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## **Mechanical GPCR activation by traction forces exerted on receptor N-glycans**

Stefano Marullo<sup>1\*</sup>, Stephane Doly<sup>2</sup>, Kusumika Saha<sup>1</sup>, Hervé Enslin<sup>1</sup>, Mark G.H. Scott<sup>1</sup>, Mathieu Coureuil<sup>3</sup>

1. Université de Paris, Institut Cochin, Inserm U1016, CNRS UMR8104, Paris, France

2. Université Clermont Auvergne, INSERM, NEURO-DOL, 63000, Clermont-Ferrand, France

3. Université de Paris, Institut-Necker-Enfants-Malades, Inserm U1151, CNRS UMR 8253, Paris, France

\*Correspondence: [stefano.marullo@inserm.fr](mailto:stefano.marullo@inserm.fr) (S. Marullo).

### **Keywords**

Mechanosensors, mechanotransduction, shear stress, pilins, lectins, sialic acid, host-pathogen.

## **Abstract**

Cells are sensitive to chemical stimulation, which is converted into intracellular biochemical signals by the activation of specific receptors. Mechanical stimulations can also induce biochemical responses via the activation of various mechanosensors. Although principally appreciated for their chemosensory function, G protein-coupled receptors (GPCRs) may participate in mechano-transduction. They are indirectly activated by the paracrine release of chemical compounds secreted in response to mechanical stimuli, but they might additionally behave as mechanosensors that are directly stimulated by mechanical forces. Although several studies are consistent with this latter hypothesis, the molecular mechanisms of a potential direct mechanical activation of GPCRs have remained elusive until recently. In particular, investigating the activation of the catecholamine  $\beta_2$ -adrenergic receptor by a pathogen revealed that traction forces directly exerted on the N-terminus of the receptor via N-glycan chains activate specific signaling pathways. These findings open new perspectives in GPCR biology and pharmacology since most GPCRs express N-glycan chains in their N-terminus, which might similarly be involved in the interaction with cell surface glycan-specific lectins in the context of cell-to-cell mechanical signaling.

Mechanical stimuli acting at the level of organs, tissues, individual cells or molecular complexes regulate multiple physiological and developmental processes. Mechano-transduction, the molecular process converting mechanical stimuli into biochemical signals, is elicited by the direct activation of mechano-sensors, which are the molecules that directly respond to changes in mechanical cues. Different types of plasma membrane-associated mechano-sensors have been identified, which respond to various types of mechanical stimuli and activate distinct signaling pathways.

### **“Typical” mechano-sensors**

The most extensively investigated mechano-sensors so far do not belong to the GPCR family. Extracellular matrix (ECM) stiffness provides mechanical signals regulating cell differentiation, proliferation, migration, apoptosis<sup>1-4</sup>. Rigidity sensing involves transmembrane integrin molecules that bind the ECM at their extracellular side, and the actin cytoskeleton, via adapter proteins, at their intracellular side<sup>(5</sup> for a recent review). Platelet endothelial cell adhesion molecule (PECAM)-1 present on adherent junctions forms a mechano-sensory complex<sup>6</sup> with VE-cadherin and vascular endothelial growth factor receptor (VEGFR)-2/VEGFR3. In endothelial cells this complex is activated by shear stress resulting in the phosphorylation of PECAM-1 and Src-dependent activation of phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K). This process in turn activates integrins to bind to ECM by an inside-out mechanism. Piezo family mechanically-activated ion channels<sup>7,8</sup> play a major role in vascular development and function, pulmonary function and sensory transduction (reviewed in<sup>9</sup>). In mammals, two distinct genes code for PIEZO1, principally expressed in non-neuronal cells, and PIEZO2, restricted to sensory neurons and mechano-sensory structures. These proteins are arranged as trimers. Their activation results in an influx of Na<sup>+</sup> and Ca<sup>2+</sup>. Membrane stretching and shear stress activate PIEZOs by generating a membrane tension, which causes the reorganization of lipids around the protein and channel opening<sup>10</sup>. Additional types of mechanically-activated ions channels have been discovered in lower organisms and in mammals<sup>11</sup>. Among these, two-pore-domain potassium channels (K<sub>2P</sub>) constitute a family of 15 members sharing a 4 trans-membrane (TM) domain topology. Some of them directly sense mechanical forces imparted through the lipid membrane<sup>12</sup>. Mechano-transduction also plays an important role in adaptive immune response.

