INTRODUCTION TO ENDOTHELIAL CELLS MECHANOBIOLOGY

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SUMMARY

Almost all of the cells of the human body are subjected to mechanical stresses. In endothelial cells, mechanical stresses can vary from some milli-Pascal (shear stress) to some Pascal (hydrostatic pressure). Today it is know that mechanical stresses have a decisive part in cellular physiology. However, if the main biological effects of mechanical stress are well documentated, the mechanisms between mechanical forces to physiological phenomenon remain nearly unknown (mechanotransduction phenomenon). In this work, through personal results and published works, the authors considers the effects of mechanical stresses and possible hypothesis.

Key-words: endothelial cells; mechanobiology; mechanotransduction

1. BIOMECHANICS: OLD HISTORY AND DEFINITIONS

The interest of man for the explanation of the movements of the body has always been sharp. Famous names marked out the history of Biomechanics among them, without being exhaustive: Aristote (384-322 before JC) with the movement of the animals, Archimede (287-212 before JC) and Galien who were undoubtedly the first biomechanicians.

Since Léonard de Vinci (1452-1519) published his work on the human body movement and William Harvey (1578-1658) described the microcirculation and capillary circulation and can be considered as the first modern hemorheologists (He writed "*How does the blood manages to cross the porosities of the flesh one his way from arteries to the veins*?).

It has been then necessary to wait until the 17th and 18th centuries for a better comprehension of the physico-hemomechanics of blood circulation with Malpighi (1628-1694) who described the capillaries and red blood cells, Van Leeuvenhoeck who invented the first microscope or Malpighi and Hales (1677-1761) who measured blood pressure.

The first physiological, mechanical and hemorheological approaches date in fact from the 19th century. Jean-Marie Léonard Poiseuille (1799--1869) can be considered as one of the pioneers of modern physiomechanics. Her works allowing an approach of the complexity of the biological fluids and particularly of blood. The term of Biomechanics has been used for the first time in 1887 by Benedikt (Uber mathematische Morphologie und Biomechanick). In the same time works of Wolf on the adaptability of bone Roux on tissue remodeling, Fahraeus on the microcirculation, Burton, Copley, Scott-Blair, Born, Fung, Chien, Skalak helped to a better understanding of the role of the local forces on cell physiology and tissue remodelling.

Finally the progresses of the knowledges in Biology at the end of the 20th century by researches in Genetic and Molecular Biology, together with the development of physical instrumentations (atomic microscopy force, confocal microscopy, optical tweezers...) give a novel interest for the development of a new Biomechanics and its applications (cell and tissue Engineering, tissue and cell therapies...).

Biomechanics is the mechanic applied to life sciences. This definition is both vast and vague; it is also restrictive, because it does not include the physiological or pathological effects generated by the applied forces. Three definitions (more or less complete) can define the fields of biomechanics:

The former suggested by the application of the laws of mechanics aims to find solutions to solve medical, biological, ergonomic or sporting problems.

The second, more mechanical in its spirit, takes into account the study of mechanical properties of cells and tissues with regard to the complexity of the studied structures (ex: properties of the cardiac muscle, the wall of the vessels, the blood microcirculation, the cartilage, the bones...).

The later, more recent integrative, is interested not only in the properties of the objects studied through their structures, but also in their functions and their physiopathological consequences. This approach implements, not only the resolution of fundamental problems and development of models, but also the most recent knowledge of Molecular Biology, Genomics or Cell Biology. Its potential applications in cell and tissue engineering which, can be included under the term of "mechanobiology", seems very promising.

2. MECHANOBIOLOGY – MECHANOTRANSDUCTION

Cells in the body are permanently subjected to different forces (blood pressure, forces related to the movements...). Those vary from Pascal – Pa – (stress shear on the vascular wall) to MegaPa

– MPa – (forced on the cartilage of the hip). Recently however, it was shown that these forces were likely to influence the properties of cells (physiology, syntheses, expression of genes...) as well as biochemical modifications. One of the first observations, left a long time without continuation, was done by Wolff, a German surgeon who studied the adaptability of the bone. At the same time, Roux (1881) proposed the concept of adaptation which defined tissue remodelling.

