Caveolin-1 prevents sustained angiotensin II-induced resistance artery constriction and obesity-induced high blood pressure

Istvan Czikora,* Attila Feher,* Rudolf Lucas, David J. R. Fulton, and Zsolt Bagi
Vascular Biology Center, Medical College of Georgia, Georgia Regents University, Augusta, Georgia
Submitted 9 September 2014; accepted in final form 12 December 2014

Czikora I, Feher A, Lucas R, Fulton DJ, Bagi Z. Caveolin-1 prevents sustained angiotensin II-induced resistance artery constriction and obesity-induced high blood pressure. Am J Physiol Heart Circ Physiol 308: H376–H385, 2015. First published December 15, 2014; doi:10.1152/ajpheart.00649.2014.—The type I angiotensin II (ANG II) receptor (AT1R) undergoes internalization following stimulation by ANG II. Internalization reduces cell surface AT1R, and it is required for AT1R resensitization. In this process AT1R may interact with caveolin-1 (Cav1), the main scaffolding protein of caveolae. We hypothesized that the interaction between Cav1 and AT1R delays AT1R resensitization and thereby prevents sustained ANG II-induced resistance artery (RA) constriction under normal conditions and in experimental obesity. In rat and mouse skeletal muscle RA (diameter: ~90–120 μm) ANG II-induced constrictions were reduced upon repeated (30-min apart) administrations. Upon disruption of caveolae with methyl-β-cyclodextrin or in RA of Cav1 knockout mice, repeated ANG II applications resulted in essentially maintained constrictions. In vascular smooth muscle cells, AT1R interacted with Cav1, and the degree of cell surface interactions was reduced by long-term (15-min), but not short-term (2-min), exposure to ANG II. When Cav1 was silenced, the amount of membrane-associated AT1R was significantly reduced by a short-term ANG II exposure. Moreover, Cav1 knockout mice fed a high-fat diet exhibited augmented and sustained RA constriction to ANG II and had elevated systemic blood pressure, when compared with normal or high-fat fed wild-type mice. Thus, Cav1, through a direct interaction, delays internalization and subsequent resensitization of AT1R. We suggest that this mechanism prevents sustained ANG II-induced RA constriction and elevated systemic blood pressure in diet-induced obesity.

Obesity is accompanied by pathologic activation of both systemic and vascular renin-angiotensin-aldosterone system (RAAS), which eventually leads to the development of elevated systemic blood pressure (17). A sustained activation of type I angiotensin II (ANG II) receptor (AT1R) by ANG II, one of the main effectors of RAAS, has been proposed to contribute to increased peripheral vascular resistance in obesity-associated hypertension (33). It is known that unlike type 2 ANG II receptor, AT1R exhibits downregulation by its own ligands (19). In the short term, AT1R undergoes rapid desensitization and consequent internalization upon stimulation by ANG II, thereby decreasing the number of AT1R on the cell surface and the role of this pathway in further signaling (18, 19). This negative feedback regulation of AT1R plays an important role in preventing sustained receptor stimulation by ANG II. As a functional consequence in the vasculature, sequential administration of ANG II elicits diminished vasocstriction, a well-known phenomenon, called ANG II tachyphylaxis (22, 25, 35, 39). The problem is that under pathological conditions this normal, negative feedback regulation of AT1R signaling can be compromised (4, 6). The underlying mechanism(s) through which AT1R-mediated microvascular signaling becomes augmented and sustained remains poorly understood.

