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Review

G protein-mediated stretch reception

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Storch U, Mederos y Schnitzler M, Gudermann T. G protein-mediated stretch reception. *Am J Physiol Heart Circ Physiol* 302: H1241–H1249, 2012. First published January 6, 2012; doi:10.1152/ajpheart.00818.2011.—Mechanosensation and -transduction are important for physiological processes like the senses of touch, hearing, and balance. The mechanisms underlying the translation of mechanical stimuli into biochemical information by activating various signaling pathways play a fundamental role in physiology and pathophysiology but are only poorly understood. Recently, G protein-coupled receptors (GPCRs), which are essential for the conversion of light, olfactory and gustatory stimuli, as well as of primary messengers like hormones and neurotransmitters into cellular signals and which play distinct roles in inflammation, cell growth, and differentiation, have emerged as potential mechanosensors. The first candidate for a mechanosensitive GPCR was the angiotensin-II type-1 (AT_1) receptor. Agonist-independent mechanical receptor activation of AT_1 receptors induces an active receptor conformation that appears to differ from agonist-induced receptor conformations and entails the activation of G proteins. Mechanically induced AT_1 receptor activation plays an important role for myogenic vasoconstriction and for the initiation of cardiac hypertrophy. A growing body of evidence suggests that other GPCRs are involved in mechanosensation as well. These findings highlight physiologically relevant, ligand-independent functions of GPCRs and add yet another facet to the polymodal activation spectrum of this ubiquitous protein family.

mechanosensation; G protein-coupled receptor; angiotensin-II type-1 receptor; agonist independent

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Introduction

Cells have the ability to sense numerous chemical and physical stimuli, such as hormones and neurotransmitters, but also pain, electric fields, photons, temperature, and mechanical deformations. The capability of cells to sense physical forces and convert them into electrical and biochemical signals is termed "mechanosensation" and comprises mechanoreception as well as mechanotransduction. Mechanosensation is important for the senses of hearing, touch, and balance and for proprioception and viscerception. Mechanosensitive cells exhibit mechanosensors, e.g., in nerve endings of the skin, in skeletal muscle, and in sensory hair cells of the inner ear (13). Mechanosensation also plays a role in the regulation of blood vessel diameter. In the endothelium as well as in smooth muscle cells of small resistance arteries, mechanosensitive structures are present and harbor key molecular components for the autoregulation of blood flow in these tissues (76) without neuronal involvement. Although mechanosensitive cells are often well characterized, the identity of mechanosensitive subcellular structures and proteins is still widely unknown. A prominent example of as yet unknown molecular correlates underlying mechanosensation is represented by myogenic vasoconstriction, also known as the Bayliss effect: smooth muscle cells of small resistance arteries are mechanosensitive and have the intrinsic property to constrict in response to elevated intraluminal pressure (20). Such an inherent vascular regulatory mechanism allows for the functional plasticity of the cardiovascular system and is of prime physiological relevance, because it makes a significant contribution to basal vascular tone and peripheral vascular resistance as well as capillary hydrostatic pressure and organ perfusion. This review provides an overview of mechanosensitive proteins with a particular focus on the vascular system.

How Can Mechanical Forces Act on Mechanosensitive Integral Membrane Proteins?

It is commonly accepted that mechanical forces act on cell membranes by increasing the membrane tension, thereby influencing integral membrane proteins. Increased membrane tension is the key driving force (80) and can be observed for instance as membrane stretch. To date, two main concepts are discussed as to how integral membrane proteins might perceive mechanical force (49). First, the "tethered" model, which is

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based on the assumption that intra- or/and extracellular anchorage to other proteins acts as a molecular spring that confers mechanosensitivity to integral membrane proteins. The second idea is the "membrane" model, which supposes that altered membrane tension changes the lateral pressure profile (10) at the protein/phospholipid bilayer boundary to provoke conformational changes of integral membrane proteins.

At present, it is still unknown which model would apply best to myogenic vasoconstriction and how membrane stretch and membrane tension are maintained over quite a long time period during myogenic vasoconstriction. An increase in intraluminal pressure initially results in higher wall tension, thereby inducing myogenic vasoconstriction which in turn decreases wall tension (77). Mechanistically, the relationship between reduced wall tension and maintenance of myogenic tone is not understood. Furthermore, it is not clear whether wall tension of an intact blood vessel is equivalent to membrane tension of isolated smooth muscle cells. Moreover, it would appear rather unlikely that only one specific mechanosensitive protein is responsible for myogenic vasoconstriction rather than several different proteins that synergistically operate in the initiation, maintenance, and termination of myogenic tone.

