Université de Montréal

Normal Ventilation and NO inhalation prevent pulmonary artery endothelial dysfunction secondary to cardiopulmonary bypass (CPB)

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Présenté par:

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Dr. André Denault	Membre du Jury	_____

Mémoire accepté le: _____

RÉSUMÉ EN ANGLAIS

The aim of this study is to characterize the alterations of the signal transduction of endothelial cells in the pulmonary arterial tree and to determine the effect of ventilation and nitric oxide (NO) inhalation during CPB on endothelial cells.

Six groups of Landrace swine were compared: group 1 = control, group 2 = sham without CPB, group 3 = CPB 150 minutes + no reperfusion, group 4 = CPB 150 minutes + reperfusion 60 minutes, group 5 = CPB 150 minutes + ventilation (tidal volume 12 ml/kg) + reperfusion 60 minutes, group 6 = CPB 150 minutes + nitric oxide (NO) inhalation (with ventilation, NO 40 ppm) + 60 minutes of reperfusion. Branches of second degree pulmonary arteries were isolated and divided into rings (4mm wide; 16 rings per animal). Contractions were measured with potassium chloride (KCl) and phenylephrine (PE). Endothelium-dependent relaxations were studied using acetylcholine (ACh) and bradykinin (BK) and endothelium-independent relaxations with sodium nitroprusside (SNP).

Pulmonary reperfusion following CPB induced a statistically significant decrease in endothelium-dependent relaxations to ACh compared to groups 1, 2 and 3. Mechanical ventilation and NO inhalation during CPB prevented the reduction of endothelium-dependent relaxations to ACh. There were no statistically significant differences between the 6 groups for relaxations to BK and to SNP.

Pulmonary reperfusion after CPB causes a selective dysfunction of Gi-protein mediated relaxations, most likely through the production of activated oxygen species. Mechanical ventilation during CPB and NO inhalation prevent the pulmonary endothelial dysfunction due to reperfusion after extracorporeal circulation.

KEY WORDS: Endothelium, pulmonary arteries, cardiopulmonary bypass, ventilation and NO inhalation.

RÉSUMÉ EN FRANÇAIS

Le but de cette étude est de caractériser les altérations du signal de transduction des cellules endothéliales de l'artère pulmonaire et de déterminer l'effet de la ventilation et du monoxide d'azote inhalé pendant la CEC sur l'endothélium.

Six groupes de porcs «Landrace Large White» furent comparés (groupe 1 = témoin, groupe 2 = sham sans CEC, groupe 3 = CEC 150 min sans reperfusion, groupe 4 = CEC 150 min et 60 min de reperfusion, groupe 5=CEC 150 min avec ventilation (volume courant 12 ml/kg) et 60 min de reperfusion, groupe 6=CEC 150 min avec inhalation de monoxide d'azote (avec ventilation, NO 40 ppm) et 60 min de reperfusion). Les contractions furent évaluées à l'aide de chlorure de potassium (KCl) et de phényléphrine (PE). Les relaxations dépendantes de l'endothélium furent évaluées à l'aide d'acétylcholine (ACh) et de bradykinine (BK) alors que les relaxations indépendantes de l'endothélium le furent à l'aide de nitroprussiate de sodium (NPS).

La reperfusion pulmonaire cause une diminution significative des relaxations dépendantes de l'endothélium à l'ACh comparée aux groupes 1, 2 et 3. La ventilation et le monoxide d'azote inhalé per CEC préviennent la réduction des relaxations dépendantes de l'endothélium à l'ACh. Aucune différence significative n'a été notée entre les six groupes pour les relaxations au BK et au NPS.

La reperfusion de l'arbre pulmonaire suite à la CEC cause une altération des vasorelaxations des cellules endothéliales probablement via la production de radicaux libres. La ventilation et l'inhalation de monoxide d'azote durant la CEC préviennent la dysfonction endothéliale causée par la reperfusion des poumons après la CEC.

MOTS CLÉS: Endothelium, artères pulmonaires, circulation extracorporelle, ventilation et inhalation de monoxyde d'azote.

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LISTE DES SIGLES ET ABRÉVIATIONS

ACh :	Acetylcholine
ANA :	Antinuclear Antibodies
ANOVA :	Analysis of Variance
ARDS :	Acute Respiratory Distress Syndrome
BK:	Bradykinin
$CaCl_2$:	Calcium Chloride
CEC:	Circulation Extracorporelle
cGMP:	cyclic Guanosine monophosphate
CHF:	Congestive Heart Failure
CPAP :	Continuous positive airway pressure
CPB:	Cardiopulmonary Bypass
DAG:	Diacylglycerol
EDCF:	Endothelium Derived Contracting Factor
EDHF:	Endothelium Derived Hyperpolarizing Factor
EDRF:	Endothelium Derived Relaxing Factor
EKG:	Electrocardiogram
eNOS/NOS3:	Endothelial Nitric Oxide Synthase
ET-1:	Endothelin-1
Gi-Protein:	Gi binding protein (GTPase binding protein)
Gq-Protein:	Gq binding protein
H_2O_2:	Hydrogen Peroxide
iNOS/NOS2:	Inducible Nitric Oxide Synthase
IP_3:	Inositol Triphosphate
KCl:	Potassium Chloride
KH_2PO_4:	Potassium phosphate monobasic
LV:	Left Ventricle
M:	Muscarinic
$MgSO_4$:	Magnesium Sulfate
NaCl:	Sodium Chloride
$NaHCO_3$:	Sodium Bicarbonate
NADPH:	Nicotinamide Adenine Dinucleotide Phosphate
nNOS/NOS1:	Neuronal Nitric Oxide Synthase
NO:	Nitric oxide
NPS:	Nitroprussiate de Sodium
O_2^-:	Superoxide Anion
PE:	Phenylephrine
PEEP:	Positive end-expiratory pressure
PGI_2:	Prostaglandin I_2; Prostacyclin
PGs:	Prostaglandins
PPH:	Primary Pulmonary Hypertension
PVR:	Pulmonary Vascular Resistance
RV:	Right Ventricle
SIRS:	Systemic Inflammatory Response Syndrome
SLE:	Systemic Lupus Erythomatosus
SNP:	Sodium Nitroprusside
SPH:	Secondary Pulmonary Hypertension
TXA_2 :	Thromboxane A_2

DÉDICACE

À ma mère, Lucie Gagnon et mes grands-parents, Gemma et Joseph Gagnon, qui m'ont donné confiance en moi et qui m'ont appris que tout était possible avec de la détermination.