Properly applied mechanical forces provide a direct synergistic activation of  $\alpha\beta$  T-cell receptors (TCR), which have established a chemical bond with an antigenic peptide ligand bound to major histocompatibility complex (MHC) molecules. These forces reduce by orders of magnitude the number of peptide-bound MHC molecules sufficient to trigger a  $\text{Ca}^{2+}$  flux in T cells <sup>13</sup>.

### **GPCR participation in mechano-transduction**

Although principally known for their chemosensory function, which converts the signals conveyed by chemical ligands into intracellular biochemical signals, G protein-coupled receptors (GPCRs) can be involved in mechano-transduction (Table 1). However, although the participation of GPCRs in the transmission of external mechanical stimuli has been known for more than 25 years <sup>14</sup>, experimental evidence that mechanical forces can directly activate these receptors has only recently emerged.

Since the discovery that stretch-induced hypertrophy of cardiac myocytes was caused by autocrine release of angiotensin II (Ang II) and subsequent activation of Ang II receptors <sup>14</sup>, several GPCRs of smooth muscle cells were reported to be activated by the paracrine release of peptides, lipids, nucleotides and amines by endothelial cells in response to vascular stretch. In turn, the activated receptors stimulated their cognate G proteins such as  $G_s$ ,  $G_{q/11}$  or  $G_{12/13}$  to generate a myogenic tone <sup>15-17</sup>. Other investigations revealed that mechanical stimuli could also activate GPCRs without the involvement of their cognate agonists <sup>18-20</sup>, but the molecular mechanism of receptor activation has remained elusive in most cases. The hypothesis that GPCRs might themselves be mechano-sensors has been raised by several studies, in which GPCR conformational changes consistent with receptor activation, productive G protein coupling and  $\beta$ -arrestin recruitment, were documented in response to mechanical stimuli <sup>19</sup>. Supporting this hypothesis further, it was observed that mechanical perturbation of the plasma membrane (caused by shear stress, hypotonic stress, or membrane fluidizing agents) elicited real time ligand-independent conformational transitions of GPCRs <sup>20</sup>. A large-scale automated screening assay was conducted with a mechano-sensitive cell line that exhibits shear stress-activated calcium transients to identify shear stress-sensitive mechano-transducers <sup>21</sup>. With this assay and complementary gain- or loss-of-function experiments the authors found that GPR68, a  $G_{q/11}$ -coupled receptor expressed in

endothelial cells of small diameter vessels, was necessary and sufficient for shear stress responses in these cells. Although satisfying all criteria for a mechano-sensor (*i.e.* essential for immediate response in mechano-sensitive cells, which naturally express the receptor and responsive to the relevant mechanical stimuli when expressed in heterologous cells) it could not be formally established whether GPR68 is directly activated by mechanical forces or by a putative upstream mechano-sensor or by the local release of protons, which were also reported to activate this GPCR.