Criteria for Mechanosensitive Integral Membrane Proteins

Because proteins involved in mechanically induced signaling cascades are often indiscriminately termed mechanosensitive, although they do not directly sense membrane stretch, some specific criteria should be established to unambiguously classify directly mechanosensitive proteins (14, 61). For inherently mechanosensitive proteins, there should be a strict and obvious correlation between the amplitude of the mechanical stimulus and protein activation, e.g., single channel activity of ion channels, speed of the enzymatic reaction of membrane-bound enzymes, and G protein activation of G protein-coupled receptors (GPCRs). Furthermore, the strength of the mechanical stimulus should be reflected by the magnitude of protein activation. For example, a more intense mechanical force should cause a faster protein response. To this end, a force coefficient analogous to the Q_{10} value describing the activation rate induced by a 10°C increase in temperature should be established to characterize bona fide mechanosensitive proteins (61). A high force coefficient would indicate pronounced mechanosensitivity. In addition, mechanically induced direct protein activation must occur within the protein's typical reaction time, indicating that the mechanical stimulus is adequate and physiologically meaningful so that the activation time of mechanically stimulated proteins should be very short, that is, <5 ms for ion channels (37), <50 μs for membrane-bound enzymes (100), and <500 ms for G protein activation by GPCRs (97). Furthermore, it should be possible to suppress mechanically induced protein activation by channel blockers, inhibitors, or inverse agonists. As a more direct proof of concept, mechanically induced protein activation should result in detectable conformational changes. As the ultimate criterion for intrinsic mechanosensitivity, purified proteins should be inserted into an artificial bilayer and should still be mechanosensitive (14, 61), which was convincingly demonstrated for bacterial mechanosensitive channels (91). However, to date it is frequently difficult to discriminate between direct and indirect mechanosensitivity due to limitations of the technical resolution and the experimental designs chosen. However, it should be kept in mind that mechanosensory protein complexes may consist of several distinct components that are neatly orchestrated to sense and transduce mechanical signals (9). By this means, a given protein may not be mechanosensitive eo ipso but may still react to mechanical cues in conjunction with other proteins within macromolecular complexes. It still remains elusive whether the formation of such protein complexes simply enhances a weak inherent mechanosensitivity of single protein subunits or whether it is the macromolecular assembly as such that accounts for novel mechanosensing qualities.

Potential Mechanosensors Discussed So Far

Until now, a variety of different proteins has been suggested as direct mechanosensors (see Fig. 1). For example, intracellular structures such as dense bands are discussed as mechanosensors (54). In arterioles these membrane-associated proteins consisting of vinculin and α-actinin (88) are enriched at the abluminal aspect of the blood vessel (32). This particular localization is compatible with a sensory function of these proteins. However, dense bands may serve rather as stabilizers of vascular smooth muscle cells. Other intracellular structural proteins like desmin are not involved in myogenic vasoconstriction (55). However, they may determine the rigidity of smooth muscle cells and thus of the vascular wall (50). Furthermore, the actin cytoskeleton might play a role for mechanosensation by establishing a physical link between mechanosensitive proteins (47). There is no doubt that the actin cytoskeleton influences myogenic vasoconstriction (15, 16, 31), with short- and long-term adaption of the cytoskeleton reflecting the plasticity of small resistance arteries in response to different strains (69).

Whereas the cytoskeleton may act as a tether connecting mechanosensitive proteins, the extracellular matrix may serve as an anchor relaying external relative movements to integral membrane proteins (39). This kind of mechanosensitive protein complexes is best understood in *Caenorhabditis elegans* touch receptor neurons (9, 28).

Integrins are heterodimeric glycoproteins in the plasma membrane linking components of the extracellular matrix like fibronectin, laminin, and collagen with intracellular structural proteins like dense bands. Especially with regard to myogenic vasoconstriction, integrins are discussed as mechanosensors (19, 38, 59, 64) because of their special role as linkers of intra- and extracellular proteins (40). As integrins impact the actin cytoskeleton and vice versa (92), it is still unsettled whether they primarily act as direct mechanosensors or whether they are involved in mechanotransduction as long-term modulators. As an additional facet of cell regulation, integrins impact ion channel function (101, 108, 109), thus further increasing the difficulty to precisely define their physiological role in mechanosensation.