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Finalement, je remercie mon conjoint François Paquette qui a su me soutenir et me motiver.

CORPS DE LA THÈSE : CHAPITRE I

INTRODUCTION ET ÉTAT DE L'ART

The Endothelium

The endothelium is a monolayer of cells lining the inside of all blood vessels. Important functions of the normal endothelium include inhibition of vascular smooth muscle cell contraction, inhibition of the proliferation of smooth muscle cells and fibrocytes, and providing a nonthrombogenic surface and a selective barrier to circulating proteins and cellular components (Figure 1) [4]. However, owing to its special anatomical position at the interface of blood and organs, the endothelium is a primary target for injuries such as hypertension and ischemia. A few studies have analyzed the mechanism associated with lung injury after cardiopulmonary bypass (CPB) by examining responses of isolated pulmonary arteries to selective endothelium-dependent and independent activators [5-7]. However, no studies have assessed the effect of ventilation and NO inhalation during CPB on pulmonary artery endothelial dysfunction.

Endothelial cells play a major role in local vasoregulation by releasing a variety of vasoactive substances. Vasoactive factors released by the endothelium fall into 2 major groups depending on their biological activity (Figure 2) [8]. The first group includes endothelium-derived relaxing factors (EDRF), such as nitric oxide (NO), prostacyclin (PGI_2), and the endothelium-derived hyperpolarizing factor (EDHF) [8]. The second group includes endothelium-derived contracting factors (EDCF) such as endothelin-1 (ET-1), angiotensin II, thromboxane A_2 superoxide anion, and endoperoxides [8].

Figure 1 : The role of the endothelium.

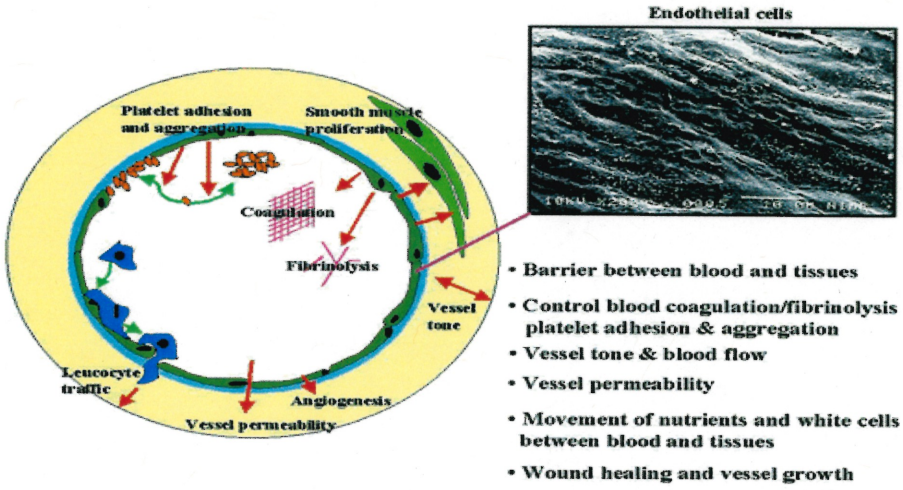
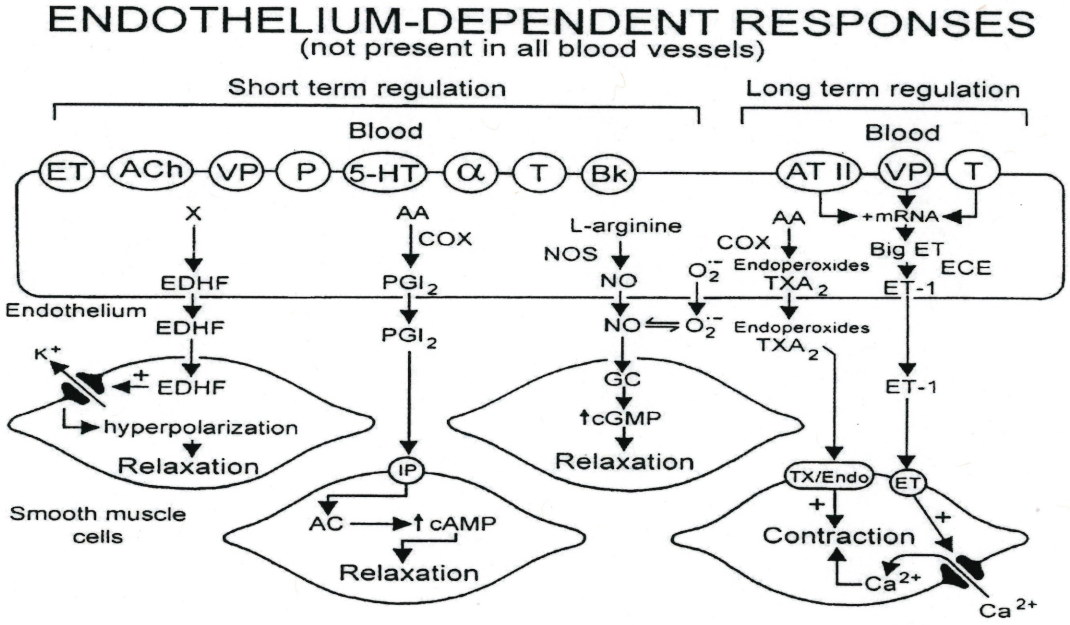


Figure 2: The 2 major groups of vasoactive factors released by the endothelium.



Endothelium-Derived Relaxing Factors

Nitric Oxide

Furchgott and Zawadzki [9], and Ignarro et al. [10] proposed NO, a labile diffusible, nonprostanoid substance that mediates the endothelium-dependent relaxation to acetylcholine (ACh), or a closely related compound as the most logical candidate for endothelium derived relaxing factor (EDRF). When NO diffuses to the vascular smooth muscle cells, it stimulates a cytosolic enzyme, soluble guanylate cyclase that leads to an increase in cyclic GMP (cGMP) intracellularly which in turn causes vascular smooth muscle cell relaxation by inhibiting calcium release from the sarcoplasmic reticulum (Figure 3) [11-13]. During inflammation and atherosclerosis, low concentrations of NO prevent apoptotic death of endothelial cells and preserve the integrity of the endothelial cell monolayer [14,15]. Likewise, NO also acts as an inhibitor of platelet aggregation, adhesion molecule expression (Figure 4) [16,17]. Altered NO production and/or decreased bioavailablility have been linked to disorders including hypertension, hypercholesterolemia, diabetes, and heart failure [18].

