ (10^{-6} M) were dissolved in DMSO, and the remaining drugs were dissolved in distilled water. All drugs were prepared in highest concentrations of stocks in their solvents daily and subsequently diluted to required concentrations.

Statistical analysis

The data were expressed as mean ± standard error of mean (SEM). The effect of concentration of acitretin and antagonists on the concentration-response curve was evaluated by two-way analysis of variance (ANOVA) test for repeated measurements followed by post-*hoc* Bonferroni test, E_{max} and EC_{50} values could not be calculated because it was not possible to obtain sigmoid concentration-response curves with maximum response due to insufficient supply of acitretin. SPSS software (Statistical Package for Social Sciences, version 25.0, SPSS Inc., Chicago, Illinois, USA) was used for the analysis the data, and the statistical significance level was accepted as $P < 0.05$.

RESULTS

Acitretin (10^{-12}–10^{-4} M) induced concentration-dependent relaxation response in both endothelium (+) and endothelium (-) aorta preparations precontracted with submaximal concentration of phenylephrine (10^{-6} M) (Figure 1). The relaxation response of acitretin at 10^{-12}–3×10^{-5} M concentration range was significantly higher in endothelium (+) preparations than that of endothelium (-) ones ($P < 0.05$; Figure 2a). When the relaxing effects of acitretin and its solvent DMSO in endothelium (+) preparations were compared; a statistically significant difference was determined only at 10^{-4} M concentration, while in endothelium (-) preparations, acitretin produced significantly more relaxation than that of DMSO in all concentrations ($P <$

153 0.05; Figure 2b, 2c).

154 There was no significant difference between phenylephrine concentration-response curves in the
155 absence and presence of acitretin, suggesting that α1-adrenergic receptors blockade had no role in
156 the vasorelaxant effect of acitretin ($P > 0.05$; Figure 3).

157 In endothelium (-) preparations, RAR antagonist AGN193109 (10^{-5} M) had no significant effect
158 on the relaxation responses of acitretin ($P > 0.05$; Figure 4), while incubation with NOS inhibitor
159 L-NAME (10^{-4} M) caused significant decrease in the relaxations induced by all concentrations of
160 acitretin (10^{-12}–10^{-4} M) ($P < 0.05$; Figure 5). The adenylyl cyclase inhibitor SQ22536 (10^{-5} M)
161 also significantly inhibited the acitretin-induced relaxations in endothelium (-) aorta preparations
162 precontracted with phenylephrine ($P < 0.05$; Figure 6a). Similarly, guanylyl cyclase inhibitor
163 (ODQ; 10^{-6} M) significantly inhibited the relaxation responses of acitretin, even producing
164 contractions at some concentrations of acitretin ($P < 0.05$; Figure 6b).

165 Incubation with the non-specific potassium channel blocker TEA (10^{-2} M) generally inhibited
166 relaxant effect of acitretin, while changing the relaxation response in the direction of contraction
167 at the concentration range of 10^{-12}–10^{-6} M ($P < 0.05$; Figure 7).

168 **DISCUSSION**

169 In this study, we primarily demonstrated that acitretin induces relaxation in the rat thoracic aorta by
170 various mechanisms independent of endothelium including activation of nitric oxide, cAMP and
171 cGMP-dependent kinases and potassium channels. Although previous clinical data proposed that
172 acitretin may have an effect on smooth muscle (Coleman and Macdonald, 1994, Csaba and Gaal,
173 1997, Reynolds, 1991, Rossi, 2009), the present *in vitro* study revealed for the first time the
174 vasorelaxant effect of acitretin and the mechanisms by which this effect may be related.

Acitretin and its solvent DMSO produced similar relaxation responses in endothelium (+) aorta preparations, whereas acitretin caused significantly more relaxation in endothelium (-) aorta than DMSO, suggesting that acitretin has a vasorelaxant effect on endothelium-denuded vascular smooth muscle, independent of its solvent. Since we obtained an endothelium-independent relaxing effect of acitretin, the mechanism of vasorelaxant effect of acitretin was examined on endothelium-denuded aorta preparations. It is remarkable that DMSO, a frequently used solvent in experimental studies, creates relaxation responses in the endothelium preserved rat aorta. This finding is consistent with the previous study by Kaneda et al. (2016) who reported that DMSO induced relaxation in endothelium-intact rat aorta by releasing NO from endothelial cells. Since it was out of the scope of the present study, this interesting effect of DMSO was not evaluated. However, on the basis of our findings and previous reports (Kaneda et al., 2016),endothelium-associated vasorelaxant effect of DMSO should be examined in further studies.

