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Increased Ang II Contraction of the Uterine Artery at Early Gestation in A Transgenic Model of Hypertensive Pregnancy is Reduced by Inhibition of Endocannabinoid Hydrolysis

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Abstract

Increased vascular sensitivity to Ang II is a marker of a hypertensive human pregnancy. Recent evidence of interactions between the RAS and the endocannabinoid system (ECS) suggests that anandamide (AEA) and 2-arachidonoylglycerol (2-AG) may modulate Ang II contraction. We hypothesized that these interactions may contribute to the enhanced vascular responses in hypertensive pregnancy. We studied Ang II contraction in isolated uterine artery (UA) at early gestation in a rat model that mimics many features of preeclampsia, the transgenic hAGN×hREN (TgA), and control Sprague-Dawley (SD) rats. We determined the role of the cannabinoid receptor CB_1 by blockade with SR171416A, and the contribution of AEA and 2-AG degradation to Ang II contraction by inhibiting their hydrolyzing enzymes FAAH (with URB597) or MAGL (with JZL184) respectively. TgA UA showed increased maximal contraction and sensitivity to Ang II that was inhibited by indomethacin. FAAH blockade decreased Ang II_{MAX} in SD UA, and decreased both Ang II_{MAX} and sensitivity in TgA UA. MAGL blockade had no effect on SD UA and decreased Ang II_{MAX} and sensitivity in TgA UA. Blockade of the CB_1 receptor in TgA UA had no effect. Immunolocalization of FAAH and MAGL showed a similar pattern between groups; FAAH predominantly localized in endothelium and MAGL in smooth muscle cells. We demonstrated an increased Ang II contraction in TgA UA before initiation of the hypertensive phenotype. AEA and 2-AG reduced Ang II contraction in a CB_1-independent manner. These RAS-ECS interactions may contribute to the enhanced vascular reactivity in early stages of hypertensive pregnancy.

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Keywords

hypertensive pregnancy; endocannabinoids; Ang II; FAAH; MAGL

Introduction

Preeclampsia is a common disorder of pregnancy that manifests with hypertension and proteinuria. The renin angiotensin system (RAS) plays an important role in the normal and pathological regulation of the female reproductive system [1] and an increased response to activation of the RAS is characteristic of pregnancies at risk of developing preeclampsia. Thus, since the pioneering work of Gant et al, an increased sensitivity to angiotensin II (Ang II), one of the main agonists of the RAS, was early recognized as a marker for the development of a hypertensive pregnancy[2-4].

The transgenic female rat containing the human angiotensinogen (*h*AGN) gene mated with the male transgenic containing human renin (*h*REN), the *h*AGTx*h*REN rat (TgA) mimics many features of human preeclampsia. This model shows increased blood pressure, proteinuria, and placenta alterations of edema and necrosis in the last half of gestation[5]. Mean blood pressure increases abruptly approximately 10 days before delivery reaching values of 160±10mmHg [5]. Among the vascular effects observed in this model, a prostanoid-mediated endothelial dysfunction of the uterine artery has been described at late gestation [6, 7].

The endocannabinoid system (ECS) is composed of mediators such as anandamide (AEA) and 2-arachidonoylglycerol (2-AG), receptors (CB_1, CB_2 and non-CB_1/CB_2) and enzymes in charge of synthesis or hydrolysis of these mediators: i.e. fatty acid amide hydrolase (FAAH) for hydrolysis of AEA and monoacylglycerol lipase (MAGL) for 2-AG [8]. Thus, AEA and 2-AG act as endogenous ligands for cannabinoid receptors. Endocannabinoids possess vasoactive, mitogenic, and differentiating properties, and are implicated in placentation [9] and in several pregnancy disorders including preeclampsia [10]. Endocannabinoids participate in the regulation of angiogenesis during implantation and decidualization [11], control myometrial contractility [12], and regulate uterine and umbilical blood flow [13]. All these events are determinants of a successful pregnancy and may have devastating consequences when altered [14].