Figure 3: The NO pathway.

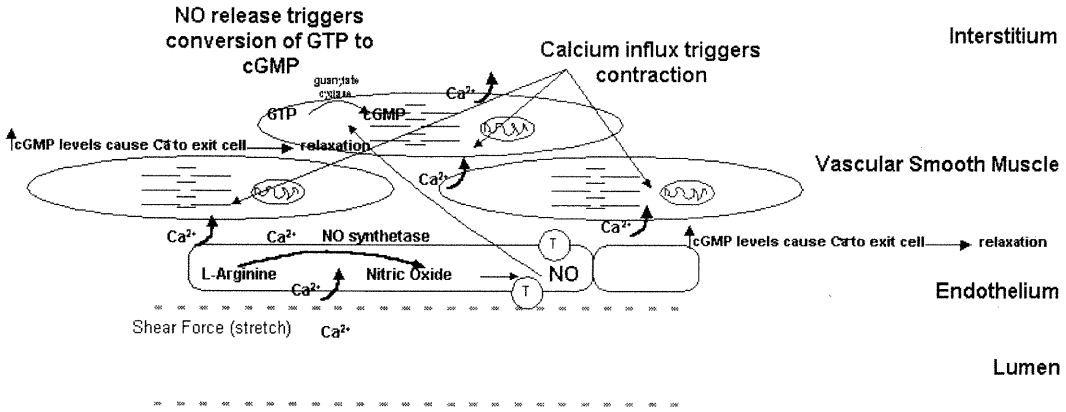
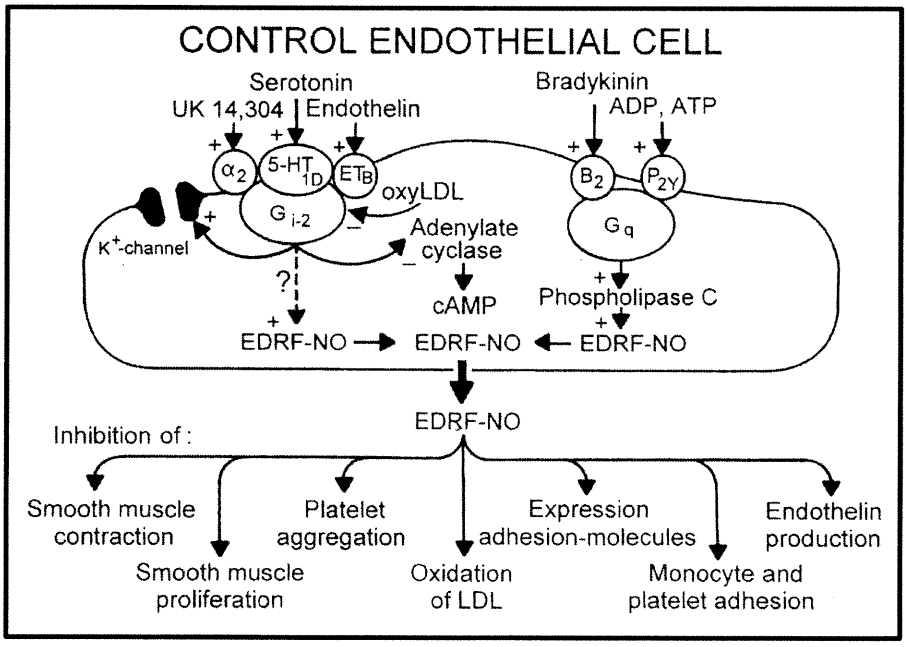


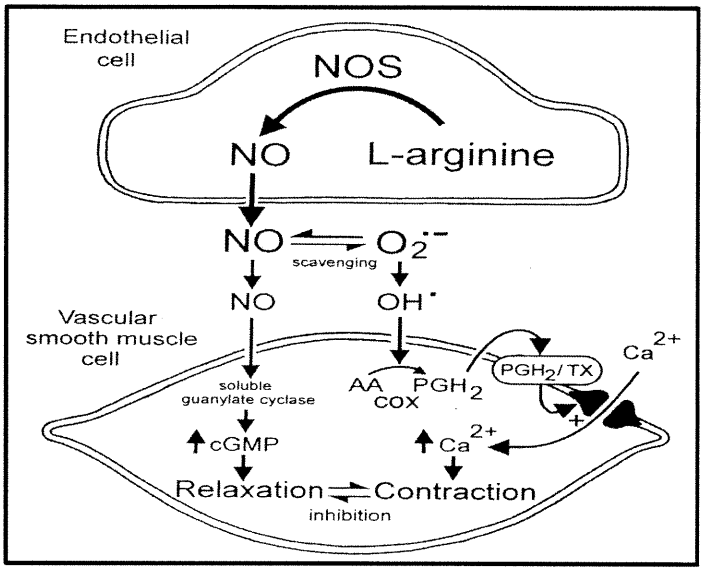
Figure 4: Control endothelial cell.



Nitric oxide is produced by a group of enzymes called nitric oxide synthases (NOS). These enzymes catalyze the production of NO and L-citrulline from L-arginine, O_2, and NADPH-derived electrons (Figure 5)[19,20]. Mammalian systems contain three well-characterized isoforms of nitric oxide synthase: neuronal NOS (nNOS, also called NOS1), inducible NOS (iNOS or NOS2), and endothelial NOS (eNOS or NOS3). The names reflect characteristics of the activity or the original tissues in which the enzymes were first described, but it is now known that each of these isoforms is expressed in a variety of tissues and cell types.

