The Role of Shear Stress on ET-1, KLF2, and NOS-3 Expression in the Developing Cardiovascular System of Chicken Embryos in a Venous Ligation Model

In this review, the role of wall shear stress in the chicken embryonic heart is analyzed to determine its effect on cardiac development through regulating gene expression. Therefore, background information is provided for fluid dynamics, normal chicken and human heart development, cardiac malformations, cardiac and vitelline blood flow, and a chicken model to induce cardiovascular anomalies. A set of endothelial shear stress-responsive genes coding for endothelin-1 (ET-1), lung Krüppel-like factor (LKL/KLF2), and endothelial nitric oxide synthase (eNOS/NOS-3) are active in development and are specifically addressed.

Fluid Dynamics

Blood flow is an important epigenetic factor in heart development (55, 56, 60). It generates a frictional force on the vessel wall, shear stress, which is parallel to the direction of the blood flow. Endothelial cells comprise the inner layer of the cardiovascular system and are, therefore, the first cells that are exposed to shear stress. Although pressure is also an important hemodynamic trigger for biological responses, this will not be discussed here. Volumetric fluid flow rate is one of the parameters determining shear stress, as can be demonstrated with a modification of the Hagen-Poiseuille law:

\[ \tau = \frac{4\mu Q}{\pi R^4} \]

In this formula, \( \tau \) is the shear stress, \( \mu \) is the viscosity of the fluid, \( Q \) is the volumetric flow rate, and \( R \) is the lumen radius. Although the Hagen-Poiseuille law concerns flow through a cylindrical tube with constant circular cross section, meaning stiff walls, it can be used for incompressible Newtonian fluids (fluids with a constant viscosity at all shear rates) at laminar flow conditions and at a constant temperature and pressure. The viscosity is dependent on the chemical composition of the fluid if the fluid is not a pure substance. Adult blood is a non-Newtonian fluid since viscosity varies at different shear rates, since blood encompasses blood cells and proteins. Non-Newtonian blood viscosity is characterized by the fact that when the shear rate decreases the resultant viscosity increases (reviewed in Ref. 67). The Fähræus-Lindqvist effect (plasma skimming) (39) describes the decrease in apparent viscosity that occurs when a suspension, such as blood, flows through a tube of small diameter (<300 \( \mu \)m). Due to the particulate nature of red blood cells, an almost cell-free layer of plasma with reduced viscosity is present near the vessel wall. Blood plasma is a Newtonian fluid. Consequently, the Hagen-Poiseuille law is applicable for approximations of the wall shear stress levels in small vessels of the adult and embryonic cardiovascular system.

A measure for the characteristics of flow is the Reynolds number (Re). It represents the ratio of inertia forces to viscous forces. Inertia is the tendency of matter to resist changes in motion (speed and direction). If the Reynolds number is high (Re >> 1), inertia forces are dominant, and viscous forces are negligible. When Re is about unity (Re ~1), the inertia forces equal viscous forces. Flow with a Re above approximately 2,100 might become turbulent. Laminar flow exists when Re is below approximately 2,100, but in these cases inertia still exists, and laminar vortices may occur. In the embryonic situation, Re is low (<1), indicating that it is a very viscous flow, where inertia forces become small and blood flow stops when propulsion (heart beat and windkessel effect) terminates (108). Due to the low Re, embryonic blood will have its maximum velocity shifted to the inner curvature in a vessel (142). In adults, Re is higher, blood velocity will peak closer to the outer curvature of a vessel, and laminar vortices or even turbulence can occur, e.g., behind valves and bifurcations, resulting in areas with shear gradients with low mean shear as well as flow reversal and the subsequent formation of atherosclerotic plaques (96, 131, 132). In contrast to the adult situation, pulsations due to cardiac rhythm in the small embryonic vasculature do not influence the distribution patterns of flow and shear stress, since the Womersley number (70, 148) is usually well below unity, e.g., \( Wo(\alpha) = 0.31 \) in the heart of a 2-day-old chicken embryo. In the Hagen-Poiseuille law, the volumetric blood flow stands in the numerator, indicating that increased blood flow causes increased shear stress. The lumen radius is in the denominator, which signifies that the smaller the
lumen the higher the shear stress. The heart has a complex geometry with various diameters, and during development it changes constantly (see below), implying that flow and shear patterns are also complex.