At the interface between Physics and Biology, cell mechanics knew a conceptual revolution during the 20 last years with the possibility to measure and apply forces of picoNewtons and nanometric deformations. It was then possible to better understand the relation between local mechanical parameters and cell functions (concept of Mechanobiology).

If the biological effects of mechanical forces on cells and tissues are now relatively well described, the mechanisms explaining the passage from a mechanical stimulus to a physiological phenomenon (ex: secretions, receptor expression, gene activation...) remain difficult to understand. It is widely admitted today that these phenomena proceed in 4 steps.

a) *mechanical coupling* which induces transformation of the applied force into a detectable force by the cells or induction of a physical phenomenon (ex: pressure on a bone which induces a circulation of fluid in the canicular system or appearance of an electrokinetic potential of flow).
b) *mechanotransduction* itself which corresponds to the action of forces on specific structures. The transduction of mechanical signals could affect the properties of the cells.
c) *transduction of signal*: conversion of mechanical signals into intracellular physiological signals.
d) *cell response*: regulation of genes, release of autocrine or paracrine factors, expression of specific receptors...

If steps 3 and 4 are almost well described, knowledge of steps 1 and 2 requires development of models and experimental approaches specific to each type of cell studied (ex: distribution of forces on and into the cell, polymerization and orientation of the cytoskeleton...).

3. TRANSDUCTION INDUCED BY THE MECHANICAL FORCES ON VASCULAR ENDOTHELIAL CELLS (EC)

3.1. Mechanical forces acting on the vascular wall

The mechanisms by which endothelial cells identify the mechanical forces (shear stress or pressure) and convert them into physiological and biochemical signals remain generally unknown. Molecules at the cell surface are ideal candidates because they have a direct interaction with circulating blood. These molecules can be directly activated by a physical movement (conformational change) or indirectly by molecular gradients (which change ligand-receptor interactions). These membrane structures or “mechanoreceptor candidates” include integrins, ion channels and G proteins, tyrosine kinase receptors... These mechanoreceptors can trigger biochemical cascades in the cytoplasmic side of the plasma membrane through to second messengers release (centralized mode of the mechanotransduction). Thus activation of the proteins kinases is followed by stimulation of cytosolic transcription factors, and/or regulation of the gene transcription in the nucleus.

Another way to transduce the signal induced by stretch or shear stresses is related to the interaction of mechanoreceptors with cytoskeletal elements themselves activated by flow. By using a “decentralized” mode of mechanotransduction, transmission of signal can be done by connection with the cytoskeleton (focal adhesion, cell-cell junctions, membrane receptors) and leads to a

greater diversity of cell responses. Some of these responses induced by molecules of intracellular signalization (second messengers) are fast, of about a second or a minute (changes of the ion permeability, production of inositol triphosphate, intracellular release of Ca^{2+}, activation of the adenylate cyclase...), while other responses are developed in hours following the signal (genes modification of the cytoskeleton, changes of the cell shape and orientation...).

In vivo the vascular endothelial cell (EC) is subjected to 3 types of mechanical forces whose intensities are variable according to the vascular bed: shear stress (τ, some mPa), hydrostatic pressure (p, some Pa) and periodic parietal deformation (ε, 0 to 3 Hz).

The shear stress (τ) induced by the blood flow acts tangentially on the EC. It is generally determined by the relation of Poiseuille: $\tau = 2Q\,\mu / \pi D$, where Q is the flow rate μ the dynamic viscosity and D the diameter of the vessel.

The hydrostatic pressure acts perpendicular to the surface of the endothelium. It operates the extracellular matrix as well as on the endothelium. At the macroscopic scale, the blood flow in the arteries is generally laminar. However, the geometry of vessels near vascular singularities (junctions, stenosis...) predispose with separation of the flow and appearance of swirls. In these areas, the shear stress and the pressure present great fluctuations in amplitude and direction on short distances. At the microscopic scale, it should also be noted that the distributions of the shear stress and of the pressure on the surface of each EC are not uniform and are dependent on the topography of cell surface (Waché and Al, 2000).