The three main isoforms share structural similarities and have nearly identical catalytic mechanisms [21,22]. They all require a number of cofactors including reduced nicotinamide-adenine-dinucleotide phosphate (NADPH), and 5, 6, 7, 8 tetra-hydrobiopterin (BH_4) and flavin adenin mono- and dinucleotides (FMN/FAD) that are needed for dimerization and NO production [19,20]. The three NOS isoforms display a number of differences related to their individual functions. For example, calmodulin functions differently in each of the NOS isoforms and the transcriptional regulation and post-translational regulation of the catalytic activity is distinct for each isoform. Neuronal and endothelial isoforms are expressed constitutively and react to agonist that increase intracellular Ca^{2+} which stimulates the binding of NOS to calmodulin activating the enzyme and intiating NO synthesis at small concentration (10^{-9} M) [23]. On the other hand, the transcription of the inducible isoform is performed by macrophages which initiate NO production several hours after being stimulated by cytokines [23]. The activated macrophage produce NO at micromolar levels (10^{-6} M) until the enzyme is degraded by proteolysis [23].

Figure 5: L-arginine to NO.



Cells that express eNOS include vascular endothelial cells and cardiomyocytes [24]. In blood vessels, NO produced by the eNOS of endothelial cells has a vasodilatory effect thereby regulating blood flow and pressure [17]. Expression of eNOS is constitutive but regulation occurs in response to factors such as shear stress, exercise training, chronic hypoxia, and heart failure [25,26]. Within the endothelial cell membrane, small invaginations characterized by the presence of proteins called caveolins serve as sites for the sequestration of signaling molecules such as receptors, G proteins and protein kinases. The oxygenase domain of eNOS contains a motif that binds to caveolin-1, and calmodulin competitively displaces caveolin resulting in eNOS activation [25]. Bound calmodulin is required for activity of eNOS, and this binding occurs in response to transient increases in intracellular Ca^{2+} [21]. Thus, eNOS is found at sites of signal transduction and produces short pulses of NO in response to agonists. Within the cardiovascular system, eNOS generally has protective effects. Studies with eNOS knockout mice clearly indicate

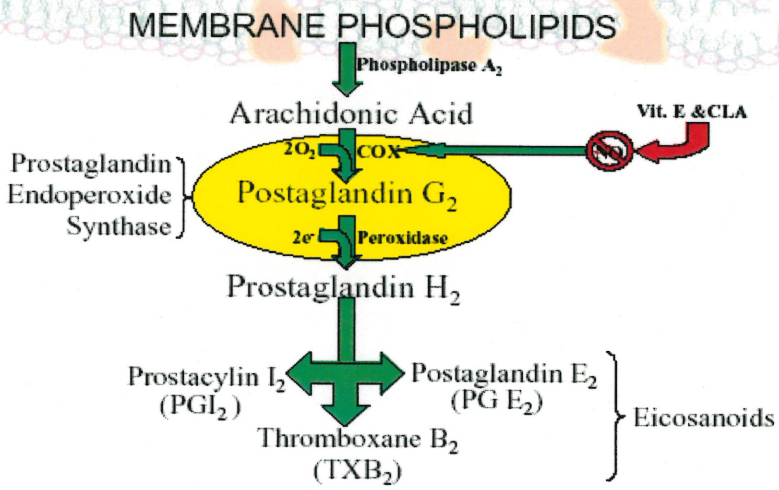
that eNOS plays a protective role in cerebral ischemia by preserving cerebral blood flow [27].

The activity of iNOS is induced by pro-inflammatory cytokines, lipopolysaccharides, endotoxins and bacterial products [28,29]. Cells that expresses iNOS include macrophages, hepatocytes, smooth muscle cells, and cardiac myocytes [29]. This inducible isoform of NOS is regulated transcriptionally and is independent of agonist stimulation and intracellular calcium levels [30,31]. Increasing evidence suggests that iNOS expression is increased in smooth muscle cells and in the neointima after vascular injury [32-34] and after allograft transplantation [35] suggesting that iNOS expression may limit the thrombotic and proliferative response in injured vessels [31]. Respiratory inflammatory disease such as asthma, acute respiratory distress syndrome (ARDS), and bronchiectasis are characterized by an increased expression of iNOS in respiratory epithelial and inflammatory-immune cells leading to a markedly elevated production of NO locally [28]. This potent increase in NO synthesis is presumably an additional host defense mechanism against bacteria, virus and tumor cells [28,29]. Sustained NO production, may be an advantage over other isoforms, but concern remains that continuous high activity could also increase superoxide production, leading to peroxynitrite formation [36]. The overall contribution of NO to inflammatory conditions of the lung is not easily predicted despite extensive research on both the pro-inflammatory and anti-inflammatory actions of NO and seems to depend on many factors such as the site, time and degree of NO production in relation to the local redox status and the acute or chronic nature of the immune response [28].

Prostacyclin

Arachidonic acid by the action of cyclooxygenase yields cyclic endoperoxide PGG_2 [37]. PGG_2 is then rapidly modified by the peroxidase moiety of the cyclooxygenase enzyme to add a 15-hydroxyl group, esssential for biologic activity, and yields PGH_2 [37]. PGH_2 then yields the prostaglandins, prostacyclin via PGI synthase (Figure 6) [37]. Prostacyclin is synthesized mostly in endothelial cells but also in the media and advential tissue in response to hypoxia and sheer stress and is a poweful vasodilator and inhibitor of platetet aggregation [37]. Prostacyclin causes the relaxation of the vascular smooth muscle by activating adenylate cyclase thus causing an increase in the production of cyclic 3'-5' adenosine monophosphate (cyclic AMP) [38]. The relaxant effect of prostacylin is essentially additive to NO, although the cGMP formed when NO is released, may act as an endogenous inhibitor of the phosphodiesterase that breaks down cAMP [2]. Hence, NO indirectly increases the half-life of cAMP, the second messenger of prostacyclin [2]. Finally, subliminal concentrations of prostacylin and NO are strongly synergistic causing a profound inhibition of platelet aggregation [39].

Figure 6: The cyclooxygenase pathway.



Endothelium Derived Hyperpolorizing Factor (EDHF)

Electrophysiologic studies of various blood vessels have shown that the endothelium-dependent relaxation to acetylcholine and other vasodilator drugs such as bradykinin is associated with membrane hyperpolarization of the underlying vascular smooth muscle, for which neither NO, nor prostacyclin are responsible. Another, as yet unidentified substance is involved: endothelium-derived hyperpolarizing factor (EDHF). It tends to act mainly in smaller blood vessels, in contrast to NO which is more active in large arteries [40]. However, when the synthesis of NO is inhibited, EDHF can mediate close to normal endothelium-dependant relaxation in large vessel. Hence, EDHF could be an important backup mechanism in the regulation of the vascular smooth muscle cells when the production of NO is decreased although its exact role in physiological and pathological states is unknown [41].