**Cardiac Development**

In humans, development of the heart starts from the third week of gestation and is completed at the beginning of the eighth week. After completion of morphogenesis, the heart will still expand considerably. In comparison, in the chicken embryo, it starts at embryonic day 2, or Hamburger and Hamilton (HH) (49) stage 8, and is finished at day 13 (HH39), whereas the chicken hatches after 21 days (HH46). Cardiac development is a complex process. First, the heart is an almost straight tube that is formed by the fusion of two cardiogenic plates (24) and consists of an outer myocardial layer and inner endothelial layer, which are separated by the cardiac jelly, an extracellular matrix (88). Then, it loops into the characteristic C shape because of the rightward rotation of its cranial portion; the subsequent twist into the S shape is driven by a leftward rotation of its caudal portion (34, 85, 86). This first stage of looping is regulated by a cascade of genes essential in left/right signaling in embryos (105). This keeps the venous and arterial poles in relatively close proximity and occurs in the human within the first 23 days after fertilization and in the chicken from approximately HH10 (day 2) to HH16 (day 3) (73). In the second phase of looping, the outflow tract (OFT) is brought in front of the atria, which is eventually followed by the wedging of the aorta in between the atrioventricular (AV) orifices, which requires, e.g., the remodeling of the inner curvature (36). Between 22 and 28 days after conception (human; comparable to HH11–HH15 in the chicken), the heart is a single-chambered pump. At the end of this time window, the endocardial cushions develop, with which the separation of the heart into the four chambers (35, 36) begins. While the endocardial cushions develop into the AV and semilunar valves, the atrial (HH19) and ventricular (HH20) septa begin to form. The ventricular septum is completed at HH34 (57) (day 46 in human). The atrial septum is completely developed at approximately HH33-HH34 (day 45 in human), except for the foramen ovale, which closes after birth together with the ductus arteriosus and ductus venosus. The septation of the common OFT into the aorta and the pulmonary trunk (35, 36) is finished around HH33-HH35 (day 51). The pharyngeal arch artery system develops symmetrically at first with the paired first, second, and third pharyngeal arch arteries. However, differences exist in the diameter of vessels at the left and right side. At approximately HH17 in the chicken embryo, the first pair disappears and the fourth pair develops, of which the left will regress around HH28, resulting in an asymmetrical system. Around HH21, the second arch arteries disappear and the sixth develop. Finally, the arch arteries will encompass the two brachiocephalic arteries (left and right third), an aortic arch (right fourth), and the pulmonary arteries and ductus arteriosus (left and right sixth) (5, 69).

**Blood Flow in the Chicken Embryo**

At HH8 of chicken development (day 22 in humans), the heart begins to contract (30), which is just before looping starts (HH10/11). However, effective laminar blood flow starts at HH12 (day 24 in humans). Before HH12, blood flow is irregular with backflow and mixing (54). Although the heart pulsates and geometrical irregularities exist, such as the trabeculations, the endocardial cushions, and the developing septa, embryonic blood flow in the heart remains laminar because of the low Re. Hogers et al. (54) have described the changes in patterning of the vitelline vessels draining the yolk sac between stages HH10 and HH17. All the changes in the vitelline veins result in alterations in the intracardiac route, with a common principle: blood from specific yolk sac regions follows a specific stage-dependent intracardiac route (54). This indicates that the development of the vitelline vessels is related to the intracardiac flow patterns. Furthermore, the alterations in the intracardiac flow pattern during development are important for normal cardiogenesis. Although the first looping of the heart occurs independently from blood flow (84), the importance of blood flow in cardiac development has been demonstrated (55, 60). Recent high-speed optical techniques involving micro-particle velocimetry (138) allow study of the composite flow patterns in the developing vasculature in detail, defining also the near-the-wall aspects of flow and ensuing shear stress. This demonstrated that, in the OFT of HH15 chicken embryos, the maximum flow velocity is approximately 26 mm/s, which corresponds to a wall shear stress of 50 dyne/cm² or 5 Pa. These values fall well within the range that has been described in literature (13, 61) and are comparable with human arterial shear stress levels, which can be up to 7 Pa (81).