Evidence that a mechanical stimulus can directly activate a GPCR also comes from studies on receptors belonging to a special sub-family of GPCRs, namely adhesion GPCRs (aGPCRs, see <sup>22,23</sup> for recent reviews). aGPCRs possess a long extracellular N-terminus with adhesive properties that anchors the receptor to the extracellular matrix or to opposed cell surfaces via cognate ligands. They also contain a juxta-membrane GPCR auto-proteolysis-inducing (GAIN) domain, which during protein maturation catalyzes receptor cleavage in N and C-terminal fragments (NTF and CTF) that remain non-covalently associated. aGPCRs function as adhesion molecules due to the NTF and as classical GPCRs through the CTF. Receptor activation exposes a cryptic tethered agonist ligand that activates the receptor. The latrophilin/dCIRL aGPCR, for example, is found in mechano-sensory neurons of *Drosophila* chordotonal organs and modulates ionotropic receptor currents. Whereas auto-proteolysis of the GAIN domain is not essential for dCIRL activity, the authors found that progressive lengthening of dCIRL's N-terminal fragment gradually reduces mechano-sensory neuronal responses. These findings are consistent with a model in which mechanical tension applied to the receptor determines the extent of its activity <sup>24</sup>.

A different mechanism of mechanical GPCR activation has been reported in a very recent article investigating the activation of endothelial histamine H<sub>1</sub> receptors (H<sub>1</sub>Rs) by shear stress and membrane stretch <sup>25</sup>. Mechanical activation of H<sub>1</sub>Rs was found to be agonist-independent, insensitive to inverse agonists, and G<sub>11/q</sub> protein dependent, resulting in NO production. Conformational changes promoted by shear stress and stretch were distinct from those elicited by receptor agonists. Interestingly, the mechanically activated pathway was strictly dependent on the presence of an 8<sup>th</sup> helix (H8) in the C-terminal tail of the receptor: transfer of the H8 to non-responsive GPCRs conferred mechano-sensitivity to non-responsive GPCRs, whereas H8

removal in H1Rs abolished it. These observations suggest that the mechanical elongation of H8 would activate the G protein.

### **GPCR activation by pathogen pulling on N-terminal glycan chains**

Meningococcus (*N. meningitidis*) is a human-specific Gram-negative bacterium, which colonizes the mucosa of nose and pharynx. However, when meningococci enter into the bloodstream, they rapidly spread and become pathogenic. After blood brain barrier crossing they can invade meninges, causing cerebrospinal meningitis <sup>26</sup>. When bacterial load is high, infection spreads to peripheral vessels causing septic shock, the most severe life-threatening form being *purpura fulminans* <sup>27</sup>. The interaction of the pathogen with endothelial cells initiates peripheral vascular lesions <sup>28</sup> and is responsible for the opening of the blood brain barrier <sup>29</sup>. This interaction involves long bacterial filamentous organelles known as Type IV pili (Tfp), which mediate the adhesion of virulent capsulated meningococci to endothelial cells *in vitro* and *in vivo* <sup>29,30</sup>. Tfp interact with two host cell receptors. The first is CD147, an IgG-family single TM receptor, which does not transduce any signal but that is essential for early adhesion of bacteria to the endothelial cells <sup>31</sup>. The second is a GPCR, the catecholamine  $\beta_2$ -adrenergic receptor ( $\beta_2$ AR). Interaction of Tfp with the N-terminus of the  $\beta_2$ AR induces an allosteric beta-arrestin ( $\beta$ arr)-biased signalling cascade in host endothelial cells, which is not blocked by the antagonist propranolol <sup>32</sup>. Although the  $G_s$ /cAMP pathway is not affected upon bacterial binding to endothelial cells, bacteria induce a GRK-dependent recruitment of  $\beta$ arrs, leading to the activation of ERK and Src, and the subsequent recruitment under bacterial colonies of various cytoskeletal (actin and ezrin) and junctional proteins. Interestingly, all these signalling events are necessary for two essential processes of meningococcus pathophysiology, namely the stabilization of growing bacterial colonies under blood-flow <sup>33</sup> and the opening of endothelial junctional spaces, which become loose and ultimately allow bacteria to penetrate into tissues <sup>34</sup>, such as meninges. The interaction of the pathogen with these two distinct receptors is facilitated by the fact that the  $\beta_2$ AR and CD147 are pre-assembled in endothelial cells forming heteromeric structures stabilized by the cytoskeletal protein alpha-actinin-4 <sup>35</sup>. Although these studies well outlined the principal pathophysiological events permitting meningococcus to penetrate into meninges, a major issue remained unresolved for

several years, namely how a GPCR activated by non-soluble bacterial proteins is able to transduce a signalling cascade, which is usually induced by cognate receptor ligands.

