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RESEARCH HIGHLIGHT

Membrane stretch and angiotensin II type 1a receptor: causes and role in the myogenic response

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The ligand-independent activation of Angiotensin II type 1 receptors following vascular stretch plays very important (patho) physiological roles. Indeed, recent studies have implicated this mechanism in cardiac hypertrophy under conditions of pressure overload and it has shown to be indispensable in the regulation of the myogenic response in smooth muscle cells of small resistance arteries, as well as mesenteric and renal resistance arteries. The information discussed in here will highlight the involvement of the mechanoactivation of the Angiotensin II type I receptors in the development of the myogenic response and the molecular mechanisms modulating them following activation.

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Several (patho) physiological processes including the myogenic response require the transduction of a mechanical stimulus through a process known as mechanotransduction. The myogenic response refers to the adjustment of blood flow during variations of perfusion pressure through a change in vessel diameter of small arteries and arterioles. It is characterized by an increase in global intracellular calcium concentration [Ca^{2+}] in vascular smooth muscle cells (VSMCs), which is mediated by a depolarization-induced activation of L-type calcium channels ($Ca_V1.2$). The depolarization is elicited by membrane stretch, but the molecular mechanism is not completely understood yet. Earlier studies in heterologous systems had suggested that members of the G protein-coupled receptors (GPCRs), including Angiotensin II type I Receptors (AT1R), contribute to the myogenic response by activating TRPC6 channels through the $G_{q/11}$ pathway without the binding of the endogenous ligands, including Angiotensin II. This ligand independent signaling, however, requires the activation of the β-arrestin pathway through a recently uncovered mechanism. With the use of AT1R biased agonists and fusions of the AT1R and G_q, as well as AT1R and β-arrestin 2, Tang W. *et al*. were able to demonstrate that membrane stretch attributes specific conformations to the AT1R, which allows only β-arrestin to be activated and not G_q [1]. The study found that in comparison to wild-type AT1R, the balanced agonist Angiotensin II had only 3.1 fold more affinity to the AT1R-βarrestin 2 fusion protein in isotonic media, whereas the β-arrestin 2 biased agonist TRV120023 bound with 9.7 fold more affinity. Interestingly, however, the presence of hypotonic osmotic stretch caused a 3.5 fold increase in the binding affinity of TRV120023 to both the WT AT1R and AT1R-βarrestin 2, whereas Angiotensin II was unaffected. Their conclusions were further supported by the ability of TRV120023 to potently trigger ERK1/2 phosphorylation and the use of a BRET β-arrestin 2 biosensor, which can differentiate between conformations of β-arrestin 2 stabilized by Angiotensin II or mechanical

stretch. This mechanism of activation is of high importance to understand as recent data from our laboratory has shown that AT1aR are the major AT1R subtype, which are essential for myogenic constriction in mesenteric and renal arteries through a ligand independent mechanism [2].

We used mice deficient in the Angiotensin II receptor subtype 1a (AT1aR), since AT1aR mediates constriction in small arteries, while AT1bR is more involved in constricting large vessels of mice. We studied contractile responses using videomicroscopy for small mesenteric and renal resistance arteries and a perfusion system for isolated kidneys. Though a pressure-induced constriction starting at about 40 mmHg was observed in control animals (*Agtr1a*$^{+/+}$), this constriction was almost completely absent in *Agtr1a*$^{-/-}$ mice. Similar contraction predominated in isolated kidneys, where the flow-induced increase in pressure was lower in Agtr1a-/- mice. Following application of phenylephrine and U46619 (vasoconstrictors), further constriction occurred thus confirming the functionality of the $G_{q/11}$ signaling pathway. The lack of response of the isolated arteries and kidneys of *Agtr1a*$^{-/-}$ mice to Angiotensin II application confirmed the absence of the receptor and suggested AT1aR as the main mediator of Angiotensin II-induced vasoconstriction in those tissues. Two pieces of evidence helped to support the ligand-independent activation of the receptor: 1) Losartan (AT1aR antagonist) had no effect when perfused into isolated kidney in *Agtr1a*$^{-/-}$ mice, and 2) mice deficient in Angiotensin II also displayed a normal myogenic response. These first results, thus suggested the AT1aR as indispensable mechanotransducers in small arteries. We then sought to unravel the mechanism of the depolarization (discussed above) by analyzing potential cation channels downstream of the GPCR pathway. Our studies refuted the idea that TRPC6 channels operate as the downstream target effectors of the myogenic response and rather pointed to an XE991 sensitive K_v channel as the culprit. Hence, in contrast with the cerebral arteries, mice deficient in TRPC6 displayed also a regular myogenic response in the mesenteric and renal arteries as well as the kidneys. Our focus therefore was the voltage-dependent potassium channels (K_v), which are inhibited (closed) *via* depletion of phosphatidylinositol 4,5-bisphosphate (PIP_2) following the activation of $G_{q/11}$ proteins. K_v7 channels are expressed in blood vessels, with $K_v7.4$ and $K_v7.5$ presumably being predominant. Anecdotal evidence using pharmacological blockers and heterologous expression systems had implicated these channels in the modulation of the myogenic tone. We used mice deficient in $K_v7.3$ (*Kcnq3*$^{-/-}$), $K_v7.4$ (*Kcnq4*$^{-/-}$), $K_v7.5$ (*Kcnq*$^{dn/dn}$) and $K_v7.4$ and 7.5 (*Kcnq4*$^{-/-}$/*kcnq5*$^{dn/dn}$), to explore our hypothesis. Surprisingly, arteries from all models showed normal myogenic tone. In addition, isolated single VSMCs from mesenteric arteries from all the knockouts exhibited a