**Cardiac Malformations**

Because of the tight regulation and intricacy of heart development, looping and septation processes can easily be delayed, resulting in cardiac defects. Congenital heart disease (CHD) represents 10% of all congenital malformations and is, therefore, the most common major congenital defect in live births. It occurs in approximately 1 in 100 live births (100). Most common is the ventricular septum defect (VSD), which is the result of incomplete fusion of the endocardial cushions with the muscular septum and conus ridges (36) at approximately 7 wk of gestation (HH34 in chicken). The defect may be present in the muscu-
lar or in the membranous portion of the septum (36), and can be isolated, or in a component of a complex cardiac defect. Another endocardial cushion anomaly is the atrial septum defect (ASD) (35). This is frequently associated with other cardiac and extracardiac abnormalities. A few other examples of CHD are transposition of the great arteries, coarctation of the aorta, interruption of the aortic arch (type B), cardiac valve anomalies, hypoplastic left heart syndrome, and tetralogy of Fallot. The etiology of congenital heart defects may be genetic, e.g., in relation with Down, DiGeorge, Turner XO, or Marfan syndromes (2, 3, 18, 79). Environmental factors can also play a role in the etiology, such as hormones (8, 65), teratogens (9, 12), and, as recently shown in a chicken model, altered blood flow (56). Nevertheless, it is often multifactorial. The heart is functional early in development, and the cardiovascular system is, therefore, exquisitely sensitive to the intra- and extra-embryonic environment, and virtually any disturbance in the environment (including the uterus) has cardiovascular consequences (69).

**Venous Clip Model**

Since the intracardiac blood flow pattern is important for normal heart development (54), a model was generated in which the effects of altered blood flow patterns during development can be studied. This model is called the venous clip model (55), in which the right lateral vitelline vein of a chicken embryo is ligated. Several other intervention models have been developed, such as right atrial clipping, conotruncal banding (CTB), left atrial ligation (LAL), and right vitelline artery ligation (VAL). Right atrial clipping increases the ventricular preload and leads to an increase in left ventricular and left atrial myocyte proliferation (21). With CTB, the embryonic ventricular afterload is increased, which results in ventricular hyperplasia, increased ventricular mass, increased contractile force, altered transmural myofiber angle distribution, and an increase in Cx40-positive Purkinje fibers (16, 48, 95, 116, 129, 130). LAL reduces filling of the left ventricle (preload) and results in a left ventricular hypoplasia, reduced ventricular pressure, altered ventricular growth and myofiber angle distribution, increased ventricular stiffness, and reduced stroke volume and cardiac output (80, 115, 116, 129, 130). VAL acutely increases arterial resistance, but embryos maintained arterial pressure by decreasing the stroke volume and cardiac output (80). These intervention models do not have changes in shear stress as the primary effector, but alterations in pressure are the main cause of effects. With ligation of the right lateral vitelline vein in chicken embryos, blood from the right yolk sac region reroutes immediately via a new vein through the caudal plexus to the left side, which prevents the embryo from becoming deprived of blood and oxygen (56). The preload might be decreased transiently, but most importantly the ligation results in altered blood flow patterns through the heart (55) and eventually to a range of cardiovascular malformations, such as VSDs, semilunar valve anomalies and pharyngeal arch artery malformations (56). Besides these morphological anomalies, hemodynamic defects have also been demonstrated, showing a decrease in heart rate and an increase in mean dorsal aortic blood flow (11). Hemodynamic changes occur immediately after venous clipping (124) but also have long-term effects, including increased ventricular stiffness due to reduced contractility and reduced compliance (123, 135), as demonstrated with pulsed echo-Doppler analyses and videoscopically determined pressure-volume loops. The effects are demonstrated even extending to several days after the clip challenge (reviewed in Ref. 53). It is now clear that blood flow is involved in the development of the heart, and by disturbing it, cardiovascular malformations will arise.

This leads to the question of how cells can sense changes in flow and how they can respond to this biologically, so that the final result is a more complex heart in both form and function.

**Shear Stress and Gene Expression**

We postulate that altered shear stress is the primary factor that causes the cardiovascular malformations by means of alterations in gene expression. Therefore, the mechanism of shear sensing, the regulation of gene expression with emphasis on three selected shear stress-responsive genes, are discussed.