Another family of proteins, tyrosine kinases of the Src family, was also touted as mechanosensors (84) or mechanotransducers (103, 107) because of their impact on the actin cytoskeleton via integrins (30). However, in HEK293 cells neither endogenous nor overexpressed receptor tyrosine kinases were mechanosensitive (62), leaving the role of tyrosine kinases in mechanosensation ill-defined.

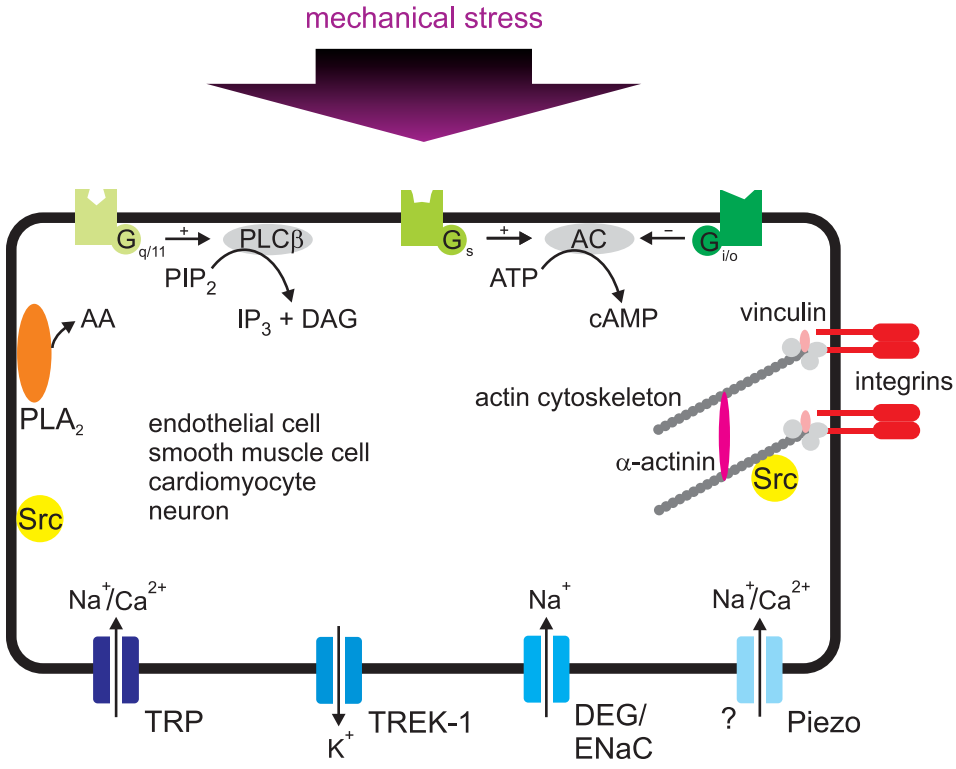

Fig. 1. Possible mechanosensors. Various proteins that have been discussed as potential mechanosensors in different cells are displayed. These proteins have been proposed to be activated by membrane stretch or by fluid shear stress. Possible mechanosensors are highlighted in color and nonmechanosensitive proteins in gray. PLCβ, phospholipase Cβ; PLA_2, phospholipase A_2; AA, arachidonic acid; AC, adenylyl cyclase; PIP_2, phosphatidylinositol 4,5-bisphosphate; IP_3, inositol trisphosphate; DAG, diacylglycerol; Src, Src tyrosine kinase; cAMP, adenosine 3′,5′-cyclic monophosphate; ATP, adenosine-5′-triphosphate; Piezo, piezo1 and piezo 2, a new family of mechanosensitive cation channels; TRP, transient receptor potential; TREK-1, TWIK-related potassium channel; Deg, degenerin; ENaC, epithelial sodium channel.

Two membrane-associated enzymes, phospholipase A_2 (PLA_2) and phospholipase C (PLC), have been convincingly implicated in mechanosensation. Slight changes of osmolarity entailed enhanced PLA_2 enzymatic activity (53), and direct mechanical stimulation of skeletal muscle cells potentiated PLA_2 but not PLC activity (94). On the contrary, PLC could be mechanically activated in mesangial cells (46). Especially in blood vessels, mechanical activation of PLC was observed (43, 60, 70, 78). While PLC can be classified as indirectly mechanosensitive (62), it is not clear at present whether PLA_2 reacts directly or indirectly to mechanical stimuli (41). However, there is strong experimental support of the notion that both proteins play a key role in mechanotransduction.