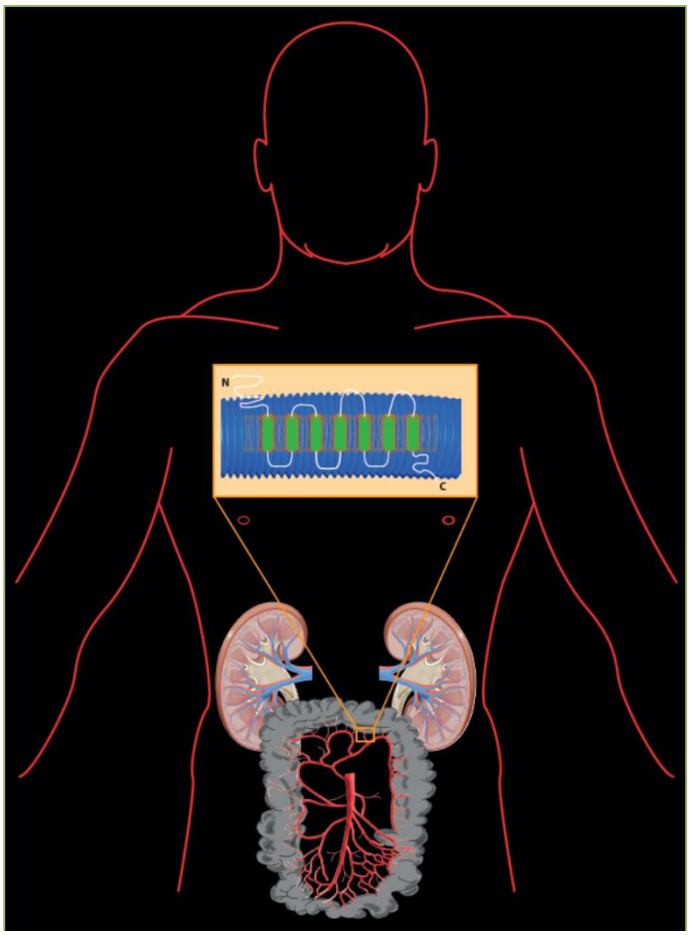

Figure 1. The myogenic response in small vessels is an important mechanism to regulate the blood flow following pressure changes. The AT1aR—a GPCR mediates the myogenic responses of small mesenteric and renal arteries independently of any ligand activation. Activation of the AT1aR through membrane stretch represents a novel mechanism for the control of the myogenic response in mesenteric and renal arteries in the absence of angiotensinogen or TRPC6 channels.

XE991-sensitive current with similar density. This current could be inhibited in WT VSMCs by activation of AT1R as well as by hypoosmotic challenge. These results suggested that $K_v7.3$-5 were not the K_v7 family subtype responsible for the regulation of vascular tone. However, as we did not explore the entire family, there might still be another K_v channel, inhibited by XE991, involved in the generation of myogenic response. One possibility is the K_v7 family subtype $K_v7.1$, which is also expressed in blood vessels, although presumably at lower levels than the other channels.

The advent of biased agonists has renewed the fervor of researchers to disentangle the complex family of GPCRs. The β-arrestin pathway in particular has received a lot of attention. Recent evidence shows that arrestin engages the GPCR using a biphasic mechanism [3] and signaling through this pathway can regulate the turnover of phosphorylated myosin light chain following stimulation of the AT1aR [4].

Importantly, β-arrestin is implicated in the ligand-independent activation of the AT1R following mechanical stretch activation. Mechanotransduction through the AT1aR is necessary for myogenic constriction in the small mesenteric and renal arteries although the cation channels involved downstream from AT1aR activation remain to be elucidated.

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Conflicting interests

The authors have declared that no competing interests exist.

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