Muscarinic M1 subgroup receptors are membrane receptors responsible for the release of EDHF induced by acetylcholine [42]. The release of EDHF by endothelial cells is controlled by the cytosolic calcium concentration and is inhibited by calmodulin agonists [42]. EDHF appears to act endogenously on smooth muscle by opening potassium channels which are not ATP-dependent [19]. Furthermore, EDHF is formed from the pre cursor arachidonic acid via the cytochrome P-450 and has some of the characteristics of epoxyeicosatrienoic acids (EET) in certain blood vessels [43]. The cytochrome P-450 CYP9 has recently been identified as the principal producer of EDHF [44].

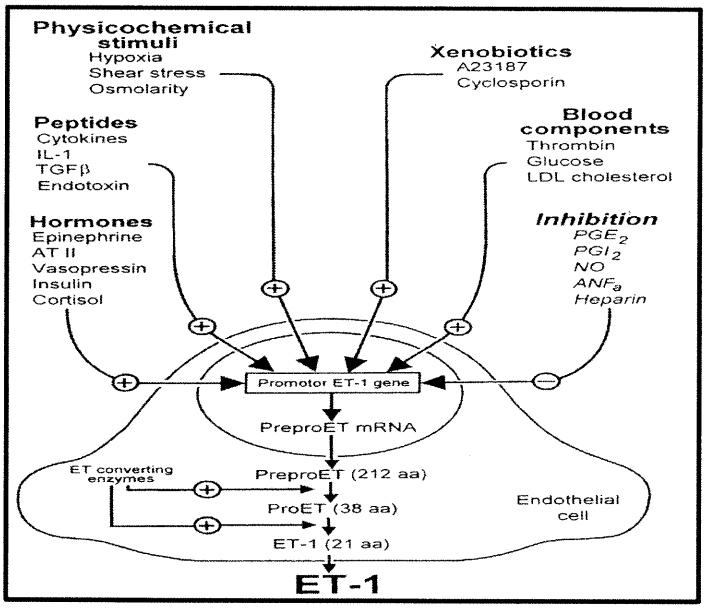
Endothelium-Derived Contracting Factors

Endothelial dysfunction, as defined by an abnormal increased tendency to vasoconstriction or loss of the vasodilatory capacity of a vascular bed, may be due to an impairment in endothelium-dependent relaxation, caused either by the reduced release (or activity) of endothelium-dependent relaxation factors, and/or a greater propensity to evoke endothelium-dependent contractions. Currently, the EDCFs that have been identified include superoxide anions, endoperoxides, thromboxane A_2 and the peptide ET-1 [8]. In endothelial dysfunction, the decrease of NO production by the endothelium [45] may increase the generation of ET-1 and initiate a vasoconstriction response via the angiotensin II pathway.

Endothelin

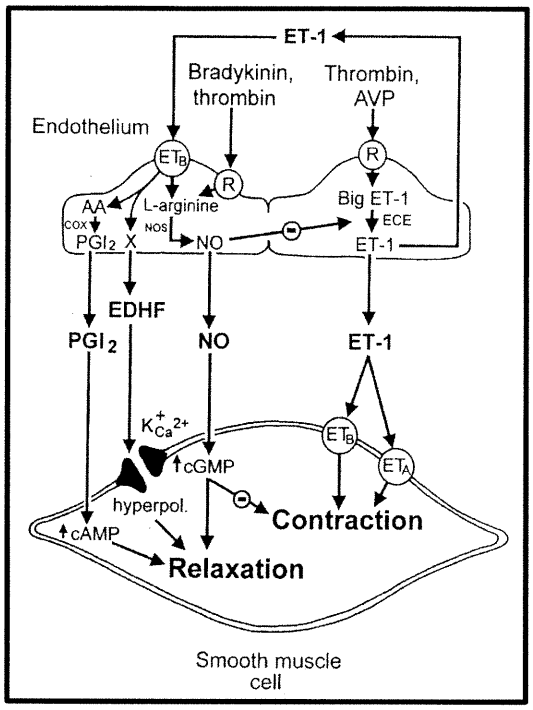
Big-endothelin is produced by endothelial cells and is converted via the endothelin converting enzyme (ECE) in endothelin-1 (ET-1). Endothelin is a 21-residue peptide released by cultured endothelial cells [46]. ET-1 is a potent arterial and venous vasoconstrictor which exerts long term effects on vascular smooth mucle tone. Thrombin, angiotensin II, catecholamines, vasopressin, interleukin-1, growth factor β1 and platelets products can activate endothelin receptors thus releasing ET-1 preferentially towards the vascular smooth muscle (Figure 7). However, the stimulated production of ET-1 causes an increase in NO production via a negative feedback control which can in turn inhibit the synthesis of endothelin-1 by endothelial cells [2].

Figure 7: The factors promoting ET-1 synthesis.



There are two ET receptor subtypes: ET_A-Rs and ET_B-Rs. In the vasculature, ET_A receptors are located on the vascular smooth muscle cells where they mediate both vasoconstriction and growth [47]. In contrast, ET_B receptors located primarily on vascular endothelial cells mediate vasodilation via the release of NO and prostacyclin while the ET_B receptors located on vascular smooth muscle cells mediate vasoconstriction (Figure 8). ET_B receptors also play an important role in ET-1 clearance [48]. ET_A-Rs and ET_B-Rs are linked to phospholipase C which leads to the formation of inositol triphosphate (IP_3) and diacylglycerol (DAG) [49]. These second messengers lead to the intracellular release of calcium and activation of protein kinase C [49]. In addition, endothelin receptors are coupled via Gi-proteins to voltage operated calcium channels [49].

Figure 8: The ET-1 pathway.