Upon stimulation AT1R is internalized primarily by clathrin-coated vesicles, in a dynamin and β-arrestin dependent manner, although other mechanisms, such as internalization by noncoated vesicles, have been demonstrated (19). It has been long recognized that internalization directs AT1R into the endosome, where it is dephosphorylated by protein phosphatases, resensitized, and recycled to the plasma membrane (2, 24). Interestingly, earlier studies showed that the recycling of AT1R to the cell surface is quite rapid (37). Given that, it is possible that a rapid internalization of AT1R enables a quick recycling, whereas mechanisms that prevent AT1R internalization delay the reactivation of AT1R. In support of this scenario, it has been shown that caveolin-1 (Cav1), the main scaffolding protein of caveolae, interacts with AT1R in vascular smooth muscle cells and that upon ANG II stimulation AT1R moves to caveolae-enriched membrane microdomains (20). There is some evidence that a direct interaction of between AT1R and Cav1 also contributes to the exocytic trafficking of AT1R (43). The role of AT1R-Cav1 interaction in affecting ANG II-induced constriction of resistance arteries and systemic blood pressure is not well understood.

Previously, we demonstrated that Cav1 plays an important role in the maintenance of resistance artery dilator function in a rodent model of high-fat diet (HFD)-induced obesity (14). We also found that in patients with diabetes the normal function of Cav1 is compromised, which leads to an impaired, flow-mediated dilation in coronary resistance arteries (8). In this study we raised the novel hypothesis that the interaction between AT1R and Cav1 is mainly to delay AT1R reactivation and hence prevents sustained ANG II-induced constriction in resistance arteries. This normal, physiological regulatory mechanism can be compromised in obesity and may contribute to the development of obesity-associated hypertension. To test these hypotheses we assessed AT1R-mediated constriction in intact rodent skeletal muscle resistance arteries, in which ANG II was administered in a repeated fashion, before and after

* I. Czikora and A. Feher contributed equally to this article.
Address for reprint requests and other correspondence: Z. Bagi, Vascular Biology Center, Medical College of Georgia, Georgia Regents Univ., Augusta, GA (e-mail: zbagi@gru.edu).
interfering with Cav1 and also after HFD-induced metabolic challenge.

METHODS

Pressure myography of isolated skeletal muscle resistance artery. All protocols were approved by the Institutional Animal Care and Use Committee at Georgia Regents University. Male Wistar rats (weighing about 300 g, n = 12, purchased from Charles River) and male Cav1 knockout mice (weighing about 25 g, n = 20; purchased from Jackson Laboratories) were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg). Under anesthesia, the gracilis muscle was excised and placed in ice-cold, oxygenated Krebs solution. Euthanasia was then performed by additional intraperitoneal injection of pentobarbital sodium (150 mg/kg). With the use of microsurgical instruments and an operating microscope, a resistance artery (90 and 150 μm in internal diameter, in mice and rats, respectively) running intramuscularly was isolated and cannulated in the pressure myograph chamber. The cannulated artery was connected with silicone tubing to a pressure servo control system (Living Systems Instrumentation) to adjust the intraluminal pressure to 80 mmHg. Vessels were observed with videomicroscopy, and the diameter was measured with a microangiometer (7, 13).

Protocols for assessment of AT1R-mediated, ANG II-induced resistance artery constriction. In the first series of experiments cumulative concentrations of angiotensin II (ANG II, 10 pM-10 nM; Sigma) were administered to the rat gracilis muscle artery and change in diameter was measured. After washout and 30 min of time period ANG II administration was repeated. Repeated norepinephrine (NE)-induced arteriolar responses were assessed. In separate experiments gracilis muscle arteries were isolated from wild-type and Cav1 knockout mice and ANG II- and NE-induced arteriolar responses were measured in similar protocols.

In situ proximity ligation assay. Rat vascular smooth muscle cells (VSMCs; SV40LT-transfected; American Cell Type Culture Collection) were grown on Ibidi Slide IV0.4 to reach 20–30% confluence, and cytosolic AT1R was determined in cultured VSMCs before and after transfection with ANG II- or NE-induced vasomotor constriction. Subsequently, vasoconstriction, also known as ANG II-induced constriction upon the repeated administration of ANG II resulted in a subsequent diminishment of ANG II- and NE-induced vasomotor responses. In separate experiments gracilis muscle arteries were isolated from wild-type and Cav1 knockout mice and ANG II- and NE-induced arteriolar responses were measured in a repeated-measures ANOVA (agonist induced responses) or one-way (comparing 2 or more experimental groups) followed by Tukey’s post hoc test. Statistical analysis was performed by repeated-measures ANOVA (agonist induced responses) or one-way (comparing 2 or more experimental groups) followed by Tukey’s post hoc test. P < 0.05 was considered statistically significant. Data are expressed as means ± SE.