In addition to the shear stress and the pressure, the arterial endothelium is submitted to a periodic deformation of the wall related to cardiac pulsations. The deformation (□) wide is classically measured is the average circumferential deformation, i.e. the relative variation of diameter: $\square = (D\text{-}D_0 / D_0$ with $D\text{-}D_0$ diameter between systole and diastole).

3.2. Effects of the shear stress on the EC: mechanotransduction

In 1968, Fry described modifications of the vascular endothelium in relation to the wall shear stress. In 1981, Dewey and al. showed the dynamic response of EC with regard to shear stress and Nerem and al. suggested that the morphology of the EC could be an indicator of the local hemodynamic conditions. During the last ten years, the influence of the local hemodynamic conditions on the EC, raised and increasing interest. The most recent works underlined the determining role of local flow conditions in their properties (Reinhart 1994, Davies and al. 1997 a), Ballermann and al. 1998). These modifications are described under the term of mechanotransduction. It is admitted that endothelium is the main actor in the processes of signal transduction that control vasomotion and various functions of vascular wall (Davies and al. 1997 b).

3.3. Distribution of mechanicals forces

The variations of the distribution of shear stresses can help to explain the various responses of endothelial cells to flows. Full-course of the endothelial cells being “rough”, the sensitivity of a cell can be considered by the fraction of surface exposed to a force above a critical value.

In addition to the variations of the distribution of forces between cells, the distribution of subcellular forces is variable in space. In this case, the localization of mechano-receptors or sensitive elements on cell surface can be a key factor. One can imagine that the sensitivity of a cell is determined by its form as well as by the localization and activation of mechano- elements on cell surface. Moreover, if we agree with the hypothesis of the presence of mecano-receptors on the membrane surface, their distributions are also a determining factor. A membrane receptor would be sensitive to the flow depending to the level of the forces in the areas. Thus, a particular flow would

not cause a cell response if its receptors are localized in a region where the force is lower than a defined threshold (Satcher and al. 1992, Barbee and al. 1995, Davies and al.. 1997-a and b).

3.4. Responses of EC to shear stress

a) Morphology of cells EC

The morphological changes corresponding to the lengthening and the orientation of EC parallel to the direction of the flow and the reorganization of the cytoskeleton are responsible for motility and cell adhesion (Dewey and al. 1981, Nerem and al. 1981, Ookawa and al.. 1993, Thoumine and al. 1995, Cucina and al. 1995). With low shear stress, in vivo and in vitro as well, the EC shows a polygonal form and are lengthened in comparison with the EC subjected to a high shear stress (Franke and al. 1984, Walpola and al. 1993, Drenckhahn and Ness 1997). In addition, the morphological changes of ECs are variable according to their localization (connections, junctions) and the conditions of flow (laminar periodic or disturbed flows, presence of vortex, etc.) (Davies and al. 1986, Helminger and al. 1991, Sato and Ohshima 1994, Truskey and al. 1995, Chiu and al. 1998).

All the elements of the cytoskeleton (actin filaments, microtubules, intermediate filaments...) undergo a reorganization during the application of shear stress, influenced by the amplitude and the period (Sato and Ohshima 1994, Girard and Nerem 1995, Galbraith and al. 1998).

At rest, EC show dense peripheral bands (DPB) with the stress fibbers ending in DPB areas. Proteins related to the cytoskeleton, such as the vinculins, Zyxins, VASP (vasodilatator stimulated phosphoprotein) etc. are associated at the ends of actin filaments to the periphery. Following a prolonged shearing, the EC are oriented in the direction of the flow. In cell, the stress fibbers and microtubules, as well as the intermediate beam alignment of filaments are aligned according to direction of the flow. This reorganization of the cytoskeleton will have a direct action on various cell signalling, in particular on protein phosphorilisation (such as the VASP) known to be the substrates of the proteins kinases A (PKA), or enzymes implicated into mechanotransduction. It will also have an effect on the properties of the endothelial cells, such as adhesion, release of Von Willebrand factor,...

b) Phosphorylation of the Vasodilator-stimulated Phosphoprotein (VASP)