Low blood concentrations of ET-1 in physiological conditions activate ET_B receptors on the surface of endothelial cells resulting in the release of NO and prostacyclin provoking vasodilatation [1,50]. On the other hand, high blood concentration of ET-1 in physiological conditions activates ET_A receptors on vascular smooth muscle cells provoking prolonged contractions [51]. An overproduction of endothelin-1 in physiological conditions is counteracted by a high NO production causing the immediate inhibition of ET-1 regeneration and of its vasoconstrictor effect [1]. This retroactive inhibition of endothelin production and action in not only due to NO release, but also to prostacyclin and EDHF release [52].

The ratio of ET_A and ET_B receptors in human resistance and conduit pulmonary arteries is approximatively 9:1 [47] and the net effect in pulmonary arteries is constriction. Endogenous production of endothelin plays a role in the development

of pulmonary hypertension. In a human in vitro model of denuded pulmonary arteries, ET-1 induced a concentration-dependent contraction that was inhibited by the ET_A selective antagonist BQ-123 [53]. Likewise, other studies of human pulmonary resistance arteries and endothelium denuded intralobar pulmonary arteries have confirmed that ET-1 causes pulmonary artery vasoconstriction predominantly via ET_A receptors, although there is some evidence that ET_B receptors may contribute at low ET-1 concentrations [54]. ET-1 stimulated proliferation of human pulmonary artery smooth muscle cells is mediated via ET_A receptors [55]. NO produced by the pulmonary vascular endothelium may inhibit the expression or activity of ET-1 [56].

Angiotensin II

Endothelial cells express angiotensin II (ANG II) [57] receptors in certain vascular beds. AT_1 endothelial receptors are coupled to phospholipase C which inhibits the vasoconstrictor effects of ANG II and the release of NO and prostacyclin [2]. Activation of AT_2 leads to endothelium-dependent relaxation to bradykinin [58]. In the vascular smooth muscle, ANG II has several effects : vasoconstriction and promotion of cellular proliferation by AT_1 receptors [59]. Angiotensin II can also stimulate free radical production, by the NADH/NADPH oxidase in vascular smooth muscle, which scavenges NO [60]. It also increases endothelin expression and release by smooth muscle cells thus producing an even greater vasoconstriction [2]. ANG II stimulates ET-1 release in endothelial cells, but NO inhibits ET-1 expression and production. In the presence of endothelial dysfunction, NO production is reduced which favors ET-1 generation and initiates a vasoconstrictor response via the ANG II

pathway [45]. Thus, ANG II acts not only through the endothelin pathway, but also through an increased in oxidative stress, an important mechanism in the development of injury [61].

Thromboxane

Arachidonic acid by the action of cyclooxygenase yields cyclic endoperoxide PGG_2 [37]. PGG_2 is then rapidly modified by the peroxidase moiety of the cyclooxygenase enzyme to add a 15-hydroxyl group, esssential for biologic activity, and yields PGH_2 [37]. PGH_2 is transformed into the prostaglandins (PGs) and thromboxane A_2 via the TXA synthase [37]. Thromboxane (TXA_2) causes undesirable effects such as platelet aggregation and vasoconstriction of vascular smooth muscle cells. On the other hand, platelet aggregation and vasoconstriction mediated by TXA_2 become useful when bleeding is present. However, nitric oxide, through a negative feedback mechanism, can minimize inducible cyclooxygenase expression by inhibiting nuclear factor-κB and nuclear factor interleukin-6 activation and may be important for limiting excessive or prolonged PGs production in pathological events [62].

Oxygen Free Radicals

Free radicals form hydrogen peroxide (H_2O_2) and subsequently hydroxyl radicals cause endothelial dysfunction by scavenging endothelial derived NO [63]. Superoxide anion (O_2^-) can interact with NO to form peroxynitrites that decomposes into hydroxyl radicals which are the main effectors of oxidative injury, i.e., lipid peroxidation [64]. Lipid peroxides activate phospholipase A_2 which triggers the endothelial expression of adhesion molecules, the release of proinflammatory mediators, the release of cytokines leading to the adhesion of leukocytes to endothelial cells and the activation and infiltration of polymorphoneutrophils (PMN) [7]. Free-radical scavengers and antioxidants attenuate pulmonary endothelial dysfunction resulting from oxygen free radicals [65].

Endothelial production of superoxide anions can contribute to a reduction of the vasodilatory capacity by scavenging NO [66] and may promote vascular smooth muscle cell growth and vascular damage in hypertension [67]. In chronic experiments, reactive oxygen species may also directly impair the G-protein-dependent signal transduction process and the oxidative stress could be a result of a generalized dysfunction of G-protein mediated relaxations [68].

Pulmonary Endothelial Dysfunction

Although the exact mechanism behind CPB-induced pulmonary hypertension is not fully elucidated, previous studies have shown that pulmonary vascular endothelial dysfunction plays an integral role. After cardiac surgery with CPB, pulmonary dysfunction is commonly observed [69]. This dysfunction has multiple causes. In part, it is caused by the absence of pulmonary blood flow during total CPB and by its near absence during partial CPB. This state of reduced pulmonary perfusion followed by the restoration of normal perfusion results in an ischemia-reperfusion injury of the sensitive pulmonary endothelium. Reoxygenation produces reactive O_2 reactive products resulting in lipid peroxidation with endothelial damage [70]. It is documented that ischemia-reperfusion injury causes endothelial dysfunction manifested by impairement of endothelium-dependent vasodilatation to neurohumoral agents such as acetylcholine and serotonin [71,72].

Adhesion molecules, important in the initiation of activated leukocytes injury in the pulmonary endothelium, have also been shown to increase in an ischemia-reperfusion rat lung model and are likely contributors in the pathogenesis of ischemia-induced pulmonary injury [73,74]. The decreased pulmonary blood flow during CPB results in very low shear stress in the pulmonary capillaries which accentuates neutrophils activation as neutrophils appear to be exquisitely sensitive to sheer stress [69]. Activated neutrophils release neutral serine, elastase, matrix metalloproteinases and oxygen radical species which damage the alveolar-capillary basement membranes and the extracellular matrix [75]. After cardiac surgery, there is an increased permeability of the alveolar-capillary barrier due to the systemic

inflammatory response associated with cardiopulmonary bypass promoting the development of pulmonary edema [69].