Besides their passive “hooking” role, Tfp also generate mechanical forces. Studies in *Neisseria* species demonstrated that pilus retraction driven by the bacterial ATPase PilT produces forces *in vivo* that allow bacteria to move over surfaces<sup>36,37</sup>. Moreover, bacteria growing on the endothelial cell surface are constantly submitted to forces produced by blood flow, which can be propagated to endothelial cell plasma membranes via Tfp attachment. Although Tfp binding to host endothelial cells is necessary to engender pulling forces, the molecular plasma membrane tether involved in this process remained elusive. Studies recapitulating the hemodynamic conditions existing in capillaries, showed that the  $\beta_2$ AR is involved in the signaling cascade that allows small meningococcal colonies to remain stable under flow<sup>32</sup>. A possible mechanistic explanation of the above findings would be that the  $\beta_2$ AR is simultaneously a mechano-sensor and a transducing receptor for meningococcal Tfp.

The hypothesis that the  $\beta_2$ AR is a mechano-sensor for Tfp-mediated traction was confirmed recently and represents the first demonstration that a “classical GPCR” can directly be activated by mechanical forces<sup>38</sup>.

In the attempt of characterizing the binding site of pilins on the  $\beta_2$ AR, a chimeric receptor approach with the angiotensin II receptor AT1R was used in a fully controlled reconstitution cell system. AT1R, also present in endothelial cells, is incompetent for *N. meningitidis* signaling. Substituting its N-terminal region with that of the human  $\beta_2$ AR generated a chimera, which could be activated by *N. meningitidis in vitro*, suggesting that pilin binding to the  $\beta_2$ AR N-terminus is sufficient to mediate  $\beta$ arr-biased activation<sup>32</sup>. Direct binding of purified pilins to this receptor region, consisting of 27 amino-acid residues, was subsequently demonstrated by homogeneous time-resolved FRET. Although only humans are infected by *N. meningitidis*<sup>26</sup>, this region is conserved in mammals and is almost identical between human and monkeys. Moreover, whereas human HEK-293 cells reconstituted with mouse  $\beta_2$ AR could be activated by meningococcus, mouse 3T3 cells and mouse C166 endothelial cells expressing exogenous human  $\beta_2$ AR were not activated by the bacterium. These data indicated that the capacity of *N. meningitidis* to bind and activate the  $\beta_2$ AR in human cells involves human host cell-specific post-translational



modification of the receptor. The  $\beta_2$ AR displays two asparagine-branched glycan chains in its N-terminus<sup>39</sup>. It was then investigated whether these glycans might be involved in receptor activation by meningococcus. Alanine substitution of either Asn residue reduced signaling under meningococcal colonies to control levels. In a gain-of-function strategy, two glycosylation sites, nine residues distant as in the  $\beta_2$ AR, were produced by mutagenesis in the AT1R: this mutant reconstituted meningococcus-promoted signaling *in vitro*, whereas mutants expressing only one of the glycan chains (after substitution with Ala) failed. These data supported a model where both glycan chains of the  $\beta_2$ AR are necessary and sufficient to permit receptor activation by *N. meningitidis*. Using a panel of lectins to inhibit meningococcus-promoted signaling *in vitro* it could be determined that terminally exposed  $\alpha_{2-3}$  branched sialic acids play an important role in the activation of the  $\beta_2$ AR by the pathogen.