The vasorelaxant effect of acitretin observed in the present study is in line with a previous study (Wang et al., 2013), reporting that all-trans retinoic acid (ATRA), a naturally occurring derivative of vitamin A (retinol), induces relaxations in rat mesenteric artery. However, it is noteworthy that in contrary to Wang et al. reporting the ATRA-dependent relaxations in endothelium-preserved artery, the relaxing effect of acitretin on the aorta was independent of the endothelium in our study.

Pharmacokinetic studies indicated that plasma concentration of acitretin after long-term therapy may reach to 500 µg/L (1.5×10^{-6} M) (Vahlquist and Rollman, 1987). In the present *in vitro* study, vasorelaxant effect of acitretin started at a concentration of 10^{-9} M. Therefore, it may be proposed that some unwanted smooth muscle-related effects of acitretin treatment may be caused by its relaxant effect on vascular smooth muscles, which needs to be confirmed in further translational

198 and clinical studies.

199 The experiments to reveal the mechanism of acitretin-induced vasorelaxant effect in
200 phenylephrine-precontracted rat thoracic aorta have shown that neither blockade of alpha-1
201 adrenoceptors nor activation of RAR play a role in this relaxation. In contrary, the vasorelaxation
202 responses of ATRA in rat mesenteric artery have been reported to completely diminish after
203 application of RAR antagonist (BMS492), suggesting that RAR may play a role in ATRA-
204 mediated relaxation responses (Wang et al., 2013).

205 However, relaxation responses with increasing concentrations of acitretin were significantly
206 inhibited in the presence of NOS inhibitor (L-NAME), adenylyl cyclase inhibitor (SQ22536),
207 guanylyl cyclase inhibitor (ODQ), and non-specific potassium channel blocker (TEA)
208 suggesting that pathways involving nitric oxide, adenylyl and guanylyl cyclase enzymes and
209 potassium channels may be involved in the relaxing effect of acitretin on vascular smooth
210 muscle. The complete elimination of the acitretin-induced relaxations in endothelium (-) aorta in
211 the presence of L-NAME suggested that neuronal NOS rather than endothelial may participate in
212 these relaxation responses. Previous studies proposed that neuronal NOS can be an alternative to
213 endothelial NOS for nitric oxide generation, leading to vascular smooth muscle relaxation. The
214 neuronal NOS has been shown to be expressed in vascular smooth muscle in addition to various
215 neuronal and non-neuronal tissues (Forstermann and Sessa, 2012, Chen et al., 2008, Kashiwagi et
216 al., 2002, Suematsu et al., 2003). Arce et al. (2017) showed that among various rat blood vessels, only
217 rat aorta contains neuronal NOS. It has also been shown that the neuronal NOS can compensate the
218 loss of endothelial NOS in knockout mice, and vasodilation can still be maintained by nitric
219 oxide (Huang et al., 2002, Talukder et al., 2004). The presence of neuronal NOS in the vascular
220 tissue of wild-type mice and in the endothelium of coronary arteries in endothelial NOS knockout

mice have also been revealed by using immunohistochemical techniques (Huang et al., 2002, Talukder et al., 2004). However, it was reported that ATRA reduced endothelial NOS expression and nitric oxide synthesis in bovine aorta endothelial cells (Cho et al., 2005). In contrary, AM580, an ATRA and RAR agonist, significantly increased endothelial NOS phosphorylation, phosphoinositide 3-kinase (PI3K) activity, and nitric oxide production in human dermal microvascular endothelial cells, human umbilical artery endothelial cells, and rat lung vascular endothelial cells, which was inhibited by L-NAME, suggesting that ATRA increases nitric oxide production by endothelial NOS phosphorylation as a result of RAR-mediated PI3K/Akt pathway activation in vascular endothelial cells (Uruno et al., 2005). On the other hand, based on our findings and previous studies showing the expression of neuronal NOS in vascular smooth muscle of rat aorta (Arce et al., 2017, Forstermann and Sessa, 2012, Chen et al., 2008, Suematsu et al., 2003, Kashiwagi et al., 2002, Huang et al., 2002), we propose that acitretin could induce a relaxation response via cGMP/guanylyl cyclase mediated by nitric oxide synthesized by neuronal NOS in rat thoracic aorta preparation.