Once generated, the actions of AEA and 2-AG are terminated by specific reuptake [15] and subsequent degradation by either FAAH [16] or MAGL [17], respectively. The ability to manipulate *in vivo* endocannabinoid levels by blocking their degradation makes these enzymes attractive therapeutic targets for several pathologies where AEA and 2-AG are involved [18-20], hence emphasizing the relevance of studying FAAH and MAGL blockers [21]. Notably, the localization and expression pattern of FAAH in the blastocyst suggests a role for this enzyme in limiting AEA levels as a protection for a successful implantation [22]. Data also suggest that these metabolizing enzymes regulate endocannabinoid levels in maternal tissues during pregnancy [23].

Recent evidence of interactions between the RAS and ECS suggests a regulatory role for endocannabinoids in modulating Ang II vascular responses. Thus, the contraction to Ang II in mice gracilis arteries is increased by blockade of the CB_1 receptor suggesting a vasodilatory role of endocannabinoids modulating contraction to Ang II in arteries from the systemic circulation [24].

In this study, we compared the Ang II-mediated contraction of isolated uterine artery from TgA and SD rats at early gestation and the effects of blocking AEA and 2-AG hydrolysis, by inhibiting FAAH or MAGL. We hypothesize that prostanoids have a greater impact in modulating Ang II contractions in preeclamptic rats and that blocking endocannabinoid degradation antagonizes Ang II contraction.

Material and Methods

Animals

Female *h*AGN transgenic rats, when mated with a *h*REN transgenic male (hAGN×hREN), develop hypertension and proteinuria in the second half of pregnancy [5]. Seven days pregnant preeclamptic *h*AGN×*h*REN (TgA, n=9) and 7 days pregnant Sprague-Dawley (SD, n=6) rats were used. All experiments were performed in accordance with the guidelines of the Wake Forest School of Medicine Institutional Animal Care and Use Committee. (See online-only Supplemental Data for details).

Vascular Reactivity Experiments

Segments of the main uterine artery (UA), maximum of 2 mm in length, were mounted between an isometric force transducer (Kistler Morce DSC 6, Seattle, WA, USA) and a displacement device on a myograph (Multi Myograph, Model 620M Danish Myo Technologies, Aarhus, Denmark) using two stainless steel wires (diameter 40 μm), as described previously[25]. In a subgroup of arteries from the control and TgA rats the endothelium was destroyed by passing a human hair through the lumen [26]. (See online-only Supplemental Data for details).

Response to Potassium Chloride (KCl)

After equilibration, in order to test the viability of the arterial preparations and determine the response to non-receptor mediated contraction, UA segments were exposed to 75 mmol/L KCl in KHB during 5 min, after washing the incubation was repeated twice. Contraction measured at the third incubation was recorded as maximal contraction to KCl (K_{MAX}).

Response to Ang II

After washing and resting for 30 min, UA segments were exposed to a cumulative concentration-response curve of Ang II by exposing arteries to eleven (10^{-11}–10^{-8}M) increasing concentrations in fourth-log steps, with each subsequent dose being introduced only after a steady response had been reached (2 min). Since at higher concentrations of ligand the Ang II contraction decreased as a result of desensitization, only doses as high as 10 nmol/L were used. In parallel experiments, different arterial segments were denuded or pre-incubated for 15 min with the cyclooxygenase inhibitor indomethacin (10^{-5} mol/L) or

the nitric oxide synthase inhibitor L-NAME (10^{-4} mol/L). Additional arterial segments were pre-incubated for 15 min with the FAAH blocker URB597 at $1x10^{-6}$ mol/L or the MAGL blocker JZL184 at $1x10^{-6}$ mol/L. Some TgA UA were pre-incubated with the CB_1 receptor blocker SR141716A at $1x10^{-6}$ mol/L or SR141716A at $1x10^{-6}$ mol/L plus URB597 at $1x10^{-6}$ mol/L.