The VASP are closely related to the reorganization of the cytoskeleton in the development and the repair of the endothelium, this phenomenon is important in the comprehension of vascular diseases. Phosphorilation of the VASP, triggered by a mechanical force, results in binding of VASP to the adhesion focal points of molecules of the cytoskeleton, such as vinculin or actin. Study of the phosphorilation of proteins in cells subjected to shear stresses of various durations and amplitudes as well as the analysis of the interactions between VASP and actin fibbers allowed us to demonstrate the existence of various phenomena and the presence of interactions between cytoskeleton and VASP.

c) Localization of adhesion molecules

We observed, also that EC activation, involves an increased expression of adhesion molecules such as ICAM-1, (ligant of leucocyte integrin) which support firm adhesion. In addition, during the application of a shear stress, ICAM-1 migrated towards the apical pole of the EC via the cytoskeleton to create an open area accessible to circulating adhesive cells (leucocytes...).

d) Regulation of Von Willebrand factor (VWF)

The von Willebrand factor is a multimeric glycoprotein (GP) wich is involved in of endothe-

lial lesion events, in platelet adhesion... It is also the transporter for the procoagulant factor VIII. It is synthesized in the Weibel-Palade bodies and is considered as a good marker of EC. It also plays a significant role in the atherosclerosis process which is itself in direct relationship to the mechanical forces of shearing on the wall. We showed an increase of vWF synthesis in cells exposed to a strong shear stress (1,0Pa) during 24h, but not in cells exposed to a low force (0,2Pa) showing that the local hemodynamic conditions (variation of the shear stress in vascular stenosis, etc) play a determining role in thrombosis and the atherogenesis via regulation of the release and the synthesis of vWF whereas the TNF-α induced a release of vWF simply.

e) Translocation of Caveolin-1

The caveolae are constituted by membrane microdomains implied in the mechanisms of mechanotransduction, due to their localization at the level of the ion channels and their properties of transport of macromolecules. Caveolin-1, constitutive protein of these microdomains, play a role as element of activation of the transduction molecules. Variations in their expressions were studied following to various mechanical and biological stimuli (TNF-α). We thus found a significant modification in the distribution and expression of caveolin-1 in EC exposed to a laminar flow and change in the spatial distribution according to time. More precisely, the caveolin-1 concentration was higher in the areas of high shear stress. Moreover, caveoline-1 expression increased following 24h shearing. On the other hand, TNF-α induced a reduction in the expression of caveolin-1 following 24h stimulation and inhibition of F-actin polymerisation blocked the redistribution of caveolin-1. These results show that the shear stress induces a translocation of caveolin-1 and that there is a correlation between this redistribution of caveolin-1 and the organization of F-actin in the EC.

It is possible that the molecules responsible for the transduction of mechanical signal are activated during their separation with caveolin-1. Consequently, changes of conformation or localization of caveolin-1 by shear stress could play a significant role in mechanotransduction.

f) Intracellular Responses

Since about years, the response of EC to mechanical forces was largely studied and the variations of a high number of cell functions were reported (electrophysiology, biochemistry, receptors, regulation of gene, etc) (Reinhart 1994, Davies and al. 1997-a). The effects of brutal or chronic shearing were studied *in vitro* (Ballermann and al. 1998, Braddock and al. 1998). The various responses of EC to mechanical forces can be classified according to the reaction time, although they are often simultaneous (Table I). For example, the fast electrophysiological changes in the membrane potential (of about a second) and the activation of the biochemical cascades occur in a similar characteristic time. G protein activation, release of NO, mobilization of derived the phosphoinositides, release of intracellular CA^{2+}, phosphorylation of cyclic nucleotides, etc, require longer times.

g) Gene regulation

Regulation of gene expression of molecules synthesized by the EC, like ET-1 (Morita and al. 1994), PAF (Diamond and col. 1990), PDGF A and B (Hsieh and al. 1991), MCP-1 (Shyy and al. 1994), adhesion molecules (ICAM-1, VCAM-1) (Nagel and al. 1994, Sampath and al. 1995, Ando and al. 1994), is influenced by the flow. Thus, expression of PDGF-B and FGF are increased in the EC and in the muscle cells smooth (CML) vascular when subjected to forces of shearing. Resnik and al. showed that the expression of PDGF-B in EC is dependent on a sequence of 12 nucleotides (SSRE) in the