This finding contributes to shed some light on the mechanism of meningococcus species selectivity. Two forms of sialic acids are predominant in mammals, Neu5Ac and Neu5Gc, which differ by a single oxygen atom<sup>40</sup>. Only Neu5Ac is produced in humans, contrasting with the predominant presence of Neu5Gc in other mammals<sup>41</sup>. Indeed, Neu5Gc is synthesized from Neu5Ac by the cytidine monophosphate-N-acetylneuraminic acid hydrolase (CMAH)<sup>42</sup>, which is absent in humans, due to a mutation in the *cmah* gene. The observations above raised the hypothesis that species selectivity of *N. meningitidis* for humans might be explained by the requirement of 2 terminally exposed Neu5Ac at a particular distance<sup>38</sup>. Consistently, it was found that deletion of *cmah* in mouse cells restored meningococcal signaling via the  $\beta_2$ AR, while mouse  $\beta_2$ AR receptor was activated by meningococci when expressed in human cells.

From the above data a plausible scenario emerged postulating that traction forces applied by bacterial Tfp bound to sialic acid residues would activate the  $\beta_2$ AR (**Figure 1**). To test this hypothesis, wild type and mutant meningococci deficient in PilT activity (referred to as  $\Delta T$ ) were compared for their ability to induce signaling in endothelial cells (**Figure 2**). Signaling of  $\Delta T$  meningococci was significantly impaired compared to wild type bacteria under basal, static conditions. Next, adherent meningococci were submitted to orbital rotation to apply a centrifugal force on bacteria. Rotation promoted signaling under meningococcus colonies both in wild

type and  $\Delta T$  meningococci, but the effect was superior for wild type colonies, indicating that maximal signaling depends on added effects of PilT-induced pilus retraction and exogenous forces applied to bacteria. Interestingly, sialic acid-selective lectin-coated beads submitted to the same orbital rotation recapitulated the signaling features observed with meningococci. Traction forces specifically applied on the  $\beta_2$ AR via its N-glycan chains can therefore induce  $\beta$ arr-biased activation and downstream ezrin recruitment independently of bacteria. Moreover, whereas meningococcal activation of the  $\beta_2$ AR was strictly dependent on the presence of two glycan chains at a particular distance, probably reflecting structural constraints, such as pilin orientation or spacing within the bacterial pilus,  $\beta_2$ AR mutants lacking either N-glycosylation site maintained their capacity of being activated by lectin-coated beads<sup>38</sup>. Thus a force applied via a single N-glycan chain is potentially sufficient to promote the mechanical activation of a GPCR.

Recent structural and molecular studies have identified the events, which control GPCR function at the molecular level<sup>43</sup>. For small molecule-activated class A GPCRs, the class to which the  $\beta_2$ AR belongs to, the orthosteric binding site used by endogenous agonists to activate the receptor, is situated in the middle of the seven-transmembrane helical bundle, between the extracellular loops and the middle plane of the plasma membrane. Allosteric agonists bind to the receptor in varying positions outside the orthosteric site. For example, a small allosteric modulator of the  $\beta_2$ AR was found to bind to a pocket formed by the cytoplasmic ends of TM 1, 2, 6 and 7, the intracellular loop 1 and the helix 8<sup>44</sup>. An allosteric modulator of the M2 muscarinic receptor, instead, binds above the orthosteric site and establishes molecular interactions with the extracellular vestibule, which includes the second extracellular loop<sup>45</sup>. The exosite for the aryloxyalkyl tail of salmeterol, a selective  $\beta_2$ AR long-acting agonist was investigated in a recent study. The salmeterol tail extends toward the extracellular surface of the receptor, filling a cleft formed by amino-acid residues belonging to extracellular loops 2 and 3 and to the extracellular ends of the TM 6 and 7<sup>46</sup>. These findings, and the observation that the orthosteric site for of peptide-activated GPCRs can involve the N-terminal region and is stabilized by extracellular loops<sup>47</sup>, suggests a mechanism in which the traction applied via meningococcal pili bound to  $\beta_2$ AR glycan chains engages the helical bundle region, through the recruitment of the N-terminal region, which creates interactions with the transmembrane core. This model is supported by the fact that

applying traction forces on the  $\beta_2$ AR, via beads that are coated with lectins specific for the exposed sialic acid of receptor glycan chains, reproduces a pili-like activation