Immunohistochemistry

Expression of FAAH and MAGL in UA was detected by immunostaining using commercial antibodies. For details see online-only Suplemental Data. Images were acquired at 400 × magnifications using a Leica DM 4000B upright microscope (Leica Microsystems, Bannockburn, IL, USA). Illumination settings were held constant for image capture sessions (Retiga 1300R CCD Digital camera, QImaging, Surrey, BC, Canada; SimplePCI v6 software Cranberry Twp., PA). Regions of interest (ROI) were defined using the open source Fiji software (ImageJ, NIH) (http://fiji.sc/Fiji) covering smooth muscle and endothelial layers in each arterial segment. Intensity of the staining in five ROI's per segment was quantified following the reciprocal intensity method [27].

Data Analysis

All data analysis was performed using GraphPad Prism v5 statistical analysis package (GraphPad Software Inc., La Jolla, CA). See online-only Suplemental Data for details. Data are expressed as mean ± SEM. One-way ANOVA with Bonferroni's multiple comparisons was used to determine significant differences. A $p< 0.05$ was accepted as an indication of statistical significance.

Results

Blood pressure in TgA transgenic rats at early gestation

Mean blood pressure values were not different between SD (n=6) and TgA (n=7) animals at 7 days of gestational age (98.8±2 *vs.* 97±3 mmHg, $p>0.05$).

Contractile response to Ang II in UA at early gestation in control rats

Optimal diameters of the isolated UA segments used in these studies were not different between control and TgA (339±13 *vs.* 317±4 μm, $p>0.05$). Maximal response to KCl was increased in TgA compared to control UA (4.6±0.5 *vs.* 3.3±0.3 mN/mm, $p<0.05$). In control arteries from SD rats Ang II (10^{-11}-10^{-8} mol/L) elicited a dose-dependent contraction that reached a plateau around 90% of K_{MAX}. Arterial denudation increased maximal Ang II contraction and sensitivity (Figure 1A, Table 1, $p<0.05$) whereas pre incubation of intact arteries with indomethacin increased Ang II sensitivity (Figure 1A, Table 1, $p<0.05$). Blockade of NO production with L-NAME in intact arteries increased maximal contraction and sensitivity to Ang II (Figure 1A, Table 1, $p<0.05$).

Contractile response to Ang II in TgA UA at early gestation

In uterine arteries from TgA rats the contraction to Ang II was increased compared to SD controls. Maximal response and sensitivity values were higher in TgA UA (Figure 1B, Table

1, $p<0.05$). In denuded arteries, contraction was similar to intact arteries whereas pre-incubation of intact arteries with indomethacin diminished maximal response and sensitivity as compared to TgA intact. Compared to intact arteries, sensitivity to Ang II in indomethacin-treated arteries was lower in TgA (Figure 1E, Table 1, $p<0.05$). Blockade of NO production with L-NAME in intact arteries did not alter Ang II contraction in TgA (Figure 1F, Table 1, $p<0.05$). Ang II contraction in SD and TgA UA was completely abolished by pre-incubation with losartan 10^{-6} mol/L (data not shown).

Effects of blocking endogenous production of AEA and 2-AG on Ang II contraction

Pre-incubation with the FAAH blocker URB597 10^{-6} mol/L reduced maximal response to Ang II in SD UA, whereas pre-incubation with the MAGL blocker JZL184 10^{-6} mol/L had no effect (Figure 2A, B and Table 2). In contrast, both FAAH and MAGL blockade inhibited maximal response and sensitivity to Ang II (Figure 3A, B and Table 2) in TgA UA. The inhibitory effect of blocking FAAH on Ang II in TgA UA was not modified by concomitant blockade of the CB_1 receptor with SR141716A 10^{-6} mol/L. We observed no effect of blocking CB_1 receptor alone on Ang II contraction (Figure 4, and Table 2).

Expression of MAGL and FAAH in control and TgA UA at early gestation

Immunolocalization of FAAH and MAGL revealed the presence of both enzymes in UA. FAAH was localized predominantly in the endothelium (Figure 4B and E), whereas MAGL was detected predominantly in smooth muscle cells (Figure 4C and F). A similar intensity of FAAH and MAGL signals was observed in both control and TgA arteries with stronger staining for MAGL than FAAH in both groups. (Supplemental Figure S1).