RESULTS

Cav1 prevents sustained ANG II-induced constriction in the rodent resistance artery. It has been long recognized that sequential activation of AT1R by ANG II results in a subsequently diminished vasoconstriction, also known as ANG II tachyphylaxis (22, 25, 35, 39). In the present study we first confirmed this phenomenon by showing that in the skeletal muscle resistance arteries (~150 μm in internal diameter) isolated from the normal Wistar rat repeated administration of ANG II (30 min apart) resulted in reduced constrictions (Fig. 1A), whereas constrictions to α1-adrenergic agonist norepinephrine (NE) were essentially maintained upon the repeated administrations (Fig. 1C).

To examine the role of membrane caveolae and Cav1 in AT1R-mediated vasomotor responses, ANG II-induced constrictions were assessed in the presence of methyl-β-cyclodextrin (mβCD) and also in resistance arteries (~90 μm) of Cav1 knockout mice (CavKO), similar to our earlier studies (8, 14). In the presence of mβCD, ANG II elicited constriction upon the first application with a similar magnitude as seen without mβCD treatment (Fig. 1B). We found that the magnitude of

References

1. doi:10.1152/ajpheart.00649.2014 • www.ajpheart.org

Downloaded from http://ajpheart.physiology.org/ by 10.220.33.6 on April 18, 2017
constrictor response, however, remained substantial to the repeated ANG II administration in the presence of mβCD (Fig. 1B). A similar observation was made when resistance artery responses were assessed in CavKO mice, in which ANG II-induced constrictions were essentially maintained upon repeated ANG II administrations (Fig. 2B), compared with responses of wild-type controls mice exhibiting the normal ANG II tachyphylaxis (Fig. 2A). No significant effects of mβCD treatment or the lack of Cav1 were found on arterial constrictions to repeated NE administrations, which remained the same under these conditions (Fig. 1D and Fig. 2D). These findings indicated a significant regulatory role for Cav1 in preventing a sustained AT1R activation and ANG II-induced constriction in the rodent resistance artery.

Direct interaction between Cav1 and AT1R in VSMCs. According to our hypothesis upon ANG II stimulation a direct interaction between Cav1 and AT1R prevents the internalization of AT1R and therefore reduces the number of active receptors availability for the second, repeated agonist stimulation. To test this hypothesis and examine the role of the AT1R-Cav1 interaction in regulating the initial phase of AT1R trafficking, we used cultured VSMCs. To follow and dynamically assess the interaction between AT1R and Cav1, a highly sensitive PLA was used (8). In brief, in this assay PLA probes create a positive and amplified signal (shown in red dots in Fig. 3, A and C) only when the epitopes of the targeted proteins are in close proximity (<40 nm). Using this technique we quantified the level of AT1R-Cav1 interaction under basal condition and also after stimulating VSMCs with ANG II for a short (2 min) and a longer (15 min) periods of time. We found that AT1R interacted with Cav1 even in unstimulated VSMCs and observed that the level of interaction was unaltered by short-term (2-min) exposure to ANG II, which was significantly reduced by a longer-term (15-min) ANG II administration (Fig. 3, A and B). In situ PLA also allowed us to assess subcellular, i.e., cell surface versus cytosol, distribution of AT1R-Cav1 interactions. We found that the percentage of cell surface PLA signals significantly reduced after 15-min ANG II exposure (Fig. 3, C and D). We interpreted these findings to mean that upon stimulation with ANG II AT1R interacts with Cav1 within the membrane compartments, which is then followed by a delayed, subsequent internalization of AT1R.