Table 1 – Main responses of the EC to shear stresses

Time	Responses	Physiological significance	Authors
seconds	Activation of the channels potassic	– Selective opening of the channels K^+ – The entrée of calcium supports ??	Olesen and al.
	Activation of the seconds secondary messengers (IP_3, DAG, $CA^{2+,}$ PKC, protein-G...)	– activation of the transduction of signal (e.g. activation of the way Ca^{2+}- dependent)	Prasad and al. Bhagyalakshmi and al. Shem and al. Helmlinger and al. Hsieh and al. Berthiaume and Frangos Kuchan and al.
	Release of NO	– Vasorelaxtion flow – dependent	Kuchan and Frangos Gooch and Tennant
minutes	Release of PG	– Vasodilatation and anti-thrombosis	Frangos and al.
	Activation of MAP Kinase	– Transduction of the signal	Tseng and al.
	Activation of NFκ b Regulation of SSRE-dependent PDGF-B	– Activation of the transcription – Régulation cell multiplication	Mohan and al. Hsieh and al.
>1 h	SSRE-dependent Regulation (PDGF, OUR, tPA, TGFβ1, ICAM-1, c-fos, MCP-1 etc)	– Regulation of the growth cell – Vasorelaxation flow – dependent – Increase in the activity fibrinolytic – Cell adhesion – Transduction of thesignal – Recruitment of monocytes	Hsieh and al. Xiao and al. Diamond and al. Ohno and al. Nagel and al. Sampath and al. Hsieh and al. Shyy and al. Stoltz et al
>6 h	ET-1: increase (weak shearing); reduction (>0.6Pa)	– Vasoconstriction	Kuchan and Frangos
	VCAM-1: reduction	– Celle adhesion	Ando and al.
	Rearrangement of Cytoskeleton Alignment of the sites of focal adhesion Increase in the connexine 43 Rearrangement of organelles cell (MTOC, Golgi, etc).	– Mechanisme in the variations of morphology	Galbraith and al. Davies and al. Davies
	Cell prolifération Increase: turbulent disturbed No change or reduction: laminar	– Prolifération of CE	Davies and al. Gooch and Tennant
	Change of morphology (>12h) Rearrangement of Fn	– Adaptation to flow	Davies Barbee and al. Wechezak and al.
	Negative regulation of Tm	– Anti-thrombosis	Malek and al.
>24 h	Increase in the rigidity of cell surface	– Reduction in deformability cell surface	Sato and al.
	Alignment et elongation of the CE.	– Morphology adapted to flow	Girard and Nerem Galbraith and al.

promoter region of PDGF-B gene while is distinct from sequences of interaction with well know factors transcription. This same team showed that in another gene, coding for ICAM-1 which is involved in inflammation, increased when EC were subjected to forces of shearing: Whereas expression of VCAM-1 and ELAM-1, whose promoter region are deprived from SSRE sequence, was not affected under the same conditions. These observations suggest that the SSRE sequence was not affected under the same conditions. These observations suggest that the SSRE could be present in the EC and activated by mechanisms of mechano-transduction. The other transcription factors which take into the activation of promoters by shear stress are the nuclear factor kappa B (NF-KB), the activating protein-1 (AP-1), the early-1 growth promoter (Egr-1) c-fos, c-jun, c-myc and of stable (Sp-1) (Resnick and Gimbrone 1995, Gimbrone and al. 1997). The variations observed in gene regulation suggest that two types of gene elements sensitive to shear stresses (positive and negative) may exist end moreover, of the multiple regulations in company of the other factors transcriptional.

The response of gene expression of the EC to flow forces can be classified in tree types: early transitory increase, continuous increase expression of m RNA and biphasic regulation: Table II summarizes the different levels of regulation of molecule or gene transcription (level of ARNm) of molecules by the shear stress.