Discussion

For the first time we report that the transgenic *h*AGTx*h*REN rat, a rodent model that mimics many features of preeclampsia, displayed increased contraction to Ang II in the uterine artery at early gestation, before the onset of blood pressure rise. Herein, we also present additional novel findings of functional interactions between RAS and ECS in the uterine vasculature, with an increased role for the endocannabinoids AEA and 2-AG in reducing Ang II contraction in this preeclamptic model.

Increased vascular sensitivity to Ang II was reported in human pregnancies with an increased risk of developing hypertension[2-4]. We observed this effect at early gestation in the TgA rat before the hypertensive phenotype is established. Since increased sensitivity to Ang II constitutes a hallmark for the development of a hypertensive pregnancy our results contribute to the characterization of the *h*AGTx*h*REN rat as a suitable model of preeclampsia. The presence of agonistic autoantibodies to the AT1 receptor (AT1-AA) is another characteristic of human preeclampsia that this model replicates [28]. AT1-AA have been shown to modulate vascular responses in systemic and placental vessels [29, 30], and increase Ang II sensitivity in pregnant rats [31]. The effects of AT1-AA on blood pressure and Ang II sensitivity in isolated vessels of late pregnant rats required the presence of both AT1-AA and Ang II[32], and these vascular responses are blocked by the epitope peptide AFHYESQ[32]. Since AT1-AA appear to be secondary to a hypoxic/ischemic vascular

disorder [33], it is less probable that AT1-AA are playing a role in the increased Ang II sensitivity in early gestation TgA UA before the pathogenic syndrome is established.

The effects of endothelium denudation or inhibition of NO production on Ang II contraction were lower in TgA than controls suggesting an endothelium-derived dysfunction in TgA UA, consistent with a previous report in this model at late gestation [6]. Ang II contraction was dependent on prostaglandin generation. Indomethacin pre incubation shifted Ang II contraction to the left in arteries from control animals and to the right in arteries from TgA animals. This suggests a change in the type of prostanoids being generated in UA; from vasodilatory prostanoids in control UA to vasoconstrictor prostanoids in TgA arteries. This observation is consistent with the reported imbalance in the production of prostanoids described in preeclampsia: endothelial production of prostacyclin PGI_2 is decreased, whereas thromboxane TXA_2 levels are increased [34, 35], an effect proposed to be mediated by increased ROS generation in preeclamptic pregnancies. Interestingly, PGI_2 levels decreased months before the clinical onset of preeclampsia [36]. TgA rats appear to reproduce this imbalance before the installation of the hypertensive phenotype. Recent reports also support the contribution of CYP epoxygenase to the effects on blood pressure, albuminuria, and vascular function observed in the TgA rat at late gestation [7].

The actions of endocannabinoids in the vasculature are complex and not explained by a single mechanism or target tissue [37]. An endothelium-dependent vasodilatory action involving a CB_1 receptor-dependent pathway, as well as NO generation and K^+ channels activation has been demonstrated for endocannabinoids [38]. Although the development of highly specific blockers of FAAH and MAGL has allowed the study of the mechanisms mediated by AEA and 2-AG [39, 40], one limitation of our study is that our approach to examine endocannabinoid -mediated responses relies exclusively on pharmacological blockade. Using this approach however, an efficient catabolism of AEA and 2-AG by their corresponding hydrolyzing enzymes was demonstrated in vascular tissues [38, 41]. Thus, in the mesenteric artery AEA and 2-AG are able to relax pre-constricted arteries, an effect that is potentiated by blockade of the hydrolases FAAH and MAGL [38]. We used the enzyme blockers, URB597 and JZL184, at concentrations previously shown effective in vascular studies; i.e. URB597 10^{-6} M abolished AEA vascular actions in rat gracilis arteries [42]. Our observations of a reduced Ang II contraction in TgA UA upon blockade of FAAH or MAGL suggest the induction of a vasodilatory mechanism or the attenuation of vasoconstrictor mediators. Studies on coronary arteries showed that the vasodilatory effects of endocannabinoids, particularly AEA, are mediated by their catabolism to arachidonic acid and subsequent conversion to vasodilatory eicosanoids such as prostacyclin or epoxyeicosatrienoic acids [43]. Interestingly, these effects in coronary arteries are not mediated by the CB_1 receptor [43], in agreement with the absence of effects of CB_1 receptor antagonism on the reduction of Ang II contraction induced by FAAH blockade in TgA UA. Vasodilatory effects of AEA in rat aorta [44], as well as AEA-mediated nitric oxide production in endothelial cells [45], have both been described as being independent of CB_1 or CB_2 receptors, making it possible to explain the involvement of non-CB_1/CB_2-mediated vasodilatation in the reduction of Ang II contraction that we observed after blockade of endocannabinoid degradation.