Cav1 directs AT1R to membrane compartment in ANG II-stimulated VSMCs. To detect changes in ANG II-induced subcellular distribution of AT1R, cell fractionation approach was also used in cultured VSMCs. We have found a trend toward an increased membrane associated AT1R in ANG II-exposed VSMCs (Fig. 4D). More importantly, the membrane associated AT1R was markedly reduced after siRNA knockdown of Cav1 (Fig. 4D). Correspondingly, we observed an increase in the cytosolic AT1R content after Cav1 deletion, both in control and ANG II-exposed VSMCs, a trend, which
did not reach statistical significance (1-way ANOVA, Fig. 4E). Neither Cav1 knockdown nor the ANG II-exposure changed significantly the AT1R content in the whole cell lysates (Fig. 4C). Similar observation was made after 2 min and after 15 min exposure to ANG II. We interpreted these findings that Cav1 play a role in the maintained membrane localization of AT1R after ANG II stimulation.

HFD is accompanied by elevated blood pressure and sustained resistance artery constriction to ANG II only in the Cav1 knockout mice. Based on our results we assigned an important regulatory role for Cav1 in preventing sustained AT1R activation in skeletal muscle resistance artery under normal condition. It is possible, however, when this normal regulatory function of Cav1 is compromised in disease, such as we found earlier in obesity and diabetes (8, 14), it may affect ANG II responsiveness of resistance arteries and also alter systemic blood pressure. To test this hypothesis in this study wild-type and Cav1 knockout mice were fed a HFD for 3 mo and vascular reactivity of ex vivo skeletal muscle resistance arteries and systemic blood pressure were measured and compared, in similar protocols as we did in our previous studies (5, 14, 21). When fed a HFD, both wild-type and Cav1 knockout mice gained weight compared with those mice on normal chow diet. We found that the weight gain was significantly greater in wild-type than in Cav1 knockout mice (Fig. 5A). There was significantly increased weight of epididymal and retroperitoneal fat pads in HFD fed wild-type mice than in Cav1 knockout mice (Fig. 5B). These findings correspond to the data in the literature (34) and suggest that Cav1 knockout mice are somewhat resistant to weight gain when challenged with a HFD. It is known that Cav1 knockout mice on HFD have normal fasting glucose levels (10), but the mice are characterized by a marked increase in serum triglyceride, free fatty acid, and cholesterol levels, which is due to the altered binding, transport, and/or storage of fatty acids and cholesterol (34). In this study we found that Cav1 knockout mice had significantly higher serum total cholesterol levels, either with or without HFD (Cav1KO: 166 ± 10 vs. WT: 78 ± 12 vs. WT + HFD: 99 ± 8 mg/dL).

It is of particular interest that only HFD-fed Cav1 knockout mice exhibited a significantly increased systolic and diastolic blood pressure than wild-type animals either on normal chow diet or HFD (Fig. 5C). In similar protocols as described above resistance artery responses to ANG II and NE were obtained in isolated, pressurized skeletal muscle resistance arteries of HFD-fed wild-type and Cav1 knockout mice. We found a clear trend toward an increase in the vascular protein expression of Cav1 between normal, wild-type, and HFD-fed mice (Fig. 5D).
chow or HFD, arteries of Cav1 knockout mice exhibited an augmented constriction to ANG II (Fig. 6A), which remained at the same, augmented level even after repeated ANG II administration (Fig. 6B). Arteries of wild-type mice fed a HFD exhibited only a trend toward augmented constrictions to repeated ANG II administrations, when compared with those mice on normal chow diet (Fig. 6B). No significant changes were found in resistance artery constrictions to repeated NE administrations between the experimental groups (Fig. 6, C and D).