In another study, it was reported that adenylyl cyclase-6 plays a key role in the vasodilation mechanism and in regulating the membrane potential of vascular smooth muscle cells (Nelson et al., 2011). Since acitretin-induced vasorelaxation was inhibited by the specific adenylyl cyclase inhibitor (SQ22536), we propose that in addition to nitric oxide pathway, adenylyl cyclase enzyme and/or its product, cAMP, contribute to relaxation responses of acitretin. This finding is in line with another study in which it was reported that all retinoids increase cAMP formation in guinea pig keratinocyte cell lines (Wilkinson and Orenberg, 1983).

The relaxation responses caused by ATRA in isolated endothelium (+) rat mesenteric arteries have been shown to be inhibited *in vitro* by the guanylyl cyclase inhibitor, and intraperitoneal

ATRA administration caused a significant decrease in blood pressure in spontaneously hypertensive rats (Wang et al., 2013). Similarly, in the present study relaxation responses of acitretin in endothelium (-) rat isolated thoracic aorta preparations were abolished with ODQ pre-administration, thus, we suggest that guanylyl cyclase enzyme and/or cGMP contributes to the vasorelaxant effect of acitretin.

Finally, our findings indicated that potassium channels may also participate in acitretin-induced vasorelaxation in endothelium (-) aorta preparations. The blockade of calcium-activated potassium channels has been reported to inhibit the relaxation response obtained with ATRA in the endothelium (+) rat mesenteric arteries (Wang et al., 2013). When evaluated together with our other findings, acitretin may cause vascular smooth muscle relaxation by activating potassium channels via non-endothelial NO.

The main limitation of the study was lack of *in vivo* data to confirm the vasorelaxant effect of acitretin, or molecular data to clarify the interactions of various pathways underlying this relaxant effect. Furthermore, small sample size and insufficient supply of acitretin precluded us from reaching more definitive conclusions on the mechanisms of acitretin-induced endothelium-independent vasorelaxation. Despite these limitations, the presented study is of critical importance as it is the first study to show the effect of acitretin on vascular smooth muscle.

In conclusion, acitretin induces an endothelium-independent relaxation response in vascular smooth muscle by involvement of various pathways including nitric oxide, cAMP and/or cGMP, and potassium channels. Further studies are needed in order to clarify the exact mechanisms underlying acitretin relaxations in vascular smooth muscle and interaction of these pathways, and to explore the clinical significance of acitretin-induced vasorelaxation.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

This experimental study was approved by the Baskent University Animal Experiments Local Ethics Committee (ANNEX-1, project number: DA18 / 27) and supported by the Baskent University Research Fund (Project Approval Date: 17.09.2018). The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest.

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FIGURE LEGENDS

Figure 1. Original recording of isometric tension changes showing the relaxations induced by acitretin (10^{-12}–10^{-4} M) in endothelium (-) rat thoracic aorta preparations precontracted with submaximal concentration of phenylephrine (PE). wo, wash-out

Figure 2. (a) Effect of acitretin (10^{-12}–10^{-4} M) on phenylephrine (10^{-6} M) pre-contracted endothelium (+) and endothelium (-) rat isolated thoracic aorta preparations (n = 10). *$P < 0.05$ for endothelium (+) vs. endothelium (-). Comparison of vasorelaxant effects of acitretin and its solvent dimethylsulfoxide (DMSO) on phenylephrine (10^{-6} M) pre-contracted endothelium (+)**(b)** and endothelium (-) **(c)** rat isolated thoracic aorta preparations. * $P < 0.05$ for acitretin vs. DMSO. DMSO concentration range corresponding to 10^{-12}–10^{-4} M of acitretin concentrations is 10^{-8}-1%. Vertical deviations represent the standard error of the mean.

Figure 3. Phenylephrine concentration (10^{-8}–10^{-4} M)-response curves in the absence and presence of acitretin (10^{-8}, 10^{-7} and 10^{-6} M) in endothelium (+) **(a)** and endothelium (-) **(b)** rat isolated thoracic aorta preparations (n = 6). Vertical deviations represent the standard error of the mean.