Potentially Mechanosensitive Ion Channels

In the past, various ion channels have been implicated as molecular components of a mechanotransduction pathway. With regard to myogenic vasoconstriction, chloride channels (23, 36, 72, 110) and cation channels (105, 106) were considered as mechanosensors. Furthermore, candidate channels of the degenerin (Deg)/epithelial sodium channel (ENaC)/acid-sensing ion channel family were suggested to be endowed with mechanosensing properties in specialized protein complexes (24, 25, 95) compared with mechanosensitive complexes in *C. elegans*. Notably, ENaC channels were particularly receptive to shear stress when endogenously (102) or heterologously expressed (11, 87) even after reconstitution in an artificial bilayer (4), pointing to their inherent mechanosensitivity.

Lately, several members of the transient receptor potential (TRP) family of cation channels (17, 65), especially TRPC1, TRPC6, TRPM4, TRPM7, TRPV2, TRPV4, and TRPP1 (PKD2) (nomenclature according to the International Union of Pharmacology), have attracted considerable attention as potentially mechanosensitive ion channels (42, 85, 113). While TRPC1 was primarily suggested to be the endogenous mechanosensitive ion channel in *Xenopus* oocytes (58), this concept has been recently challenged (22, 35).

TRPC6 and TRPM4 were also regarded as direct mechanosensors. The latter two proteins are depolarizing cation channels involved in the constriction of resistance arteries as evidenced by a profound suppression of myogenic vasoconstriction of cerebral arteries after preincubation with specific antisense oligonucleotides (27, 105). In accord with the role of TRPC6 for myogenic responsiveness, inherent mechanosensitivity of TRPC6 channels was reported (89) but could not be verified later on (35, 41, 62). TRPM4 channel activity is tightly regulated by free intracellular calcium (73) and protein kinase C (26), and in a heterologous overexpression system, TRPM4 was also activated to membrane stretch (66), and clarification of the various mechanisms by which TRPM4 channel function is regulated will require additional studies. However, the channel protein's inherent sensitivity to membrane stretch is still a moot issue (96). Likewise, the activation of TRPM7 by mechanical stimuli has been reported to contribute to regulatory volume changes in epithelial cells (74, 75) but was subsequently called into question after thorough electrophysiological analysis of the overexpressed protein (7). There is hardly any solid experimental evidence that TRPM7 is in any traditional sense a mechanosensitive ion channel. Probably, the dilution of intracellular magnesium or magnesium nucleotides caused by hypotonic cell swelling rather than membrane stretch may have caused TRPM7 activation when investigating regulatory volume changes in epithelia (74, 75). Thus results obtained solely by hypotonic cell swelling as a mechanical stimulus have to be interpreted with great caution.

Thermosensitive TRPV2 channels may also serve as mechanosensors in vascular smooth muscle and may exhibit inherent mechanosensitivity upon functional expression of recombinant proteins (68). With regard to TRPV4, there is a considerable body of experimental evidence to support the notion that these ion channels are not directly gated by mechanical forces but are activated downstream of a signaling pathway, encompassing PLA_2 and cytochrome-*P*450 epoxygenase (99, 104) with PLA_2, possibly representing the cellular mechanosensor. Furthermore, Deg/ENaC rather than TRPV channels were recently identified as major mechanosensors in *C. elegans* nociceptor neurons (34), whereas TRPV channels are supposed to be activated downstream of Deg/ENaC channels.

Whereas homomeric TRPP1 (PKD2) channels encoded by polycystic kidney disease 2 gene (PKD2) do not appear to be directly mechanosensitive (48), TRPP1 (PKD2) proteins complexed with TRPV4 or PKD1 (previously known as polycystin-1) might represent crucial constituents of mechanosensitive protein complexes in the vasculature (48, 86). Heteromeric TRPP1 (PKD2)/TRPV4 protein complexes have been advocated as polymodal sensory complexes that mediate flow-sensitive cation influx (48). Similarly, TRPP1 (PKD2)/PKD1 complexes in the primary cilium of kidney cells were reported to mediate mechanosensation (71), whereas in vascular smooth muscle cells, TRPP1 (PKD2) does not appear to be directly mechanosensitive but may rather inhibit endogenous stretch-activated cation channels, an effect which is abrogated by interaction with PKD1 (86). In addition, TRPP1 (PKD2) proteins are supposed to associate with TRPC1 (93). However, physiological corollaries of these TRPP1 (PKD2)/TRPC1 complexes remain elusive. Thus the proposed mechanosensitivity of TRPP1 (PKD2) is far from being understood mechanistically and presently lacks conceptual consistency.