**DISCUSSION**

The present study indicates that a direct interaction between AT1R and Cav1 delays AT1R reactivation after ANG II stimulation and thereby prevents sustained ANG II-induced constriction in skeletal muscle resistance arteries. We also show that this interaction may protect against the development of obesity-associated increased resistance artery constriction and elevated systemic blood pressure. These conclusions are supported by our results showing that Cav1 interacts with AT1R in VSMCs and the interaction is reduced only after a prolonged exposure to ANG II. After genetic deletion of Cav1 ANG II-induced AT1R translocation from membrane to cytosol fraction is augmented. In addition, in Cav1 knockout mice, but not in wild-type, repeated ANG II applications result in maintained resistance artery constriction and Cav1 knockout mice are predisposed to develop elevated systolic and diastolic blood pressure when fed a HFD.

It has been proposed earlier that upon stimulation by ANG II, AT1R interacts with Cav1 in the caveolae membrane microdomains (20, 38, 43). In the present study we raised the possibility that this interaction serves mainly to prevent internalization of the AT1R, hence delaying its resensitization and recycling to the cell surface. In this context, an earlier study by Linder et al. has shown that disruption of caveolae by mβCD reduced the tachyphylactic response to ANG II in the thoracic aorta of normal rats (25). Confirming this observation and extending it to include the rat skeletal muscle resistance artery, herein we show that mβCD augments ANG II-induced constrictions to repeated administration of ANG II, but not to the first application. In addition, we show that skeletal muscle resistance arteries from mice with genetic deletion of Cav1 exhibit an essentially maintained constriction to repeated administration of ANG II. Thus it seems that Cav1 plays a fundamental role in preventing sustained ANG II-induced constrictions and contributes to normal ANG II-induced tachyphylaxis both in conduit vessels and, as we demonstrate here, in resistance arteries.

AT1R trafficking is composed of ANG II-induced AT1R internalization, receptor resensitization, and recycling to the plasma membrane (19). Ishizaka et al. (20) has studied of the initial phase of AT1R trafficking in VSMCs and showed that AT1R translocates to caveolin-rich membrane fractions after 1 to 10 min stimulation with ANG II and also that Cav1 co-immunoprecipitates with AT1R. In our present study, using in situ proximity ligation assay, we detected a direct interaction...
between AT$_1$R and Cav1 in VSMCs, which remained unchanged after short, 2-min ANG II exposure, but was significantly reduced after a prolonged, 15-min treatment with ANG II. We found that the level of cell surface localized AT$_1$R-Cav1 interactions decreased significantly after 15 min of ANG II exposure. Collectively, these data suggested that after the initial interaction between AT$_1$R and Cav1 ANG II stimulation promotes AT$_1$R internalization and its dissociation from Cav1. We also found that the amount of membrane-associated AT$_1$R was significantly reduced after genetic silencing of Cav1 in ANG II-stimulated VSMCs. Taken together, based on these observations we propose that upon stimulation by ANG II, AT$_1$R moves and physically interacts with Cav1 in the plasma membrane of VSMCs and this interaction delays the internalization and subsequent reactivation of AT$_1$R. As an important functional consequence, our data indicate that the interaction between AT$_1$R and Cav1 is likely to contribute to normal ANG II-induced tachyphylaxis (22, 25, 35, 39) and prevents sustained ANG II-mediated signaling in the rodent skeletal muscle resistance artery.

Our present findings corresponds well with the literature data describing the adverse cardiovascular phenotype of Cav1 knockout mice, which comprises cardiac hypertrophy (9), impaired systolic and diastolic myocardial contractile function, and the development of pulmonary arterial hypertension (12, 42). Many of these pathological changes are believed to be mediated by overactivated RAAS and consequently enhanced ANG II signaling. Interestingly, studies assessing alteration in the systemic blood pressure, which is also affected by the activated RAAS, are controversial in the Cav1 knockout mice. For example, detailed assessment of systolic and diastolic blood pressure and variability found no differences between Cav1 knockout and wild-type mice (12). Similarly, Pojoga et al. (31, 32) showed no significant changes in systolic blood pressure of Cav1 knockout at baseline, whereas the authors reported recently a significantly elevated systolic blood pressure in these animals. In contrast, mean arterial pressure of Cav1 knockout mice was found to be significantly reduced, an alteration, which was normalized after genetically reconstituting the vascular endothelial Cav-1 (27). The possible mechanisms of these controversial observations are still debated.