Other factors causing pulmonary vascular endothelial dysfunction include the inflammatory response to CPB elicited by the contact of blood with non physiologic surfaces resulting in activation of the complement pathway with neutrophil activation and sequestration. A group of circulating glycoproteins comprises the complement system and from the basic matrix of the body's response to immunologic, traumatic, infectious, or foreign body insult [3]. Complement is activated through two interrelated cascades termed the classical and the alternate pathways. The classical pathway is usually initiated via interaction with antigen-antibody complexes. Chenoweth et al. first demonstrated in 1981 that the alternate pathway is activated by exposure of blood to foreign surfaces such as CPB resulting in the generation of C3a and C5a anaphylatoxins [76]. The C3a causes smooth muscle contraction in a wide variety of animal tissues [3]. The C5a is 10 to 20 times more active than C3a on a molar basis and has a wider biological activity [3]. C5a rapidly binds to circulating neutrophils which become activated and undergo deposition and sequestration in the lungs releasing superoxides and lysosomal enzymes which in turn produce direct endothelial damage, alterations in capillary permeability and accumulation of extravascular water [3]. Thus, leukocytes are stimulated during CPB to adhere to stimulated endothelial cells, but ischemia-reperfusion further stimulates leukocyte adherence and cytotoxicity [7].

Pulmonary Hypertension

During CPB, the absence of pulmonary blood flow favors the accumulation of neutrophils in the lungs which can cause damage to pulmonary endothelial cells and provide a favorable setting for the development of pulmonary hypertensive crises [69]. There are two forms of pulmonary hypertension: primary and secondary which are both associated with obliteration and narrowing of resistance pulmonary arteries and thickening of the intima [77,78]. The finding of fibromuscular hyperplasia of the intima in chronic pulmonary hypertension has caused increased interest in the role of the pulmonary endothelium [79]. Important differences in the levels of eicosanoids and endothelium derived nirtic oxide production between primary and secondary pulmonary suggests that the two different forms of pulmonary hypertension involve seperate underlying mechanisms and distinct patterns of endothelial dysfunction [79].

Primary pulmonary hypertension (PPH) has, by definition, no known cause. It affects young adults, women being afflicted more often than men [79]. Usually the patients afflicted by this disorder test positive to antinuclear antibodies (ANA) which is often linked to systemic lupus erythomatosus (SLE) and antiphospholipid antibody syndromes [79]. Other know triggers for plexigenic pulmonary arteriopathy include cirrhosis and portal hypertension, atrial septal defect, anorexigens, L-tryptophane, cocaine, autoimmune disorders, human immunodeficiency virus infection, obesity and thyroid disease [80]. PPH is characterized by abnormalities of the pulmonary vascular biology in each layer of the blood vessel. The lumen has a prothrombotic diathesis, the smooth muscle cells are depolarized and calcium-overloaded and the adventitia displays excessive remodeling [81]. In PPH, the endothelium produces excessive amounts of vasoconstrictor factors such as endothelin-1, develops

abnormalities in the balance of prostacyclin and thromboxane production and an increased secretion of the procoagulant plasminogen activator inhibitor [82]. In vitro studies on vessels of animals with pulmonary hypertension have demonstrated a loss of endothelium-dependent relaxation due to a decreased production of NO [83] secondary to a decrease in the expression and activity of eNOS [82]. Administration of NO directly to the pulmonary vasculature by means of inhalation has been effective in the short-term treatment of pulmonary hypertension [84,85]. Besides NO inhalation other vasodilatory treatments include: adenosine, calcium channel blockers, steroids, L-arginine (minor vasodilation), prostacyline and prostaglandins [80,86]. The specific lack of response to L-arginine in patients with PPH is compatible with a fundamental defect in the expression or activity of NOS which cannot be overcome by substrate loading [82].

Secondary pulmonary hypertension (SPH) affects patients with congestive heart failure (CHF) and patients with alveolar hypoxemia from chronic obstructive lung disease. SPH due to CHF is the consequence of pulmonary arterial wall remodeling leading to abnormalities of elastic fibers, intimal fibrosis, and medial hypertrophy that result in vascular stiffness and reduced vasodilator responsiveness [87]. Secondary pulmonary hypertension is also associated with a decreased pulmonary clearance of endothelin-1, a decreased intracellular availability of L-arginine, a decreased production of endothelium derived NO and a decreased activity of eNOS [79,88]. Possible therapeutic approaches include: NO inhalation, L-arginine (major vasodilatation), prostacyclin, and oxygen therapy [82]. This favorable response to substrate loading suggests that a limitation of the intracellular availability of L-

arginine, possibly due to defective transport, might contribute to the low levels of basal nitric oxide production in the pulmonary circulation of patients with SPH [82].

Cardiopulmonary Bypass

Cardiopulmonary bypass (CPB) remains essential for the majority of cardiac operations since it allows the surgeon to work while the heart is stopped and the lungs are deflated. CPB replaces the functions of the heart and lungs by temporarily bypassing the venous blood through an extracorporeal device that oxygenates and returns the blood to the arterial system of the patient [89]. In the clinical setting, after attaining a steady state on pump most of the venous blood is deviated in the venous reservoir of the CPB machine resulting in minimal blood flow through the pulmonary circulation. Subsequently, the ventilator is turned off causing a further decrease in blood flow through the pulmonary artery.

Although extracorporeal circulation is useful and saves many lives, it causes a significant amount of stress and injury to the organism, a phenomenon known as the whole body inflammatory response, which is triggered by the contact of blood elements with the nonbiocompatible material of the bypass circuit [7]. More specifically, this systemic inflammatory response involves the activation of the alternate complement pathway, leukocytes, and endothelial cells with release of cytokines, proteases, leukotrienes, arachidonic acid metabolites, oxygen free radicals and is also associated with clumping of neutrophils which can lead to obstruction of capillary blood flow, coagulopathy, bleeding, and significant end-organ dysfunction, including neutrophil-mediated pulmonary injury [90]. Activation of the complement pathway has been incriminated as the major biochemical pathway mediating lung

injury due to the adhesion of polymorphonuclear leukocytes to the microvascular endothelium resulting in leukocyte extravasation.