Figure 4. Effect of acitretin (10^{-12}–10^{-4} M) on phenylephrine (10^{-6} M) pre-contracted endothelium (-) rat isolated thoracic aorta preparations incubated (n = 3) and not incubated (n = 10) with RAR antagonist AGN193109 (10^{-5} M). Vertical deviations represent the standard error of the mean.

Figure 5. Effect of acitretin (10^{-12}–10^{-4} M) on phenylephrine (10^{-6} M) pre-contracted endothelium (-) rat isolated thoracic aorta preparations incubated (n = 6) and not incubated (n = 10) with nitric oxide synthase inhibitor L-NAME (10^{-4} M). * $P < 0.05$ for L-NAME (+) vs.L-

358 NAME (-). Vertical deviations represent the standard error of the mean.

359 **Figure 6. (a)** Effect of acitretin (10^{-12}–10^{-4} M) on phenylephrine (10^{-6} M) pre-contracted
360 endothelium (-) rat isolated thoracic aorta preparations incubated (n = 6) and not incubated (n =
361 10) with adenylyl cyclase inhibitor SQ22536 (10^{-5} M). * $P < 0.05$ for SQ22536 (+) vs. SQ22536 (-
362) **(b)** Effect of acitretin (10^{-12}–10^{-4} M) on phenylephrine (10^{-6} M) pre-contracted endothelium (-)
363 rat isolated thoracic aorta preparations incubated (n = 6) and not incubated (n = 10) with
364 guanylyl cyclase inhibitor ODQ (10^{-6} M). * $P < 0.05$ for ODQ (+) vs. ODQ (-).

365 **Figure 7.** Effect of acitretin (10^{-12}–10^{-4} M) on phenylephrine (10^{-6} M) pre-contracted
366 endothelium (-) rat isolated thoracic aorta preparations incubated (n =6) and not incubated (n =
367 10) with potassium channel blocker (TEA, 10^{-2} M). * $P < 0.05$ for TEA (+) vs. TEA (-) * $P < 0.05$;
368 versus TEA. Vertical deviations represent the standard error of the mean.

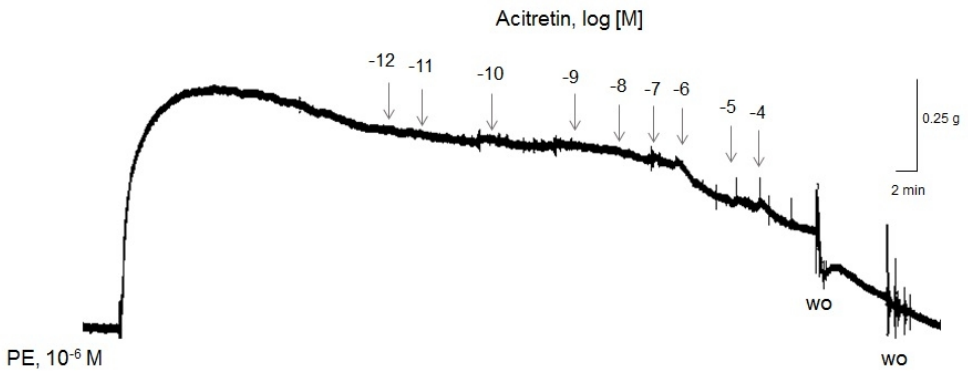

Figure 1. Original recording of isometric tension changes showing the relaxations induced by acitretin (10-12–10-4 M) in endothelium (-) rat thoracic aorta preparations precontracted with submaximal concentration of phenylephrine (PE). wo, wash-out

238x92mm (96 x 96 DPI)