Based on the aforementioned criteria for direct mechanosensitivity, no TRP channel can be classified as a direct mechanosensor up to now. However, there is circumstantial evidence to suggest that TRP channels play a modulatory role in mechanotransduction as crucial downstream effector proteins. However, there is compelling evidence of direct mechanosensitivity of a different cation channel, TREK-1, a two pore-domain background potassium channel. Membrane stretch activates TREK-1 channels with half-maximal activation at about −40 mmHg of negative pressure (56, 79). The mechanosensitivity of TREK-1 is independent of the expression model, the technical configuration, and the type of mechanical stimulation. Furthermore, TREK-1 gene-deficient mice exhibit reduced touch sensitivity (2), providing in vivo evidence for neuronal mechanosensation. Although TREK-1 was not involved in myogenic vasoconstriction in mesenteric arteries, TREK-1 plays a role for pressure-induced vasodilatation in the skin (33), highlighting distinct mechanosensory mechanisms in different tissues.

Recently, two new proteins, piezo1 and piezo2 (also known as Fam38a and Fam38b), were described as a new class of mechanosensitive ion channels (18). These integral membrane proteins are characterized by 24 to 36 predicted transmembrane segments and were found to be expressed in various tissues like bladder, colon, and lung where they may represent molecular correlates of mechanosensitive cation channels. Furthermore, piezo2, which is highly expressed in dorsal root ganglia, was found to play an important role for mechanosensation in these somatosensory neurons (15). In accord with these observations, the amphiphilic peptide GsMTx4 was found to inhibit mechanically induced piezo1 currents upon heterologous expression (5). The latter peptide is an inhibitor of mechanosensitive ion channels, yet does not appear to block these channels directly, but acts by insertion into the membrane bilayer, thus modifying the lipid composition surrounding integral membrane proteins, resulting in an altered lateral pressure profile at the phospholipide/protein interface (90). Thus future studies will have to show whether piezo proteins are in fact pore forming subunits of mechanosensitive ion channels or indispensable accessory subunits of a larger mechanosensitive protein complex.

Interestingly, voltage-gated channels appear to be mechanosensitive as well (51, 52, 67). This property may impact the regulation of heart beat, since GsMTx4 was shown to inhibit atrial fibrillation (8).

GPCRs as Mechanosensors

Recently, GPCRs, generally known as mediators of light, olfactory and gustatory stimuli, as well as of the vast majority of hormones and neurotransmitters, were appreciated as mechanosensors. GPCRs exhibit distinct functions in many physiological processes like inflammation, cell growth, and differentiation. Interestingly, the $G_{q/11}$ protein-coupled angiotensin-II type-1 receptor (AT_1R), a cardinal component of the renin-angiotensin regulatory system, was identified as the first mechanosensitive GPCR (115). Instead of autocrine angiotensin-II release (83), Zou et al. (115) demonstrated that agonist-independent mechanical AT_1R activation is one of the main mechanisms mediating stretch-induced hypertrophy of cardiomyocytes. Stretch-induced receptor activation could be completely prevented by deploying specific inverse AT_1R agonists (111, 115). Involvement of endogenous angiotensin II was ruled out using angiotensinogen-deficient mice and a neutralizing antibody directed against angiotensin II.

Apart from the involvement of AT_1Rs in the pathophysiological state of cardiac hypertrophy, AT_1Rs were identified as mechanosensors participating in the physiological process of myogenic vasoconstriction (62). Membrane stretch in a physiological range was shown to activate $G_{q/11}$ protein-coupled receptors independently of endogenous agonist (62), demonstrated firstly by employing an angiotensin-converting enzyme inhibitor to suppress endogenous angiotensin II secretion and secondly by the fact that in rat cerebral arteries, the endothelium does not express chymases which generate angiotensin II and which are present in other species (44, 63). In addition, a neutralizing antibody directed against angiotensin II did not affect mechanically induced AT_1R signaling (62). Furthermore, in the conditioned medium of mechanically stimulated myogenic arteries, angiotensin II was not detectable by radioimmunoassay and a sensitive bioassay, thus ruling out any contribution of endogenous angiotensin II on mechanical receptor activation (62). However, under physiological conditions, basal angiotensin II levels are likely to sensitize the receptor's response toward mechanical stimulation.