**Shear sensing**

The response to changes in wall shear stress in endothelial cells is provided by a mechanosensor for wall shear forces, e.g., by stimulation through the activation of integrins, G-protein receptors, tyrosine kinase receptors, or ion channels (78, 111, 132). The interaction complex involving CD31/PECAM-1, VE-cadherin, and VEGFR2/KDR/FLK-1 has been particularly documented (134). Furthermore, the glycocalyx, has been proposed to be involved in shear stress sensing (40, 147). Finally, an ultrastructurally defined organelle for mechanosensing, called a primary cilium, has been introduced (90, 121). Primary cilia are 1-5 µm nonmotile membrane protrusions, with a 9 + 0 microtubular core, that are the sensing counterparts of the motile (9 + 2) cilia found on numerous cell types, including airway epithelium and sperm cells. Both ET-1 and NO-3 have been implicated in the functioning of motile cilia of airway epithelial cells (127, 149).

Cilia comprise a specific set of proteins: the ciliome. Mutations in many of the ciliome genes lead to congenital disorders like Bardet-Biedl syndrome, autosomal dominant/recessive polycystic kidney disease, and Kartagener syndrome (26). The embryonic deter-
mation of handedness is mediated through wall shear stress sensing by primary cilia (94, 158). It is important to realize that many models targeting ciliary proteins present with situs inversus. Endothelial cells can also be ciliated (64, 136). Ciliated endothelium is locally abundant in the embryonic heart, more specifically in areas of low and disturbed wall shear stress (136), negative for KLF2 expression (43). Recent data also show ciliated endothelial cells in large vessels, such as the aorta, and on the aortic valves in adult mice with and without atherosclerosis (137). The presence of endothelial cilia in mice is associated with low and disturbed shear stress. The mechanism by which the primary cilium is involved in wall shear sensing is becoming elucidated. Bending of the primary cilium induces a calcium transient (107), probably mediated through polycystin-2, which is related to a calcium channel that is involved in polycystic kidney disease (1). Alternatively, the cilium may function as a lever to facilitate and amplify deformation of the cytoskeleton, which is likewise implicated in stress sensing (19). Primary cilia are also present on embryonic aortic endothelial cells (14), cultured senescent bovine aortic endothelial cells (10), and cultured human umbilical vein endothelial cells (HUVECs) (64). Cilia are part of the cytoskeleton, which makes them attractive for performing a central function in transducing shear forces to intracellular mechanisms (136).

**Gene Expression**

On detecting a change in shear stress by endothelial cells, a signal is sent to the nucleus by means of the activation of protein kinase C (PKC), which in turn activates a second messenger cascade, involving the family of mitogen-activated protein (MAP) kinases, most importantly extracellular signal-regulated kinases (ERK1/2), resulting in gene transcription to be up- or downregulated (reviewed in Refs. 33, 52, 132). Members of the MAP kinases have many potential substrates, including other protein kinases (Rafl-1, MEK), transcription factors, enzymes (cPLA2), and cell surface proteins (EGF receptor). Transcription factors, such as c-fos, c-jun, Egr-1, Sp1, and NF-κB are activated or inhibited and influence gene regulation. The involvement of integrins, p38 MAPK, ERK1/2, and NF-κB have recently been demonstrated in shear stress-induced MMP-9 expression in HUVECs (126). The expression of transcription factor Forkhead box protein O1A (FoxO-1) and its target genes p27kip1 and angiotensin-2 (Ang-2) were recently shown to be decreased by shear stress via activation of the serine/threonine kinase Akt, which may be implicated in the regulation of angiogenesis (15). Changes in gene expression by shear stress have been demonstrated by several independent groups (22, 83, 93, 110). In this review, the focus is on three genes: ET-1, KLF2, and NOS-3. The focus is on ET-1 because it is a strong vasoconstrictor and, therefore, involved in flow regulation. Additionally, it plays a role in cardiovascular development since ET-1 knockout (KO) mice display cardiovascular anomalies (73). Of KLF2, it has been demonstrated to be expressed in areas of high shear stress in the adult human aorta (22) and is directly regulated by shear stress (145). And NOS-3 completes the circle, being a functional counterpart of ET-1, a vasodilator, and being directly regulated by KLF2 (23, 117).