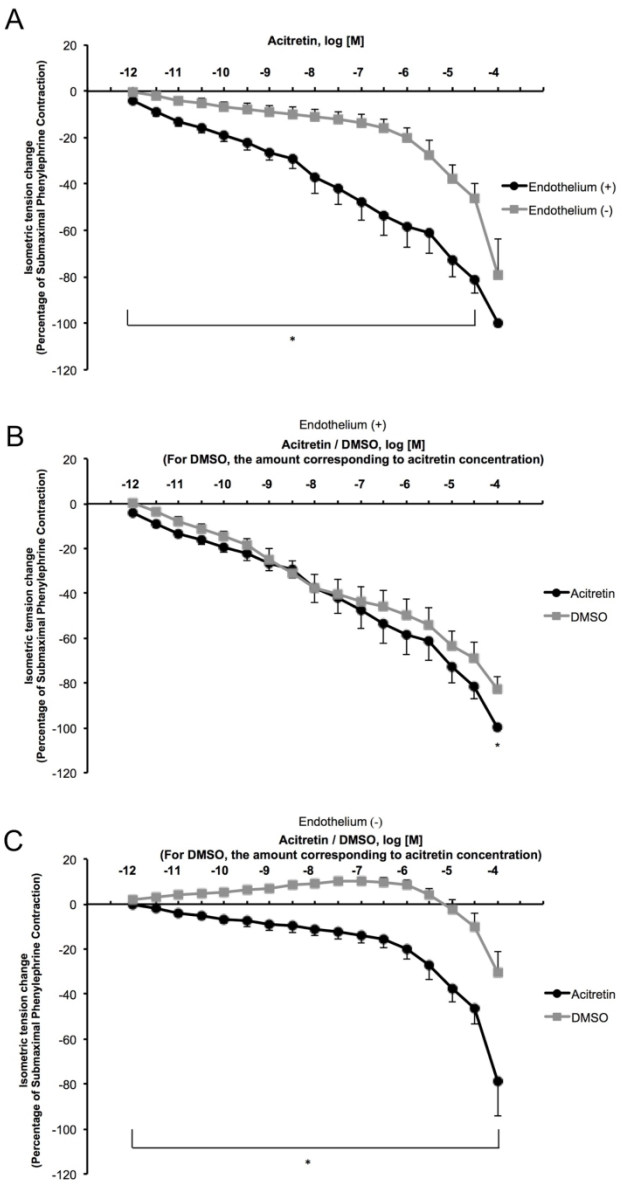

Figure 2. (a) Effect of acitretin (10-12–10-4 M) on phenylephrine (10-6 M) pre-contracted endothelium (+) and endothelium (-) rat isolated thoracic aorta preparations (n = 10). *P < 0.05 for endothelium (+) vs. endothelium (-). Comparison of vasorelaxant effects of acitretin and its solvent dimethylsulfoxide (DMSO) on phenylephrine (10-6 M) pre-contracted endothelium (+)(b) and endothelium (-) (c) rat isolated thoracic aorta preparations. * P < 0.05 for acitretin vs. DMSO. DMSO concentration range corresponding to 10-12–10-4 M of acitretin concentrations is 10-8-1%. Vertical deviations represent the standard error of the mean.

150x285mm (300 x 300 DPI)