When compared with Zou et al. (115) in analyzing very distal steps of the signaling cascade induced by receptor activation, Mederos y Schnitzler and colleagues focused on more proximal events. In the latter study mechanically induced receptor activation was PLC dependent, which is in line with

former studies showing that PLC isoforms are important elements of signaling pathways involved in mechanotransduction in myogenic arteries (43, 60, 70, 78). Stretch-induced receptor activation leads to GTP loading of $G_{q/11}$ proteins, PLC activation, and subsequently diacylglycerol-mediated TRPC channel activation, allowing for sodium and calcium influx. Beyond PLC, PLA_2 might also act as mechanotransducers potentiating TRPC6 activity by 20-hydroxyeicosatetraenoic acid production in concert with ω-hydroxylase (41). However, it is still not clear whether PLA_2 isoforms are intrinsically mechanosensitive, whereas in contrast PLCs are most probably indirectly mechanosensitive. Altogether, these findings revise the present views of GPCR function in general and they shed new light on the mechanism of myogenic vasoconstriction. In contrast to former concepts of mechanosensation, GPCRs are principally mechanosensitive and TRPC channel activation occurs downstream of mechanically induced GPCR activation, indicating that TRPC channels are only indirectly mechanosensitive. In smooth muscle cells TRPC channel activation leads to depolarization of the membrane potential, thus increasing the channel open probability of L-type voltage-gated calcium channels resulting in calcium influx, elevation of the intracellular calcium concentration, force development, and smooth muscle cell contraction (62, 98).

Mechanical GPCR activation is not restricted to AT_1Rs. Although the $G_{q/11}$-coupled endothelin type-1a (ET_{1A}) receptors were initially described as insensitive to mechanical stimulation pointing to a rather exceptional property of AT_1Rs as mechanosensors (115), Mederos y Schnitzler et al. showed that a large variety of $G_{q/11}$ protein-coupled receptors, including the endothelin ET_{1A} receptor, are directly mechanosensitive (62). Furthermore, the propensity to react to mechanical stimuli varied from the histamine H_1 receptor, exhibiting the strongest cell responses upon mechanical stimulation, followed by the AT_1R, the muscarinic M_5 receptor, and finally the vasopressin V_{1A} receptor. As yet, the structural basis for such differential mechanosensitivity is unknown.

It is reasonable to propose that the mechanosensitivity of $G_{q/11}$ protein-coupled receptors is involved in other physiological processes as well and may even be a general property of most, if not all, $G_{q/11}$ protein-coupled receptors. In smooth muscle cells, mechanosensitivity depends on receptor density (62), indicating that the specificity of cells, tissues, and organs to be activated by mechanical stimulation is regulated by the expression levels of the GPCRs.

As mentioned before, directly mechanosensitive proteins are characterized by mechanically induced conformational changes. In line with this prerequisite, the AT_1R was shown to undergo a mechanically induced conformational change based when modeled after the bovine rhodopsin crystal structure (112), in which a dislocation and an anticlockwise rotation of transmembrane domain 7 toward the agonist-binding pocket occurred upon mechanical stimulation. Interestingly, mechanical membrane stretch stabilized a receptor conformation that was significantly different from that induced by agonist characterized by dislocation and rotation of transmembrane domain 6 out of the binding pocket, thereby veering away from transmembrane domain 3 (29, 45). The different agonist- or mechanically induced receptor conformations are displayed in Fig. 2. Furthermore, a distinct inactive receptor conformation induced by binding of an inverse agonist could be discerned.