**ET-1.** ET-1, a 21-amino acid polypeptide, was first isolated in 1988 and is described as the most potent endogenous vasoconstrictor discovered to date (153). Prepro-ET-1 mRNA is translated into a 203-amino acid peptide precursor, prepro-ET-1. This is converted by endopeptidases into a pro-ET-1, big-ET-1, which is then cleaved by endothelin-converting-enzyme-1 (ECE-1) into the functional ET-1 that can exert its biological effects through its receptors. Hitherto, the receptors have been grouped into two main classes: the endothelin-A (ETA) and the endothelin-B (ETB) receptors. Besides being a vasoconstrictor, mediated by the action of ET-1 on both ETA and ETB receptors on smooth muscle cells (50), ET-1 also has vasodilator properties mediated by nitric oxide (NO) and phosphodiesterase (PDE) release through activation of the ETB receptor located in the endothelium (20, 101, 133, 139). Furthermore, ET-1 is a growth factor, involved in, e.g., the proliferation of fibroblasts and smooth muscle cells through the ETA receptor (31, 47, 155, 159), the proliferation of endothelial cells through the ETB receptor (25, 98), and hypertrophy of various cell types, including cardiomyocytes (120, 150). It is also implicated in collagen production (46). In addition, it is involved, via the ETA receptor, in the closure of the ductus arteriosus after birth (128). Interestingly, ET-1 KO mice (Edn-1/-) display similar cardiovascular defects as chicken embryos in the venous clip model (56, 73). Heterozygous mice (Edn+/-) show elevated blood pressure (74). Likewise, KO mice of genes from the ET-1 pathway, Ece-1/- and Eta-/- show similar cardiovascular malformations (17, 152).

ET-1 mRNA and protein production are regulated by wall shear stress, although the manner of response remains controversial. The majority of in vitro studies have demonstrated that ET-1 gene expression and protein levels are decreased after long-term (up to 24 h) high steady laminar shear stress levels (≥8 dyne/cm²) (82, 83, 91, 93, 97, 103, 118). Also under long-term (24 h) low shear (≤6 dyne/cm²) ET-1 mRNA has been shown to be downregulated (91, 97, 160). However, some studies report an increase in ET-1 by low shear stress (92, 157) and some an upregulation of mRNA or peptide release by high shear (109, 141, 143). In addition, there are studies that have shown that ET-1 is transiently increased by shear, followed by a chronic downregulation (82, 83, 97, 160). Another study by Wang et al. (144) demonstrated an increase in ET-1 secretion by human glomerular microvascular...
endothelial cells after 25 h of steady laminar shear with a magnitude of 5 dyne/cm², which increased even further with 10 dyne/cm², but was decreased with 15 dyne/cm². The authors discussed other factors that affected the secretion of ET-1, which were flow (steady or pulsatile), cell age, cell type, cell species, endothelial cell monolayer length, and whether the endothelial cell monolayer is connected or disconnected (144). What is striking in the different studies is that those that showed an increase in ET-1 used either microvascular cells and/or pulsatile flow, factors that are expected in living embryos. However, in vivo, vessels are not rigid, and pulsatility may not be sensed by endothelial cells as dramatic as in an in vitro situation where cells are grown on rigid plastic. In early chicken embryonic development, ET-1 is expressed along the endocardial cushions, but with increasing gestational age it becomes complementary to that of KLF2 and NOS-3, being downregulated at the high shear areas (43). Three hours after venous clip, ET-1 expression is decreased in the inner curvature and upregulated in the dorsal aorta (44), which corresponds with the decrease in flow up to 5 h after venous clip (124). All this implies that, in the embryonic in vivo situation, ET-1 is decreased by high shear and increased by low shear, which is in agreement with the majority of in vitro studies applying steady laminar shear stress on either HUVEC or bovine aortic endothelial cells (BAEC).

Further involvement of ET-1 in the abnormalities induced in the venous clip model is demonstrated by systemic application of ET-1 or antagonists of its receptors ETA and ETB, since this results in a partial phenocopy of the venous clip with respect to cardiac function and morphology (45).