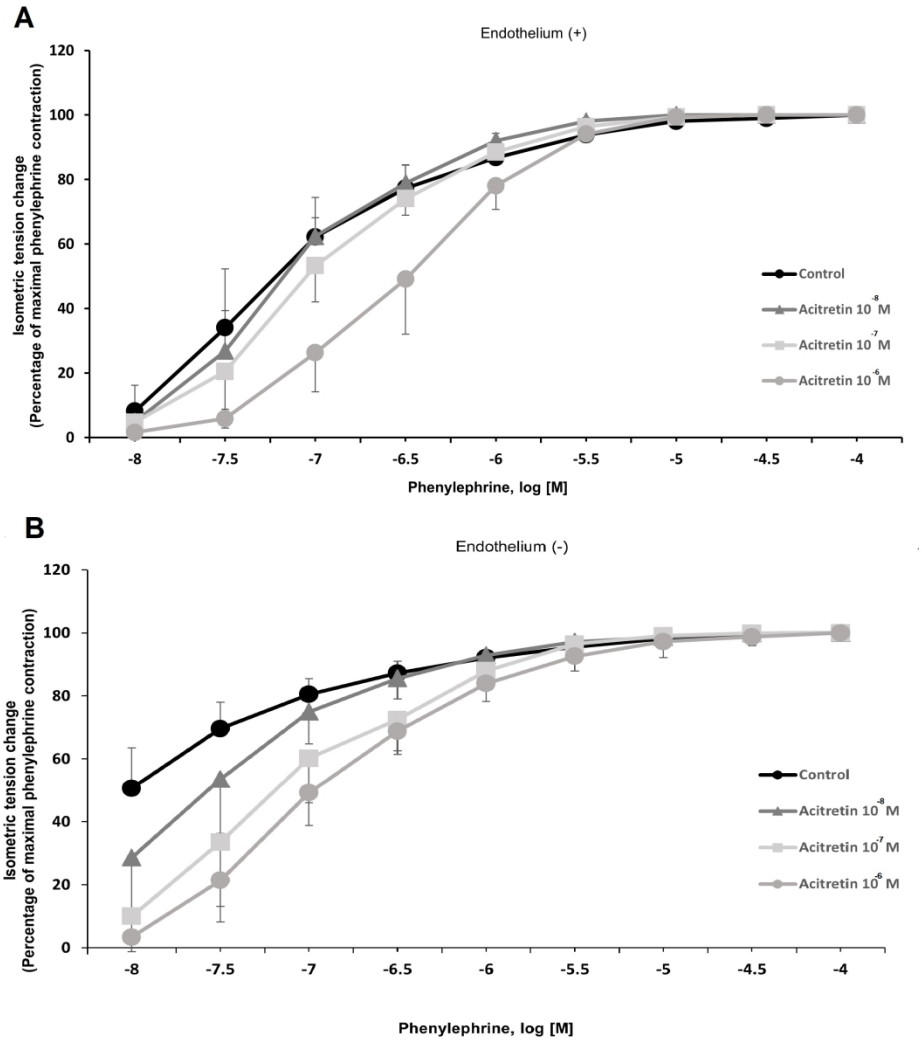

Figure 3. Phenylephrine concentration (10-8–10-4 M)-response curves in the absence and presence of acitretin (10-8, 10-7 and 10-6 M) in endothelium (+) (a) and endothelium (-) (b) rat isolated thoracic aorta preparations (n = 6). Vertical deviations represent the standard error of the mean.

546x701mm (120 x 120 DPI)

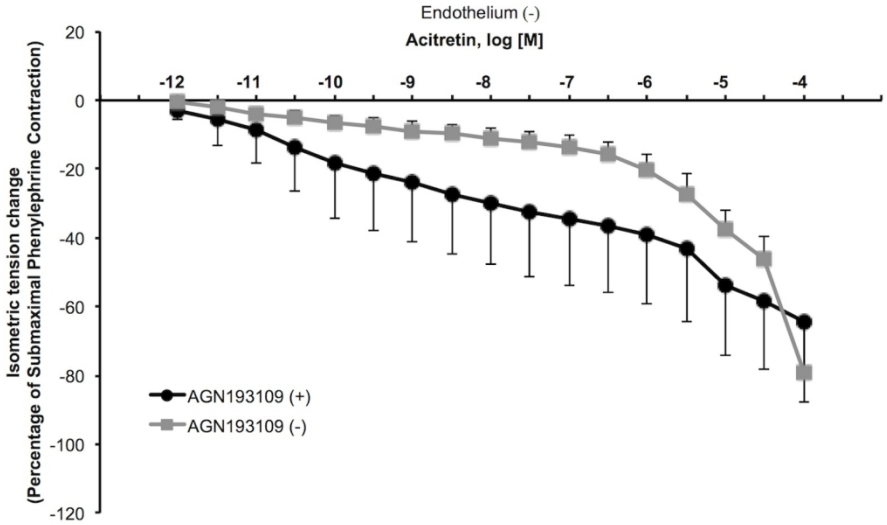

Figure 4. Effect of acitretin (10-12–10-4 M) on phenylephrine (10-6 M) pre-contracted endothelium (-) rat isolated thoracic aorta preparations incubated (n = 3) and not incubated (n = 10) with RAR antagonist AGN193109 (10-5 M). Vertical deviations represent the standard error of the mean.

150x91mm (300 x 300 DPI)