4. POTENTIAL MECHANORECEPTORS

The response of EC to mechanical stimuli relates to practically all the mechanisms dependent on growth, cell metabolism and their functionality. However, the mechanisms remain hypothetical: how and by which “sensors” on the EC re-

Table II – Regulation of the transcription (level of ARNm) of genes by a shear stress (according to Braddock and al. 1998 and Stoltz and al. 1999).

Genes	Cells used	Response of the ARN	SSRE	Other factors
ET-1	HUVEC/BAEC	Biphasic	–	AP-1
VCAM-1	HUVEC	decrease (τ strong) increase (τ low)	–	AP-1, NF-κB
ACE	RAEC	decrease	+	SSRE,AP-1,Egr-1
TF	BAEC	increase	–	Sp-1
TF	HAEC/HUVEC	increase	–	Egr-1
Tm	HUVEC	Biphasic	–	AP-1
PDGF-A	BAEC	Biphasic	+	Egr-1
PDGF-B	BAEC	Biphasic		
ICAM-1	HUVEC	increase (or biphasique)	+	AP-1,NF-κB
TGF-β	BAEC	increase	+	AP-1,NF-κB
c-fos, c-jun	HUVEC	increase precociously transitorily	+	AP-1
ENOS	HUVEC	increase	+	AP-1,NF-κB
MCP-1	HUVEC	Biphasic	+	AP-1,NF-κB

ACE – Angiotensin-converting enzyme; TF – tissue Factor; c-fos and c-jun – members of proto-oncogen family; eNOS – endothelial Nitric Oxide Synthase; AP-1 – Activator Protein-1; NF-κB – Nuclear Factor-κB; Egr-1 – Early Growth Response Factor-1; SSRE – Shear Stress Response Element.

ceive these mechanical stimuli and convert them into biochemical signals?

The molecules present at the luminal cell surface are at first sight the ideal candidates because they are in direct contact with circulating blood. These molecules can be activated directly by a physical displacement (conformational change) or indirectly by transfer of gradients (which change the interactions ligant-receptor). These membrane structures or mechanoreceptors include ion channels, integrins, G proteins related to the receptors and receptors tyrosine kinase, caveola...

These mechanoreceptors can induce cascades of response from the plasma membrane, through release of biochemical second messengers (centralized mode of mechano-transduction). Thus activation of the proteins kinases is followed by stimulation of factors of cytosolic transcription, and/or regulation of gene transcription in the nucleus (Patrick and McIntire 1995).

Another way of signal transduction can be related to the interaction of mechanoreceptors, activated by flow, with elements of the cytoskeleton. By using a "decentralized" mode of mechanotransduction, the transmission of the signal would be induced then via connection with the cytoskeleton (sites of focal adhesion, junctions cell-cell, nuclear membrane) which would lead to the great diversity of cell responses.

Some of these responses are fast, of about a second or of a minute. Other responses develop in the hours following the birth of the signal (cf Table I).

4.1. Integrins

The integrins are responsible for cell adhesion and migration on the extracellular matrix. Via their interactions with other molecules, they initiate the modulation of cytoskeleton organization. They are largely implied in the regulation of the embryonic development, apoptosis, hemostasis, recruitment and activation of the leucocytes and retraction of the blood clot. At the time of connection to integrins, the ligands bind firmly or gather the integrins while binding to the adjacent molecules on the cell surface. The connection or the assembly of integrins leads to the formation of focal adhesion where the integrins bind to intracellular cytoskeletal complexes and indirectly with the actin filaments. The displacement of a transmembrane integrin could communicate the force with the cytoskeleton through protein/protein interactions in the cytoplasmic side of membrane. For example β1 integrin receptor of extracellular matrices can induce the formation of a focal adhesion and induce a force-dependent signal. The rigidity of the cytoskeleton would increase with the applied pressure, thus would require intact microtubules as well as intermediate filaments and microfilaments.

Following this connection closes integrins with their ligands, they "integrate" the signals external given by the othercells or the components of the extracellular matrix to which they adherent, signals which they transmit inside the cell while joining the cytoskeleton and by starting signals of transduction. These signals lead to the hydrolysis of phospho-inositols and thus to the increase in intracellular Ca^{2+}, with phosphorylation of several proteins, in particular activation of tyrosine kinase of focal adhesion ($pp125^{FAK}$ and to the induction of various genes.