Krüppel-like factor 2. KLF2 is a member of the SP/KLF family of Cys2/His2 zinc-finger transcription factors (6, 106). KO mice of Klf2 die between embryonic day (E) 12.5 and E14.5 due to severe hemorrhages in the embryonic cardiac outflow tract region and in the abdomen (72, 146). The hemorrhaging is caused by rupture of the vessels due to abnormal thinning of the tunica media and concomitant instability of the vessel wall (72), demonstrating that KLF2 in endothelial cells regulates the assembly of the vascular tunica media and concomitant vessel wall stabilization during mammalian embryogenesis. The embryos are also retarded in growth and show craniofacial abnormalities and signs of anaemia (146). The latter can be explained by the fact that KLF2 is essential for primitive erythropoiesis and that it regulates the β-like globin genes (4). A recent study using cell-specific conditional Klf2 KO mice demonstrated that endothelial loss of Klf2 results in lethal embryonic heart failure at E14.5 due to a high cardiac output state at E11.5 (76). This occurs in the absence of anemia or structural vascular defects, but it is ascribed to a profound loss of peripheral vascular resistance. Animals with cardiac and smooth muscle cell-specific loss of Klf2 grew to adulthood and did not show any phenotypic abnormalities (76). The authors state that their results indicate that the lethal hemorrhage is secondary to the vascular smooth muscle defects and is not the primary cause of death in KO and Tie2-cre KO embryos.

In HUVECs, it has been demonstrated that KLF2 is upregulated by increased shear stress (68). In addition, KLF2 is expressed in the endothelium of the adult human aorta at sites of high shear stress (22). In the chicken embryo, its expression pattern (43) overlaps with the high shear distribution pattern (44), which is at the inner curvature and at narrow regions, such as the OFT and AV canal. This is also the case in the mouse embryo (76). It is, however, mutually exclusive with the presence of primary cilia (136). In the venous clip model, KLF2 expression is increased in the high shear areas. It has, furthermore, been shown that there is a direct link between flow-induced phosphatidylinositol 3-kinase (PI3K) activation, histone acetylation, KLF2 transcription, and NOS-3 induction (63). KLF2 can induce NOS-3 expression and enzymatic activity, as has been shown by SenBanerjee et al. (117), who also demonstrated that KLF2 regulates endothelial activation in response to proinflammatory stimuli. However, the latter is not of importance in early embryos, since the immune system has not yet been developed. Recently, it has been demonstrated that it can inhibit the transcription factors AP-1 and ATF2, influencing gene expression (7, 29). In addition, Dekker et al. (23) have confirmed that KLF2 induces NOS-3 expression and also demonstrated that KLF2 decreases ET-1 expression. This identifies KLF2 as a molecular switch between the production of NO and ET-1 at sites of high shear stress. Furthermore, KLF2 deficiency can be rescued by administration of phenylephrine, which raises vessel tone (76). Its own regulation of expression occurs through the transcription factor myocyte enhancing factor 2 (MEF2). KLF2 contains a single consensus MEF2-binding site in its promoter (71). Extracellular signal regulated kinase 5 (ERK5) is a highly flow-induced factor (151), and the MEF2-family is one of its best characterized targets (32, 66, 87, 154). In this way, KLF2 expression can be induced. In addition, on the KLF2 promoter, histone deacetylase-4/5 (HDAC-4/5) and p65 (a component of NFκB) can form a trimolecular complex with MEF2 that inhibits the ability of MEF2 to induce KLF2 expression (71).

Endothelial nitric oxide synthase. NOS-3, also called eNOS, is the major NOS isoform of the endothelium. It is the functional counterpart of ET-1, since it catalyses the conversion of L-arginine and molecular oxygen to L-citrullin and NO (89), the latter being a vasodilator. Therefore, endothelial NO together with ET-1 is physiologically important for maintaining vascular homeostasis (reviewed in Ref. 75).