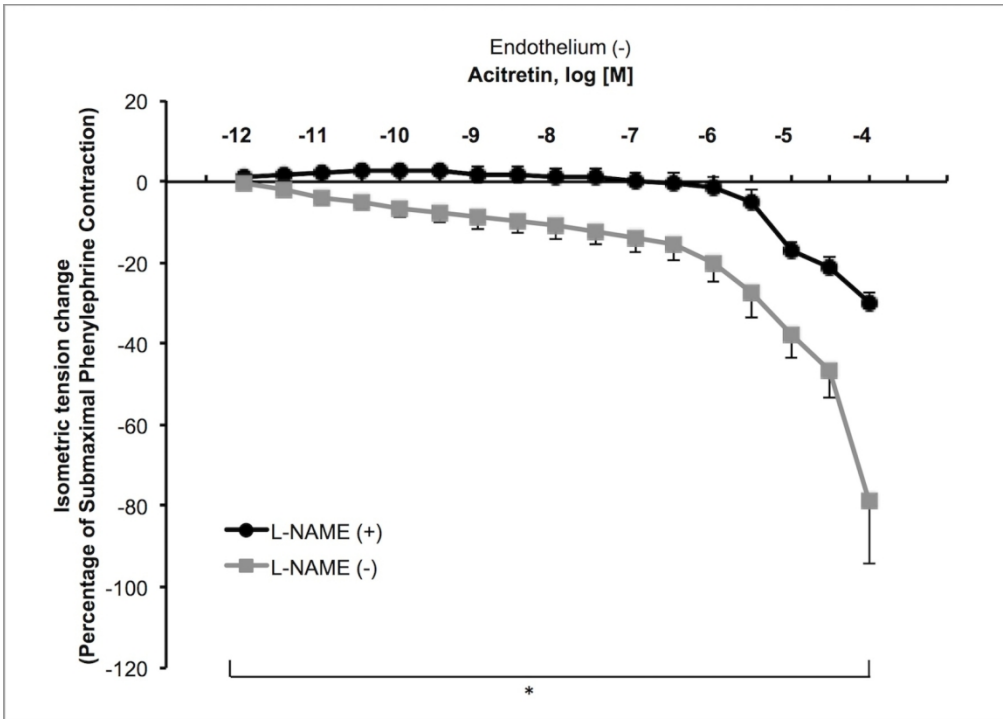

Figure 5. Effect of acitretin (10-12–10-4 M) on phenylephrine (10-6 M) pre-contracted endothelium (-) rat isolated thoracic aorta preparations incubated (n = 6) and not incubated (n = 10) with nitric oxide synthase inhibitor L-NAME (10-4 M). * P < 0.05 for L-NAME (+) vs.L-NAME (-). Vertical deviations represent the standard error of the mean.

150x107mm (300 x 300 DPI)