In this study we also examined the role of Cav1-AT$_1$R interaction in modulating ANG II-induced resistance artery constriction and its potential systemic consequence(s) on blood pressure in an experimental model of obesity. Previously, we...
found that normal function of Cav1 is required for maintenance of resistance artery dilator function in obesity (14), but when the regulatory function of the Cav1 is compromised this led to an impaired vasodilatation of coronary arteries (8). Also, it is known that in obese and diabetic patients AT1R blockers not only reduce systemic blood pressure but also prevent the development of the functional and morphological alterations of resistance arteries (11, 29). Given that, it is possible that obesity is accompanied by an augmented ANG II-mediated vascular signaling. Studies in animal models of obesity are in line with this and found an increased contraction to ANG II in aortae from obese Zucker rats (28), db/db mice (16), and in rats fed a HFD (40). In these studies a key role for enhanced Ca2+ sensitivity of contractile apparatus (28), increased cyclooxygenase-2-derived constrictor prostaglandins (16), or enhanced production of reactive oxygen species has been proposed to account for augmented AT1R-mediated vasoconstriction (40). There is some evidence that augmented AT1R signaling occurs without any change in vascular AT1R expression (28). In support of this, our earlier study demonstrated that skeletal muscle resistance arteries exposed to a short-term (1-h) high glucose concentration resulted in an augmented and sustained constriction to ANG II (6). Previously, we also found that Wistar rats fed a HFD exhibit an impaired dilation of skeletal muscle resistance arteries, which was associated with elevated mean arterial blood pressure of obese rats (13). Given that observation, in this study we raised the hypothesis that in obese mice ANG II-induced resistance artery constriction is augmented and becomes sustained, which contributes to the development of elevated systemic blood pressure. Contrary to our assumption, results from this study show that normal wild-type mice fed a HFD exhibited no significant increase in systolic and diastolic blood pressure. We found only a marginally augmented ANG II-induced constriction in skeletal muscle resistance arteries in the HFD-induced obese mice. Similar to this observation HFD-induced obesity in mice was found to be associated with only slight (5–8 mmHg) increases in mean arterial blood pressure (41). The reason why mice are resistant to develop high blood pressure in response to HFD is not clear and may be due to experimental conditions, duration of diet, and/or age of animals as well as strain differences. According to our hypothesis we raised another possibility, namely that in obesity there are mechanisms that could compensate for the impaired vasomotor regulation. Indeed, adaptation of mechanisms intrinsic to vascular wall has been documented to contribute to the maintained dilator function of coronary resistance arteries in obesity (3). We hypothesized that Cav1, through its interaction with AT1R may serve such adaptive mechanism and thereby prevent a sustained ANG II-induced vascular signaling and the development of obesity-associated hypertension. In support of this scenario we found a clear trend toward increased Cav1 protein expression in the skeletal muscle arteries of HFD-fed wild-type mice, which suggested potential adaptive role of Cav1 in this model. To examine the adaptive role of Cav1, Cav1 knockout mice were fed a HFD and vascular reactivity of ex vivo skeletal muscle resistance arteries and systemic blood pressure were measured. Interestingly, we found that Cav1 knockout mice fed a HFD, but not those on normal chow diet, exhibited a significantly increased systolic and diastolic blood pressure, when compared with other experimental groups. Of note, the HFD-fed Cav1 knockout mice showed a markedly augmented constriction to ANG II, which remained at the same augmented level even after repeated ANG II administrations. Collectively, we interpret these findings to indicate that normal mice are protected against the ability of a HFD to increase AT1R-mediated constriction in the resistance arteries and to elevate systemic blood pressure. We suggest that Cav1 through a direct interaction with AT1R prevents sustained ANG II signaling in this model of experimental obesity. In line with our present observation, we found that Cav1 plays a pivotal role in the maintenance of endothelium-dependent hyperpolarizing factor-mediated coronary artery dilation in obesity (14). It should be noted that there are also recent reports suggesting