The application of a shear stress causes the fast activation of protein kinases, among which ERK (extracellular signal-regulated kinase) and JNK (c-Jun-terminal kinase) (Li and al. 1996). Whide lead to the transcriptional activation of early genes such as those coding for MCP-1 (monocyte chemotactic protein-1) and c-fos (Shyy and al. 1994, Jalali and al. 1998). These activations are modulated by the Ras protein, whide is itself controlled by S.O.S. protein (its of sevenless). However, in response to many growth factors such as the PDGF (platelet derived growth Factor) or the EGF

(Epidermal growth Factof), the protein adaptor Shc (Src homology2/alpha collagen) is phosphorylated at the level of its tyrosines and interact with phosphor-tyrosines of receptor Tyrosine Kinase through binding to SH2 (Src homology domain-2). Following phosphorylation, it can also interact with G2b2 (Growth Factor receptor--binding protein-2) through SH2 binding. Shc--Grb2-S.O.S. provides then an alternative way of signalization in addition to Grb2-S.O.S. transduction used by the Ras protein.

Recently it has also been shown that the Shc protein is implied in the transduction signals.

4.2. Ion channels

The lipid bilayer of cell membranes has a great permeability to polar molecules of small sizes and hydrophobic molecules while it is highly impermeable to ions and charged molecules. Specialized membrane proteins (channels and transporters) are responsible for the specific transfer of ions through the membrane. The ion channels and the ion exchangers are thus potential mechanoreceptors (Davies 1995). The ion channels K^+ modified by the stretching of the membrane would have their activity modified in response to a mechanical force. Thus, Olesen and al. identified a current K^+ selective by activated by the shear stress. This polarizing membrane current is a function of the shear stress, reaching half of its maximum of activation with 0,7 mPa.s. It is quickly activated by the shear stress (a few seconds), slowly increases (in a few minutes), and completely returns to the normal when the flow is stopped. Nevertheless, it is not certain that the activation of these channels is a primary response to the shear stress.

By the same way, many channels are used by calcium which can activate many ways of signalization, among which one particularly stimulates the production of NO and consequently vasodilatation of vessels (Himmel and al. 1993). It was proposed that the mechanisms depending on intracellular calcium concentration can play a significant role in the early and transitory responses, whereas the mechanisms independent of $[Ca^{2+}]I$ changes would be significant in the late and prolonged responses.

The calcium-dependent way is likely to be responsible for the fast response and the transitory flow, such as fast activation of NOS. In addition, intracellular calcium plays a crucial role in the reoganization of the cytoskeleton and the alignment of the EC subject to a flow (Malek and al. 1996). On the other hand, the calcium--independent way induces activation of GTPases binding to GTP and stimulation of PKC and calcium-independent MAP kinase.

4.3. Receptors link with the G proteins

Stimulation of many membrane receptors is retransmitted by a class of specific proteins, that blind to GTP (Guanosine Tri-Phosphate), those are G proteins. They operate the coupling of the receptors with the intracellular effector, and for this reason exert a significant control on the transmission of the signal. The interactions between the receptors and their second messengers are mediated by enzymes or ion channels activated by the interaction with G proteins. G proteins are heterotrimeric proteins, constituted by a sub-unit α bound with the heterodimer βγ. The α sub-unit binds to GTP, hydrolysis in GDP (Guanosine Di-Phosphate) then induce the response of the majority of the effectors. A receptor coupled to a G protein at rest is activated by the binding of a specific agonist. The change in the conformation of the agonist--receptor complex, induced by this interaction, allows the activation of the exchange of GDP by GTP and thus activation of the G α and G β/γ sub--units which will control the membrane or cytosolic activity of various effectors. The release of the phosphatase activity, wihin the G α sub-unit induces the reassociation of the G α and G β/γ sub-units and leads to a return to the initial state.