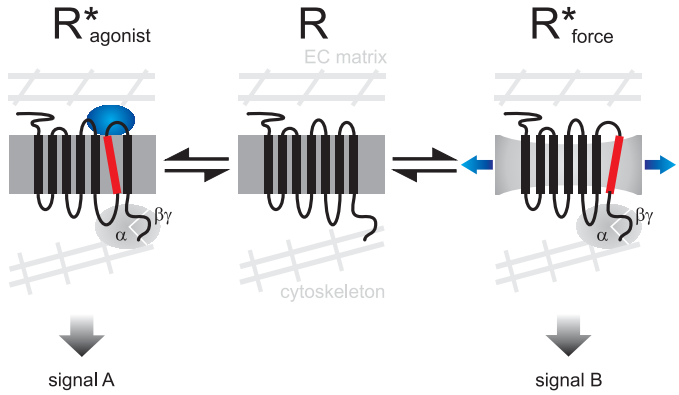

Fig. 2. Agonist and mechanically induced receptor conformations. G protein-coupled receptors possess distinct receptor conformations: inactive (R), agonist-induced active $(R_{agonist})^*$ and mechanically induced active $(R_{force})^*$ receptor conformations. Active receptor conformations are characterized by conformational changes in transmembrane domains (displayed in red) resulting in G protein activation and activation of signal transduction pathways. It is still elusive whether mechanical GPCR activation requires interaction with the cytoskeleton or with the extracellular matrix (EC matrix) as proposed by the tethered model or whether the membrane model is applicable (indicated as membrane deformation). Agonist is shown in blue, and membrane stretch is indicated by two blue arrows.

The recent successful crystallization of the β_2-adrenoceptor in its active conformation together with G_s (82) may serve as a better matrix for future receptor models. In recent years it became obvious that AT_1R and most probably also other GPCRs possess more than one active or inactive receptor conformation in analogy to the different states of ion channels. In line with this notion, different partial agonists were shown to induce several distinct active receptor conformations (116). In the performance of bioluminescence resonance energy transfer (BRET) experiments, it was demonstrated that membrane stretch favors an active AT_1R conformation that is recognized by β-arrestin (62), an observation that was recently verified by Rakesh et al. (81) in performing intermolecular fluorescence resonance energy transfer (FRET) experiments. In the latter study, G protein activation was not essential for mechanically induced β-arrestin recruitment. Of note, this study suggests the occurrence of a distinct β-arrestin conformation upon mechanical receptor activation, which is different from the one induced by agonist.

Although distinct mechanically and agonist-induced active receptor conformations could be discriminated, it still remains elusive whether mechanical receptor activation impacts agonist affinity. With a consideration that mechanical stimulation causes an increased sensitivity of the AT_1R toward agonist stimulation illustrated by a leftward shift of the concentration response curve (62), there is reason to assume that membrane stretch does at least not reduce agonist affinity. However, this issue still has to be finally resolved in the future. Moreover, it is not known whether GPCR agonists engage exactly the same signal transduction pathways as mechanical force.

Recently, Anfinogenova and colleagues (3) further analyzed the synergism between agonist- and mechanically induced receptor stimulation by measuring TRPC current activation in vascular smooth muscle cells as well as myogenic vasoconstriction. Surprisingly, after agonist stimulation with P2Y or thromboxane receptor agonists, subsequent mechanical stimulation in the presence of agonists did not enhance channel

activity or vasoconstriction, suggesting the absence of a functional synergism between these stimuli. However, it should be kept in mind that pretreatment with agonists inevitably entails receptor desensitization, resulting in a decreased secondary cell response. Furthermore, mechanosensitivity most probably depends on receptor density (62), and it is not clear whether P2Y and thromboxane receptor levels are higher in small resistance than in conduit arteries.

During the last years, some additional $G_{q/11}$ protein-coupled receptors have been demonstrated to be mechanosensitive. First of all, the $G_{q/11}$ protein-coupled bradykinin B_2 receptor exhibited mechanically induced conformational changes shown by intramolecular FRET (12). However, this study leaves open the question whether mechanical bradykinin B_2 receptor stimulation entails productive coupling to G proteins. Nevertheless, the mechanosensitivity of the B_2 bradykinin receptor may play a role in the sensing of fluid shear stress in endothelial cells.

Interestingly, many additional GPCRs were tested for their propensity to be activated by mechanical stimuli (for an overview of mechanosensitive GPCRs, see Table 1). Until now, very little is known about the mechanosensitivity of $G_{i/o}$-coupled receptors, but first evidence suggests that fluid shear stress can activate $G_{i/o}$-coupled formyl peptide-1 receptors as monitored by intermolecular FRET between G_i protein subunits (57). Although G_s-coupled β_2-adrenoceptors did not respond to mechanical stimuli (62, 115), there is growing evidence that other G_s-coupled receptors might be inherently mechanosensitive. Recently, dopamine D_5 receptors in primary cilia were described as mechanosensors (1). Furthermore, in bone cells the parathyroid hormone type-1 receptor coupling to both G_s and $G_{q/11}$ proteins was shown to be activated by fluid shear stress (114). With an intramolecular FRET approach, fluid shear stress induced a distinct conformational change of the parathyroid hormone type-1 receptor. Interestingly, increasing the fluidity of the membrane using benzyl alcohol or cholesterol extraction, two maneuvers known to affect mechanosensitive ion channels (21), also led to conformational changes monitored by increased FRET levels. The observation that changes of the lipid composition of the cell membrane