KO mice for Nos-3 are hypertensive and lack NO-
mediated, endothelium-dependent vasodilation, demonstrating that endothelial NO is an important systemic vasodilator (37, 62, 119). However, NO is not only implicated in vasodilation, it also suppresses smooth muscle cell proliferation (99, 112) and has a negative inotropic effect (38). Nos-3-deficient mice, furthermore, show mild pulmonary hypertension and hyperresponsiveness to hypoxia (27, 125). In addition, these mice were reported to display bicuspid aortic valves, heart failure, and atrial and ventricular septal defects (28, 77), implying the importance of NOS-3 in cardiac development. In contrast to the reported hypertension in Nos-3-/- mice, transgenic mice expressing large amounts of Nos-3 targeted to the vessel wall by the ET-1 promoter are hypotensive and show a reduced vascular sensitivity to NO (104). In vitro studies have shown that steady laminar shear stress increases the expression of NOS-3 mRNA and NO formation (91, 102, 161). In vivo studies in chicken embryos confirmed this by demonstrating that its expression overlaps with the high shear marker KLF2 (43) and that the expression increases after venous clip like KLF2 (44).

Interaction of Shear Stress Responsive Genes

We hypothesize that especially the change in expression of the ET-1 vasoconstrictor and growth hormone is responsible for the increased stiffness and morphological changes in the venous clip model.

The proportion of ETA and ETB receptors may differ between different vascular beds in the adult (51). In the embryo, this is also the case. At HH18, ETA recep-

FIGURE 1. Schematic drawing demonstrating the effects of venous clip
Schematic drawing demonstrating the effects of venous clip on shear stress (A), gene expression (B), and cardiovascular processes, predominantly by ET-1 (C-K), and by NOS-3 (M and N). Smaller font means a smaller effect on the particular process compared with the other receptor. +, Processes are stimulated by venous clip; -, processes are inhibited; CJ, cardiac jelly; CM, cardiomyocyte; EC, endothelial/endocardial cell; EMT, epithelial to mesenchymal transformation; FB, fibroblast; MC, mesenchymal cell; SMC, smooth muscle cell.
tor mRNA is absent in the vitelline vessel wall, whereas that of the ETB receptor is expressed in abundance in both endothelium and media of the vessel wall. In the embryonic chicken heart, both ETA and ETB receptor mRNAs are strongly present. This implies that the ET-1 pathway in the peripheral vitelline circulation is mechanistically different from the intra-embryonic circulation (45). In addition, recent studies have demonstrated that ETA and ETB receptors can exist as hetero- or homodimers (41, 42, 113, 114), which makes ET-1 signaling more complicated.

Since cardiac malformations arise after venous clip, a possible mechanism for ET-1 in the venous clipped heart is presented schematically (FIGURE 1). This description is in part specific for a region where cushions are present, because ET-1 is predominantly decreased in the inner curvature along the AV and OFT cushions after clip. In FIGURE 1, a primary cilium is shown. This does not represent the cushion areas but the low shear regions where these primary cilia are present and sense changes in flow that are transmitted to the cytoskeleton (136). The increase in shear stress in cushion areas leads to an increase in KLF2 expression (22, 136) (FIGURE 1A). Through KLF2, or directly by shear stress, NOS-3 expression is upregulated and ET-1 is decreased in the endocardial cells (23, 118, 161) (FIGURE 1B). This decrease in ET-1 mRNA also leads to a downregulation of ET-1 protein release (82, 97, 118) (FIGURE 1C1). Normally, ET-1 is predominantly secreted at the abluminal side (140, 156) toward the cardiac jelly and myocardium (FIGURE 1C1). There, it may act on its ETA and ETB receptors, but it may also be secreted at the luminal side (FIGURE 1C2) and act on the ETB receptors located on the endocardium/endothelium (122). Subsequently, it can induce processes such as contraction, dilatation, migration, proliferation, extracellular matrix production, transdifferentiation, epithelial-to-mesenchymal transformation, and, in cardiomyocytes hypertrophy, chronotropy, inotropy (contraction force), lusitropy (relaxation rate), and distensiability (FIGURE 1, D, F-J, K-N). This may partly explain the morphological and hemodynamic effects of venous clip.

Summary

The importance of mechanical forces in cardiovascular development has been largely neglected. Recent data (58-60) have identified fluid shear stress as a dominant modifier of cardiogenesis. The expression pattern of shear-induced genes correlates with the presence of primary cilia on endothelial cells. These membrane extensions projecting into the vessel lumen act probably as a lever to sensitize endothelial cells for flow-induced wall shear stress. The ciliated phenotype known from development is repeated in the adult situation under flow conditions that induce pathologies, demonstrating that biomechanical forces and endothelial fluid shear stress sensing have to be considered as important mediators for cardiovascular function and development.

References


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