The amplification of the signal triggered by an extracellular modulator follows two steps. The former is related to the activated receptor which can activates many G proteins in cascade; in the later, the α-GTP sub-unit maintains the amplified activation as long as the GTP is not hydrolyzed in GDP. Recently, it was been shown (Gudi et al. 1996) that activation of G protein is one of the earliest events in the signalization induced by flow. Following this work, a new study highlighted selective changes specific to the nature of the G protein stimulated, in correlated with changes in the signalization and the functionality of G protein (Redmond and al. 1998). Thus, receptors related to the G proteins can be also considered as potent-transducers and, downstream, as signal devices.

4.4. Receptors of tyrosine-kinase (RTK)

In fact transmembrane proteins, following stimulation, induce signalisation events. Their common characteristics is the presence of a single transmembrane segment and an intracellular field having catalytic activity of the protein tyrosine--kinase type. The interaction of ligands involves the dimerization of receptors which allows the activation of kinase carried by each of the two chains and the assembly to the adjacent sequence target also carried by the two chains. This allows intermolecular cross phosphorylation at several tyrosine residues. The phosphorylated dimmer represents the activity receptor. It contains a whole series of phosphorylated tyrosine residues which have the capacity to bind to proteins and form signalization complexes. Moreover, the dimerized and phosphorylated receptor has the potential to phosphorylate its targets. The analysis of the sequences which bind to the phosphorylated receptors showed that the majority of them, but not all, contain SH2 domains. Of other contain PTB (PhosphoTyrosinee Binding Protein) domains. Thus, assembly of the complexes of signalization depends on recruitment by phosphorylated tyrosines of the protein receptors having of a fields SH2 or PTB. Among many proteins containing of SH2 domains binding the RTK domains to form complexes. Some of these proteins themselves are phosphorylated at the end of this association.

4.5. "Mechano-sensors" and Interactions

In order to identify new interactions between various mechano-sensors, we particularly analyzed two different steps of the of mechano--transduction phenomenon: the early response, the interactions integrins-shc and a step downstream, relative to the JNK activity (Labrador and al., 2002)

With the help of specific inhibitors of the site of integrins interactions (RGD), intracellular calcium (BAPTA/AM) and G_i proteins (PTX), we confirmed the existence of interactions within the EC to allow the adaptation of the cell to mechanical forces. Indeed, the exposure of the EC to a laminar flow constant armature resulted in the fast formation (in half an hour) of new intracellular complexes between the integrins αv β3 and the protein Shc. This response uses the same process as that of the biochemical signal i.e. through interactions of integrins to their ligand. Moreover, the information of these complexes in response to flow is partly dependent on the intracellular concentration of calcium but also on the activity of G_i proteins. This step is in addition essential to the later response of the EC to shear stresses such as activation of JNK, via the integrins interaction, the intracellular presence of calcium and the catalytic activity of the G_i proteins.

Mechanical forces would activate at a first step mechano-sensitive sensors. This initial steo would be followed by with the intervention of second messengers: Intracellular Ca^{2+}, protein kinase C (PKC), G protein, Adenosine monophosphate (AMP) cyclic and guanosine monophosphate

(GMP) cyclic, etc. These changes in the balance of the second messengers then involve a change of the state of activation of "DNA binding factors" (Hsieh and al. 1992, Morita and al. 1994, Kuchan and Frangos 1994).

The final step results then in a damaging of the activity of transcription of many genes via brief replies to local shear stresses such as Shear Stress Response Element (SSRE) with positive or negative effects, or a combination of both, acting with various steps.

5. CONCLUSION

It is known today that mechanical forces induce many key events in the physiopathology of the vascular endothelium. To illustrate this phenomenon, Papadaki and Eskin proposed, in 1997, a first diagram summarizing the ways of signalization to "multiple responses" activated by shear stresses within the EC. The activation of one or several mechanoreceptors induced biochemical events which lead intracellular changes in the metabolic and gene expression of the cell, controlling thus the function. This concept, comparatively to the identification of mechanoreceptors and the responses of other cells (chondrocyte, osteoblasts) to mechanical forces should make it possible to a best understanding of mechanoregulation of cells.

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