Fig. 5. High-fat diet (HFD) elevates blood pressure in Cav1 knockout mice. Effect of 15-wk HFD on body weight (A), on the weight of various organs [liver, heart, epididymal fat (EpidF), and retroperitoneal fat (RertoF); B], and on systolic (Syst) and diastolic (Diast) blood pressure (C) in normal wild-type (WT) and Cav1 knockout (Cav-KO) mice is shown. Representative Western blot and summary of densitometry data (D; n = 4 in each group) showing Cav1 expression in skeletal muscle artery of WT mice with our without HFD are shown. Data are means ± SE. Asterisks indicate significant differences (P < 0.05).
that ANG II, when administered exogenously in an excess amount, induces pathological cardiovascular signaling through Cav1. For example, an earlier study demonstrated that while exhibiting a cardiac damage at baseline, ANG II-induced cardiac pathology is less prominent in the Cav1 knockout mice (30). In a mouse model of ANG II-induced abdominal aortic aneurism formation, Cav1 knockout mice were remarkable protected against the abdominal aortic aneurism development and rupture, but, interestingly, not against the development of increased blood pressure (36). Moreover, Cav1 deletion seemed to be protective against ANG II-induced inactivation of large conductance calcium-activated potassium (BK) channels in smooth muscle cells (26). Although these studies argued for the pathological role of Cav1 in mediating the adverse ANG II effects, the AT1R antagonist, telmisartan, treatment effectively prevented vascular and left ventricular hypertrophy and also improved cardiac pump function in the Cav1 knockout mice (23). This latter study corresponds to our present findings and suggests an augmented and sustained AT1R-mediated signaling in the absence of Cav1. Clearly, further studies are needed to solve this apparent controversy and the nature of mechanisms through which Cav1 may initially protect but at a later stage may contribute to ANG II-mediated cardiovascular pathology.

In summary, the present study demonstrates that an interaction between AT1R and Cav1 contributes to ANG II-mediated tachyphylaxis in the rodent skeletal muscle resistance artery via delaying AT1R internalization and subsequent reactivation. We propose a model, in which binding of AT1R and Cav1 delays AT1R reactivation and thereby prevents augmented vasoconstriction and elevations in systemic blood pressure in diet-induced obesity.

**GRANTS**

This study was supported by National Heart, Lung, and Blood Institute Grant R01 HL-104126 (to Z. Bagi).

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: I.C., A.F., R.L., D.J.F., and Z.B. conception and design of research; I.C., A.F., and Z.B. performed experiments; I.C., A.F., and Z.B. analyzed data; I.C., A.F., and Z.B. interpreted results of experiments; I.C., A.F., and Z.B. prepared figures; I.C., A.F., and Z.B. drafted manuscript; I.C., A.F., and Z.B. revised manuscript.

![Fig. 6. Cav1 knockout mice fed a HFD exhibit an augmented and sustained vasoconstriction to ANG II. Summary data of skeletal muscle resistance artery constrictions to repeated (1st and 2nd applications) ANG II (0.1–10 nM; n = 5 to 6 in each group; A and B) and to NE (0.1 nM–1 μM, n = 5 to 6 in each groups (C and D) in wild-type (WT) and Cav1 knockout mice (Cav-KO)]. Data are means ± SE. *Significant differences, Cav-KO HFD vs. WT HFD; #significant differences from response of WT (P < 0.05).](image-url)
REFERENCES


37. Viswanad B, Srinivasan K, Kaul CL, Ramarao P. Effect of tempol on altered angiotensin II and acetylcholine-mediated vascular responses in