It has also been described that vasodilatory prostanoids released by endocannabinoid hydrolysis would cause vasorelaxation in part via opening K^+ channels [46]. Given the important effect of blockade of prostaglandin generation we observed in TgA, it is conceivable that similar mechanisms may be operating in UA. Thus, increased endocannabinoid levels, via inhibition of their degradation, would increase vasodilatory prostanoids in UA that in turn would limit Ang II contraction.

Our immunohistochemistry data indicate differences in tissue localization for FAAH and MAGL in UA. FAAH was mainly localized to the cells lining the arterial lumen consistent with endothelial location and sparsely located in smooth muscle cells in SD and TgA arteries. MAGL was observed along the whole arterial wall spanning both endothelial and smooth muscle cells. Previous evidence indicated the presence of FAAH in bovine coronary arteries, kidney endothelial cells and human umbilical vein endothelial cells[43, 47-49]. Recently FAAH expression has also been reported in arterial smooth muscle cells[42]. The localization we observed for FAAH agrees with the endothelium-dependent effects described for the FAAH blocker URB597 [42]. As a key mediator of 2-AG degradation [50], MAGL is ubiquitously expressed [51]. In the vasculature, 2-AG relaxation of mesenteric artery has been shown to be endothelium-independent [52], in agreement with the localization in smooth muscle cells we observed in UA. In both SD and TgA UA it appears that the staining for MAGL was higher than what we observed for FAAH, and this may be related to the reported greater levels of 2-AG compared to AEA observed in the rodent uterus[53]. Thus, a higher expression of MAGL would contribute to modulate 2-AG levels. Similar intensity of immunohistochemistry signals between arterial segments from control and TgA animals suggests that the observed differences in vascular effects are not related to different levels of expression of FAAH or MAGL in arteries from preeclamptic animals.

In terms of the possible prostanoid compounds involved in the vascular responses to endocannabinoid hydrolysis, AEA and 2-AG are also substrates of the enzyme cyclooxygenase-2 (COX-2)[54]. Thus, if endocannabinoids are not able to be metabolized by FAAH or MAGL due to their blockade, more substrate would be available for COX-2. AEA and 2-AG are oxidized by COX-2 to prostaglandins-ethanolamides (prostamides) (PG-EA) and prostaglandins-glyceryl esters (PG-G) respectively [54]. These compounds do not interact with cannabinoid or prostaglandin receptors suggesting additional pathways involved in their cellular function[55]. Interestingly, the effects of URB597 on myogenic tone of mouse gracilis arteries are inhibited by indomethacin[42], suggesting the involvement of PG-EA and/or PG-G on endocannabinoid-derived vascular responses. In the mesenteric artery the vasodilatory responses to endocannabinoids are endothelium-dependent [38], however we did not test the influence of endothelium on the responses to FAAH and MAGL blockers. Thus the role of the prostanoids families of compounds in the regulation of the vascular actions of Ang II by endocannabinoids warrants further investigation.

Perspectives

We observed a functional interaction between RAS and ECS in the uterine circulation. As in the clinical manifestation of preeclampsia, an increased role of prostanoids in the vasculature may help explain the enhanced effects of endocannabinoids we observed on Ang

II contraction. The vascular response to Ang II is a clinical target for antihypertensive therapies, and since FAAH inhibitors have no hemodynamic effects under normotensive conditions[56], our results also point to the use of endocannabinoid degradation blockers as effective pharmacotherapies for hypertension.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Novelty and Significance

What Is New?