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## **Conclusion and perspectives**

Over the past 20 years an increasing number of observations supported the role of GPCRs in the transduction of mechanical signals. Although in most cases GPCR activation appears as a downstream event caused by the release of receptor agonists in response to the mechanical stimulation, recent studies support that GPCRs may function as direct mechanosensors and that mechanical forces can activate these receptors in different ways. In this context, the previously unreported mechanism of mechanical activation of a GPCR, involving traction forces applied on sialic-acid-terminated glycan chains, raises the question of its physiological relevance. It may suggest that there are supplementary functions for the  $\beta_2$ AR in the vascular system. For instance macrophages, which adhere to and roll over endothelial cells before entering into tissues, express the sialic acid-specific sialoadhesin Siglec-1<sup>48</sup>, which displays the same glycan specificity as Mal-I. Siglec-1 binding to  $\beta_2$ AR might induce some signaling in the context of cell-to-cell interactions and/or diapedesis. More generally, most (80%) GPCRs express at least one N-glycan chain in their N-terminus. These glycan chains might similarly be involved in the interaction with cell surface lectins and allosterically activated in the context of cell-to-cell mechanical signaling. However, it is difficult at the moment to evaluate how many GPCRs could actually be activated via a traction applied on their N-glycans. For example, lectin-coated beads failed to activate the AT1R, indicating that each receptor should be tested individually for its aptitude of being directly activated by mechanical forces. Also, additional studies will be necessary to establish how a traction force exerted on its N-terminus can structurally activate a GPCR.

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**Table 1. GPCRs involved in mechanotransduction**