Table 1. *Mechanosensitive GPCRs*

Receptor	Coupling	Expression System	Stimulation	Experimental Approach	Possible Function	Reference
AT_1R	$G_{q/11}$	Overexpression in HEK293 and in A7r5 cells, endogenous in vascular smooth muscle cells	Hypotonicity, direct membrane stretch, increased intravascular pressure	Whole cell measurements, Ca^{2+} imaging, inositol phosphate detection, BRET, measurements of myogenic vasoconstriction	Mechanosensor mediating myogenic vasoconstriction	(62)
		Overexpression in HEK293 and COS-7 cells, endogenous in cardiomyocytes	Direct membrane stretch, pressure overload	ERK phosphorylation, inositol phosphate detection, transthoratic echocardiography, heart morphometry, heamodynamic measurements	Pathophysiological role as mechanosensor inducing cardiac hypertrophy	(115)
		Overexpression of mutant receptors in HEK293 cells	Direct membrane stretch	Inhibition of angiotensin II binding, substituted cysteine accessibility mapping		(112)
		Overexpression in HEK293 cells, endogenous in cardiomyocytes	Hypotonicity, direct membrane stretch, pressure overload	ERK phosphorylation, intermolecular FRET, BRET	Pathophysiological role as mechanosensor inducing cardiac hypertrophy	(81)
B_2R	$G_{q/11}$	Overexpression in bovine aortic endothelial cells	Fluid shear stress, hypotonicity	Intramolecular receptor FRET	Sensors of fluid shear stress in endothelial cells	(12)
D_5R	G_s	Vascular endothelial cells	Fluid shear stress	Ca^{2+} measurements	Sensors of fluid shear stress in endothelial cells	(1)
ET_AR	$G_{q/11}$ / G_s	Overexpression in HEK293 cells	Hypotonicity	Whole cell measurements		(62)
FPR_1	$G_{i/o}$	Endogenous in HL60 cells	Fluid shear stress	Intermolecular FRET between G_i protein subunits	Retraction of pseudopods in neutrophils	(57)
H_1R	$G_{q/11}$	Overexpression in HEK293 cells	Hypotonicity, direct membrane stretch	Whole cell and cell-attached patch-clamp measurements, Ca^{2+} imaging		(62)
M_5R	$G_{q/11}$	Overexpression in HEK293 cells	Hypotonicity, direct membrane stretch	Whole cell and cell-attached patch-clamp measurements		(62)
PTH_1R	G_s / $G_{q/11}$	Overexpression in HEK293 and in murine preosteoblatic cells	Fluid shear stress	Intramolecular receptor FRET	Bone growth	(114)
$V_{1A}R$	$G_{q/11}$	Overexpression in HEK293 cells	Hypotonicity	Whole cell measurements		(62)

Mechanosensitive G protein-coupled receptors (GPCRs). Overview of mechanosensitive GPCRs described so far, main signal transduction pathways, expression systems, stimuli and experimental approaches as well as proposed physiological or pathophysiological impact. See main text for definitions of abbreviations.

profoundly affect the activity of intregral membrane proteins lends further credence to the idea of an activation model relying on changes of the lateral pressure profile.

Because GPCRs not only respond to mechanical or chemical stimuli but also sense alterations of the transmembrane potential (6), these membrane proteins appear to be endowed with polymodal-sensing capabilities to integrate a diverse array of different stimuli. This polymodal activation mechanism raises the intriguing question of whether the mechanosensitivity of GPCRs might be a general phenomenon that occurs in other mechanosensitive cells, tissues, and organs. This needs to be addressed in future studies. In addition to cardiac hypertrophy, mechanosensation and -transduction might also play key roles in other pathophysiological scenarios such as arteriosclerosis, glaucoma, chronic obstructive pulmonary disease and in the case of increased intracranial pressure.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

U.S., M.M.y.S., and T.G. drafted manuscript; U.S., M.M.y.S., and T.G. edited and revised manuscript; U.S., M.M.y.S., and T.G. approved final version of manuscript; M.M.y.S. prepared figures.

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