- Increased contraction to Ang II in the UA at early gestation in a rat model of preeclampsia
- The endogenous cannabinoids anandamide and 2-arachidonoylglycerol reduced contraction of the UA to Ang II

What Is Relevant?

- By increasing levels of endogenous cannabinoids, the inhibition of the enzymes FAAH and MAGL would counteract enhanced contractile responses to Ang II
- These effects are enhanced in a model of preeclampsia at early gestation, before the hypertensive phenotype initiates.

Summary

- A functional interaction between the RAS and the ECS was observed in the uterine circulation.

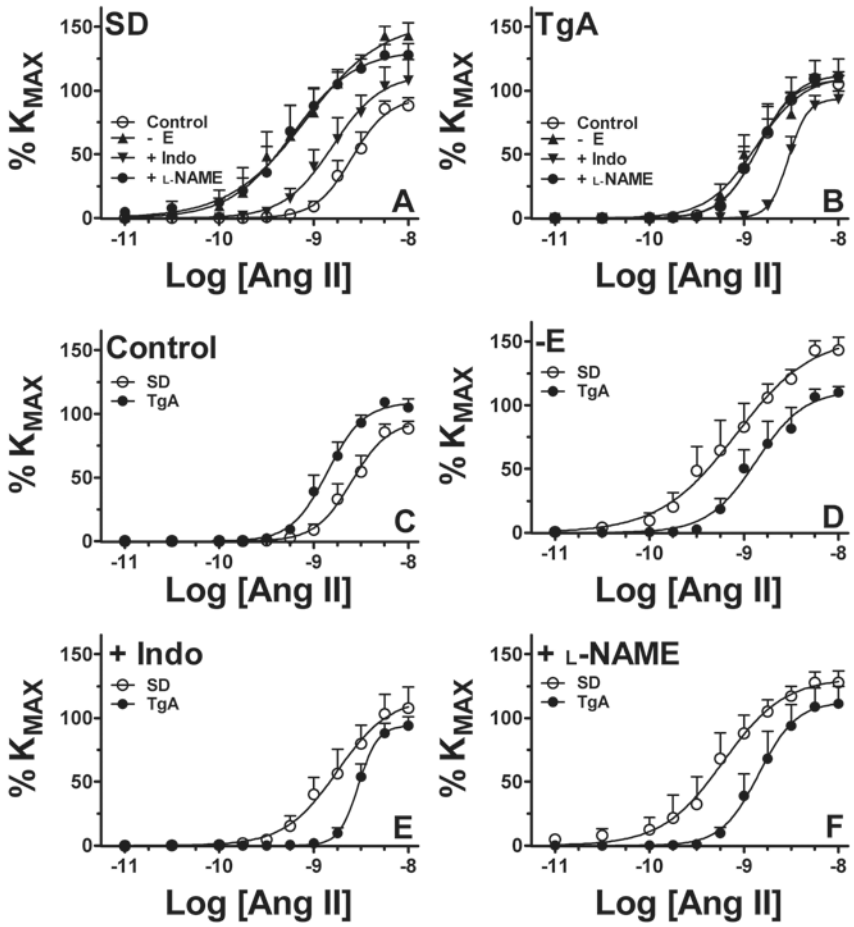

Figure 1. Contraction to Ang II in UA from SD and TgA rats at early gestation

Contraction to Ang II in isolated UA from Sprague-Dawley (SD, A, n=6) and transgenic (TgA, B, n=9) animals. Parallel experiments in intact arteries (C), denuded arteries (-E, D), intact arteries pre-incubated with indomethacin 10^{-5}M (+Indo, E) or L-NAME 10^{-4}M (+L-NAME, F) are shown. See Table 1 for analysis.