Mechano-sensory Receptor/Natural ligand	Cell type	Type of mechanical activation	Outputs, mechanisms of activation	References
Adhesion GPCRs (aGPCRs): dCIRL/tether	Sensory neurons (drosophila)	Mechanical vibrations; activation of tethered large extracellular domain (ECL), not necessitating ECL proteolysis (which is typically involved in the activation of aGPCRs)	cAMP decrease in the cell, possibly due to the activation of G $\alpha$ i; downstream alteration of the NOMPC anion channel activity; fine tuning of neuronal response	24
APJ/apelin	Cardiomyocytes Endothelial cells	Stretch Blood flow (shear stress)	Stretch signals in an APJ- and $\beta$ -arrestin dependent, G-protein-independent fashion to induce hypertrophy Hypothesis: upregulation of APJ in vascular cells is generated by the mechanosensitive properties of the receptor itself.	49 50
AT1R/angiotensin II	Cardiomyocytes, HEK293 and COS7 cells Rat aortic A7r5 cells, HEK293 cells Ventricular endothelial cells	Mechanical stretching Hypo-osmotic stretch, application of positive pressures in patch-clamp experiments Hypotonicity, membrane stretch, increased intravascular pressure stretch (left ventricle balloon stretch)	Stretch-induced hypertrophy of cardiac myocytes caused by autocrine release of Ang II Agonist-independent receptor activation (unidentified mechanism), activation of G $_{\alpha/11}$ and Jak2 Stretch-dependent, agonist-independent G $_{\alpha/11}$ activation by receptors upon antagonist-blocked conformational change (H1R>AT1R>M5R>V1AR*); secondary activation of transient receptor potential (TRP)-6 cation channel; increase of myogenic tone of vascular smooth muscle cells Coupling to G $\alpha$ i; G $\alpha$ i-dependent recruitment of $\beta$ -arrestins, EGFR transactivation and ERK phosphorylation	14 18 19 51
$\beta_2$ AR catecholamines	Human brain microvasculature endothelial cells (D3 cell line); transfected cells expressing $\beta_2$ AR and $\beta$ -arrestins	Shear stress (indirect) and traction of bacterial (meningococcus) pili	Traction forces directly applied on the N-terminus of the $\beta_2$ AR via a specific glycan (Neu5Ac). $\beta$ -arrestin-biased activation of Src-cortactin pathways and recruitment of junctional proteins under bacterial colonies causing the opening of the intercellular spaces of endothelial cells.	38
B2R/bradykinin	Bovine aortic endothelial cells	Fluid shear stress, hypo-osmotic stimulation, increase in plasma membrane fluidity	Changes in cell membrane tension and membrane fluidity affect conformational dynamics of GPCRs (agonist independent but blocked by selective antagonists)	20 19
ET1AR/endothelin	HEK293 cells	Stretch	Stretch-dependent, agonist-independent Gq/11 activation (H1R>AT1R>M5R>V1AR*); secondary activation of transient receptor potential (TRP)-6 cation channel R	19
GPR68	MDA-MB-231 Cells (breast cancer cells used for screening); murine primary microvascular endothelial cells. GRPR is naturally expressed in endothelial cells of small diameter arteries and in immune cells	Shear stress imposed by disturbed and laminar flow. Probable role in flow mediated dilation	Direct mechanical stimulation inducing receptor conformational changes or, indirectly downstream a still unknown mechanosensor; G $_{\alpha/11}$ -coupled, PLC activation, Ca $^{++}$ release from ER stores	21
H1R/histamine	HEK293 cells Endothelial cells	Stretch, Shear Stress	Stretch-dependent, agonist-independent Gq/11 activation (H1R>AT1R>M5R>V1AR*); secondary activation of transient receptor potential (TRP)-6 cation channel Traction over helix 8	19 25
M5R/acetylcholine	HEK293 cells	Stretch	Stretch-dependent, agonist-independent Gq/11 activation (H1R>AT1R>M5R>V1AR*); secondary activation of transient receptor potential (TRP)-6 cation channel	19
PTH1R/parathormone	Bone cells, murine preosteoblastic cells (MC3T3-E1)	Fluid shear stress	Changes in conformational equilibrium of the PTH1R, inhibited by the agonist. Mimicked by membrane fluidization using benzyl alcohol or cholesterol extraction	52
Receptor-independent, S1P3-coupled G protein	Human coronary artery endothelial cells (HCAECs)	Shear stress	Association between G $_{\alpha/11}$ and PECAM-1 under basal conditions; dissociation from one another in response to shear stress; direct activation of G $_{\alpha/11}$ , independent of GPCR activation (the possible role of another GPCR in heteromeric complex with S1P3 cannot be excluded)	53
SIP1/sphingosine 1-phosphate	Endothelial cells during angiogenesis	Laminar shear stress	Direct SIP1 ligand-independent activation by shear stress stabilizes blood vessels in development and homeostasis	54
V1R/vasopressin	HEK293 cells	Stretch, Shear Stress	Stretch-dependent, agonist-independent G $_{\alpha/11}$ activation (H1R>AT1R>M5R>V1AR*); secondary activation of transient receptor potential (TRP)-6 cation channel Traction over helix 8	19 25
indirect GPCR (ETRs, TXA2R, PGH2R, S1PR, P2YR,) activation by locally released ligands	Endothelial cells acting on smooth muscle cells (paracrine activation)	Vascular stretch	Myogenic tone is generated by the indirect activation of GPCRs coupled to G $_{\alpha-11}$ or G $_{12-13}$ . Stimulation occurs in response to peptides, lipids, nucleotides, amines released locally by endothelial cells. Activation of actin-myosin interaction	15 16
Indirect activation of adrenomedullin R	Endothelial cells	Shear stress	PIEZO1 mediated fluid shear stress-induced release of adrenomedullin, receptor activation, Gs-dependent cAMP production, eNOS activation	17