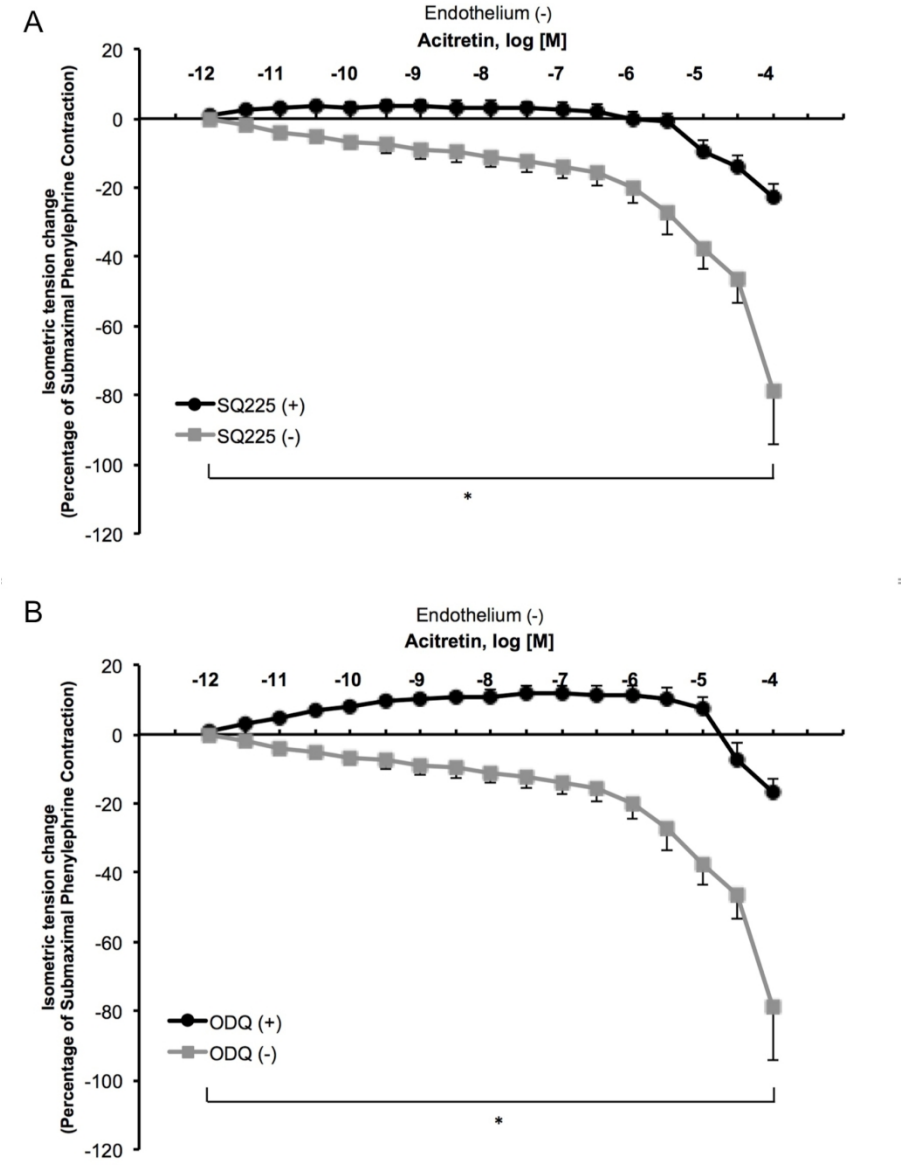

Figure 6. (a) Effect of acitretin (10-12–10-4 M) on phenylephrine (10-6 M) pre-contracted endothelium (-) rat isolated thoracic aorta preparations incubated (n = 6) and not incubated (n = 10) with adenylyl cyclase inhibitor SQ22536 (10-5 M). * P < 0.05 for SQ22536 (+) vs. SQ22536 (-) (b) Effect of acitretin (10-12–10-4 M) on phenylephrine (10-6 M) pre-contracted endothelium (-) rat isolated thoracic aorta preparations incubated (n = 6) and not incubated (n = 10) with guanylyl cyclase inhibitor ODQ (10-6 M). * P < 0.05 for ODQ (+) vs. ODQ (-).

150x199mm (300 x 300 DPI)

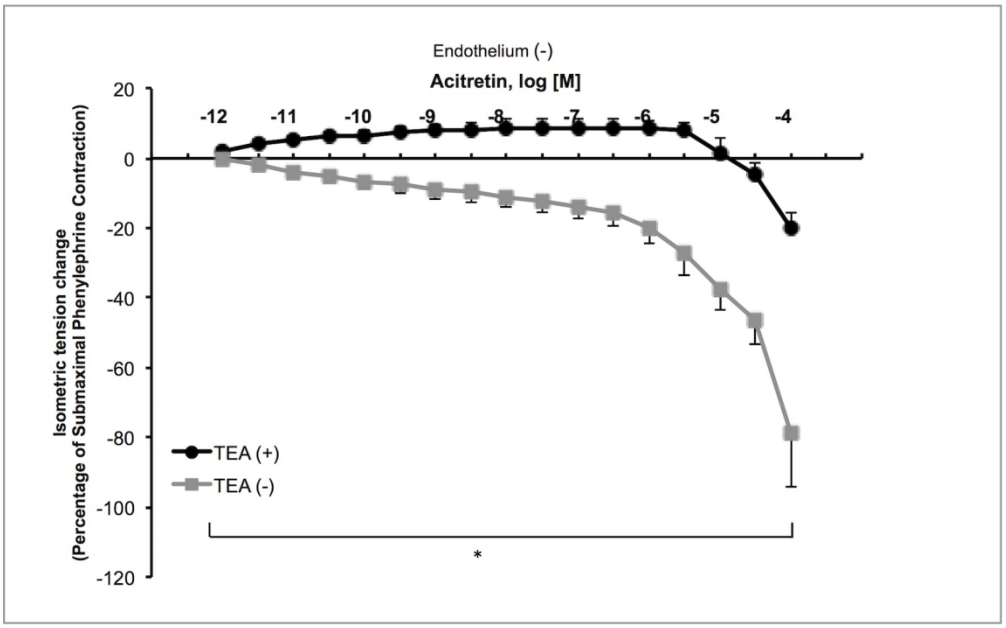

Figure 7. Effect of acitretin (10-12–10-4 M) on phenylephrine (10-6 M) pre-contracted endothelium (-) rat isolated thoracic aorta preparations incubated (n =6) and not incubated (n = 10) with potassium channel blocker (TEA, 10-2 M). * $P < 0.05$ for TEA (+) vs. TEA (-) * $P < 0.05$; versus TEA. Vertical deviations represent the standard error of the mean.

150x93mm (300 x 300 DPI)