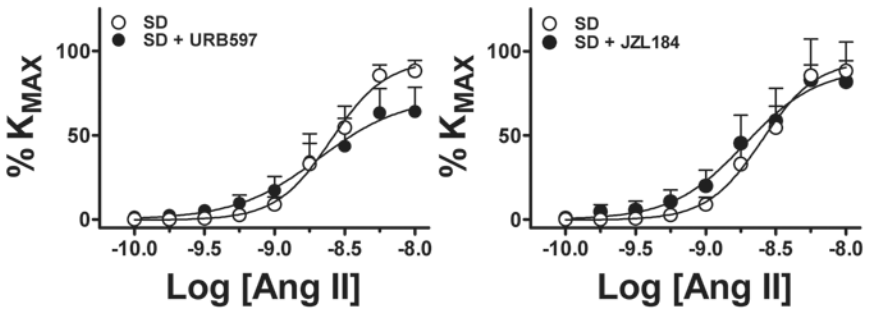

Figure 2. Effects of blocking AEA and 2-AG hydrolysis on UA Ang II contraction in SD arteries at early gestation

Contraction to Ang II in isolated UA from Sprague-Dawley rats (○, n=6) in the presence of FAAH (URB597 10^{-6}M, •, n=9, A) or MAGL (JZL184 10^{-6}M, •, n=9, B) blockade. See Table 2 for analysis.

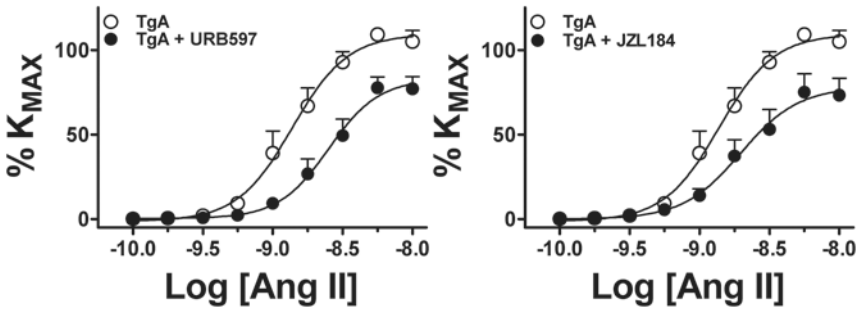

Figure 3. Effects of blocking AEA and 2-AG hydrolysis on UA Ang II contraction in transgenic TgA arteries at early gestation

Contraction to Ang II in isolated UA from TgA rats (○, n= 9) in the presence of FAAH (URB597 10^{-6}M, •, n=9, A) or MAGL (JZL184 10^{-6}M, •, n=9, B) blockade. See Table 2 for analysis.

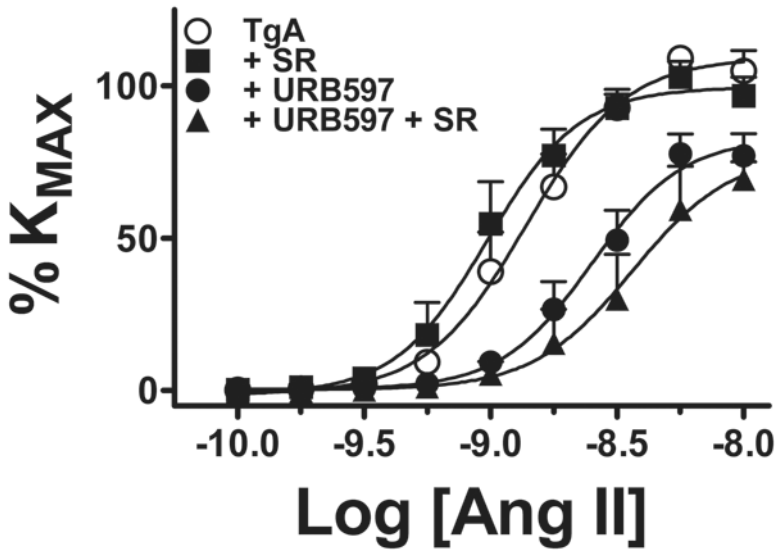

Figure 4. Effects of blocking the CB_1 receptor and AEA hydrolysis on UA Ang II contraction in transgenic TgA arteries

Contraction to Ang II in isolated UA from TgA rats (○, n=9) in the presence of FAAH blockade (URB597 10^{-6}M, •, n=9), CB_1 blockade (SR141716A 10^{-6}M (▪, n= 5) or FAAH plus CB_1 blockade (▲, n=3). See Table 2 for analysis.