## Figure Legends

### Figure 1. Chemical and mechanical activation of the $\beta_2$ AR

The  $\beta_2$ AR is principally known for its chemosensory functions (right). Agonist binding (isoproterenol in the figure) to the receptor orthosteric binding pocket induces conformational changes and coupling with its cognate  $G_s$  heterotrimeric G protein. Subsequent phosphorylation by G protein receptor kinases (GRKs) and  $\beta$ arr recruitment extend receptor signaling to additional pathways (not shown).  $\beta$ arrs are also involved in the uncoupling of the  $\beta_2$ AR with the  $G_s$  protein and in receptor endocytosis. Meningococcal pili (Tfp) mechanically activate the  $\beta_2$ AR (left). Pilus component PilE and PilV bind to  $\beta_2$ AR N-terminal glycans by interacting with terminal Neu5Ac (purple diamonds) branched on galactose residues (yellow circles). Bacteria “pull” the receptor N-terminus and induce a conformational change of the  $\beta_2$ AR, which recruits  $\beta$ arrs without coupling with the  $G_s$  G protein. This mechanical activation can also be reproduced by beads coated with lectins that recognize Neu5Ac if beads are submitted to centrifugal forces.

### Figure 2. Extrinsic and intrinsic forces concur to $\beta_2$ AR activation

Once in the blood, meningococci adhere to endothelial cells via their type-IV pili. Growing colonies at the apical surface of endothelial cells are then submitted to forces exerted by blood flow. In addition, meningococcal PilT ATPase generates traction forces via pili *in vivo* allowing bacteria to crawl over surfaces. To evaluate the respective contribution of intrinsic and extrinsic forces to the mechanical activation of the  $\beta_2$ AR by meningococci, the activation of receptor signaling was compared in wild-type and PilT defective bacteria ( $\Delta T$ ). Human endothelial cells (hCMEC/D3 cell line) were infected with wild type or mutant bacteria and ezrin recruitment under colonies (a downstream signaling event elicited by meningococcus in host cells) was analyzed under static conditions (2h incubation) or under orbital rotation (15min after initial static incubation) aimed at applying a centrifugal force on bacteria. After cell fixation, for each experimental condition the % of colonies, which recruit ezrin was counted under a fluorescent microscope. The amount of ezrin recruitment under each colony was quantified from Apotome acquired images using Image J software (expressed as

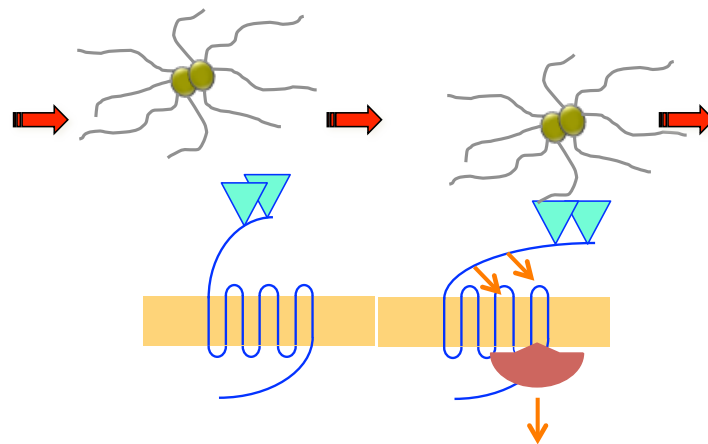
arbitrary fluorescence value per pixel). The histogram values were obtained by multiplying the % values with the median values of ezrin fluorescence.

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## Table of content graphic

### Mechanical GPCR activation by traction forces exerted on receptor N-glycans

Stefano Marullo, Stephane Doly, Kusumika Saha, Hervé Enslin, Mark G.H. Scott, Mathieu Coureuil



The application of a traction force on  $\beta_2$ AR glycan chains by meningococcal pili induces a distinct allosteric mode of receptor activation which recruits  $\beta$ -arrestin (brown) without activating the G protein. Red arrows represent the force generated by blood flow.