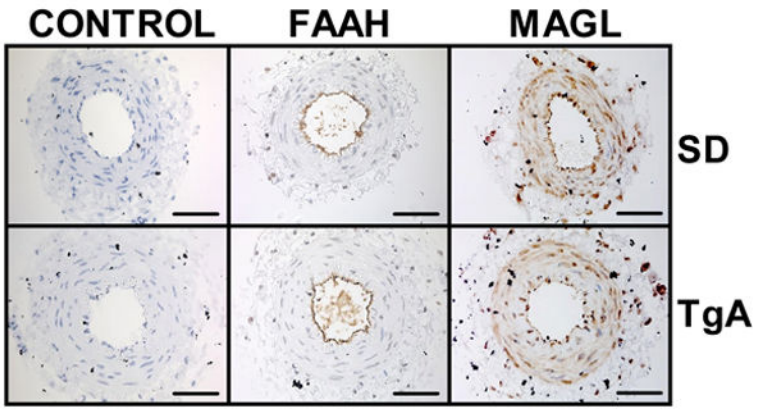

Figure 5. Immunolocalization of FAAH and MAGL in UA from control and TgA rats at early gestation

UA segments from control (SD, A-C, n=5) or TgA rats (D-F, n=5) were incubated with anti-FAAH (B and E) or anti-MAGL (C and F) antibodies. Representative pictures from each group are shown. Signals were developed by incubation with 3, 3′-diaminobenzidine (brown) and counterstained with hematoxylin (nuclei, blue). Control incubations for SD (A) and TgA UA (D) did not contain primary antibodies. Scale bar = 200 μm.

Table 1

Contractile responses to angiotensin II (Ang II) in uterine arteries from SD and TgA rats at early gestation

Maximal response to Ang II (Ang II_{MAX}) is expressed as % K_{MAX} and sensitivity as pD_2. Results are shown for intact, denuded arteries and arteries pre-incubated with indomethacin (10^{-5} mol/L) or L-NAME (10^{-4} mol/L) as indicated.

Variables measured	Intact		Denuded	
	SD	TgA	SD	TgA
Ang II_{MAX}, % K_{MAX}	91±5	109±4*	150±17†	108±4*
pD_2	8.60±0.08	8.86±0.08*	9.14±0.2†	8.87±0.13
	+ Indomethacin		+ L-NAME	
	SD	TgA	SD	TgA
Ang II_{MAX}, % K_{MAX}	111±15	92±7†	128±8†	110±13
pD_2	8.79±0.08†	8.54±0.03†*	9.28±0.17†	8.82±0.01

*p<0.05 vs SD;

†p<0.05 vs intact arteries.

Table 2

Effects of FAAH and MAGL blockade on contractile responses to angiotensin II (Ang II) in uterine arteries from SD and TgA rats at early gestation

Maximal response to Ang II (Ang II_{MAX}) is expressed as % K_{MAX} and sensitivity as pD_2. Results are shown for non-treated arteries (control), FAAH blockade (URB597, 10^{-6} mol/L), MAGL blockade (JZL184, 10^{-6} mol/L) in SD UA. In TgA UA results are shown for FAAH blockade (URB597, 10^{-6} mol/L), MAGL blockade (JZL184, 10^{-6} mol/L), CB1 receptor blockade (+SR, SR141716A 10^{-6} mol/L), and concomitant FAAH and CB1 blockade (+URB595+SR).

Variables measured		**SD**	
	Control	**+URB595**	**+JZL184**
Ang II_{MAX}, % K_{MAX}	91±5	61±12*	85±20
pD_2	8.60±0.08	8.68±0.14	8.68±0.15
		TgA	
	Control	**+URB595**	**+JZL184**
Ang II_{MAX}, % K_{MAX}	109±4	86±8*	78±10*
pD_2	8.86±0.08	8.58±0.06*	8.65±0.07*
		+SR	**+URB595+SR**
Ang II_{MAX}, % K_{MAX}		98±4	76±7*
pD_2		9.01±0.09	8.45±0.13*

*$p<0.05$ *vs* control arteries.