The Pathophysiology of Lung Injury

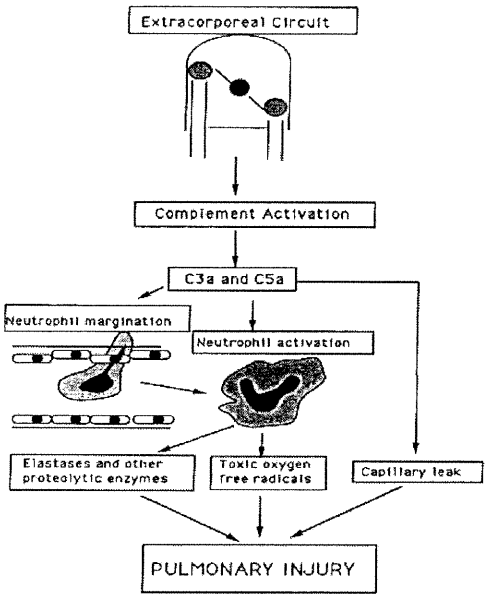
The pulmonary circulation is a low-pressure, high-capacity system [87] with a normal pulmonary artery systolic pressure of ≤ 25 mm Hg and a pulmonary vascular resistance (PVR) averaging 67 ± 30 dynes•s•cm^{-2} at sea level [91]. The pulmonary vasculature can accommodate large increases in blood flow with little increase in pressure during exercise [92] or during occlusion of either the right or left pulmonary artery [93].

The right ventricle (RV) can accommodate large increases in venous return without a rise in pulmonary arterial pressure but, in contrast, modest increase in pulmonary vascular tone can result in RV failure [87]. Thus, the pulmonary circulation is important in the modulation of right ventricular afterload and RV output. It is also a major determinant of the regulation of venous return to the left ventricle (LV) hence protecting the LV against excess preload [87].

During CPB, the standard practice is to stop mechanical ventilation of the lungs and to let them equilibrate to atmospheric pressure. In this state, the lungs are subjected to micro atelectasis, encounter surfactant washout, interstitial edema and associated lung water accumulation which result in a decrease in the static lung compliance. In particular, activation of the complement cascade and neutrophils leads to the release of cytokines, proteases, arachidonic acid metabolites, and oxygen free radicals which cause endothelial dysfunction and induce pulmonary vasoconstriction (Figure 9). The increase in pulmonary vascular resistance causes

ventilation-perfusion mismatches that lead to hypoxia and acute hypoxic vasoconstriction [69].

Figure 9: Pathophysiology of lung injury after CPB.



Lung injury remains an important cause of postoperative morbidity, despite continuing improvements in CPB techniques and postoperative care. The major causes of pulmonary morbidity post-CPB are atelectasis, acute respiratory distress syndrome (ARDS), and pulmonary hypertension. Almost all patients suffer from varying degrees of atelectasis which is the collapsing of lung tissue. ARDS also known as acute lung injury or "pump lung" is a rare complication which occurs in 1.3% of cases but results in a mortality ranging between 50% to 75% [89]. ARDS results from the systemic inflammatory response due to CPB which leads to complement activation and capillary leak, causing flooding of the pulmonary interstitium [89].

Pulmonary hypertension is present when the pulmonary artery systolic pressure exceeds 30 mm Hg or the mean pulmonary artery pressure is ≥19 mm Hg [87]. An underlying degree of pulmonary hypertension is present in up to 20% of cardiac surgery patients and its reversibility is dependent upon the degree of endothelial dysfunction and whether it is associated intimal proliferation and medial hypertrophy. The most frequent cause of lung injury post-CPB results from damage of to the endothelium barrier leading to subclinical pulmonary edema.

CORPS DE LA THÈSE : CHAPITRE II

HYPOTHÈSES ET BUTS

Few studies have analyzed the mechanism associated with lung injury after CPB by examining responses of isolated pulmonary arteries to selective endothelium-dependent and independent activators [5-7]. However, no studies have assessed the effect of ventilation and NO inhalation during CPB on pulmonary artery endothelial dysfunction.

Nyhan et al. have shown in a chronic canine model that CPB induces a selective pulmonary artery endothelial dysfunction to acetylcholine observed 4 days after CPB with recuperation of the function at 14 days after CBP [5]. This study focuses on the chronology and mechanisms of pulmonary endothelial dysfunction in the short term/acute phase, rather than in the chronic phase, since it is often immediately after weaning from CPB and in the days following surgery that severe hypoxia and pulmonary complications occur.

The objectives of the present study were to identify the mechanism of pulmonary endothelial dysfunction, specifically the role of reperfusion after CPB in the acute pathogenesis of endothelial dysfunction, to characterize the alterations in signal transduction pathway of endothelial cells in second degree pulmonary artery, and to determine whether ventilation with and without additional NO inhalation during CPB could prevent the pulmonary endothelial dysfunction.

CORPS DE LA THÈSE : CHAPITRE III

ARTICLE

Ventilation and NO inhalation Prevent Pulmonary Endothelial Dysfunction due to Cardiopulmonary Bypass

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Short Title: Ventilation and NO inhalation Prevent Pulmonary Dysfunction After CPB

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Abstract

Background. Cardiopulmonary bypass (CPB) remains the cornerstone of the majority of cardiac surgeries. Endothelial dysfunction of the pulmonary arterial tree may contribute to pulmonary hypertension and respiratory failure in the postoperative period. This objective of this study is to characterise the alterations of endothelial cell signal transduction in the pulmonary arteries following CPB and the effect of ventilation and NO inhalation during CPB on the endothelial dysfunction.

Methods. Six groups of Landrace swine were compared: group 1 = control, group 2 = sham without CPB, group 3 = CPB 150 minutes + no reperfusion, group 4 = CPB 150 minutes + reperfusion 60 minutes, group 5 = CPB 150 minutes + ventilation (tidal volume 12 ml/kg) + reperfusion 60 minutes, group 6 = CPB 150 minutes + nitric oxide (NO) inhalation (with ventilation, NO 40 ppm) + 60 minutes of reperfusion. The heart and lungs were harvested *en bloc*. Branches of second degree pulmonary arteries were isolated and dissected free of connective tissue and adventitial tissue and divided into rings (4mm wide; 16 rings per animal). Contractions were measured with potassium chloride and phenylephrine. Endothelium-dependent relaxations were studied using acetylcholine (ACh) and bradykinin (BK) and endothelium-independent relaxations with sodium nitroprusside (SNP).

Results. There was a statistically significant decrease in the amplitude of the contraction to potassium chloride (60 mM) and phenylepherine in the group CPB 150 minutes + no reperfusion and the group submitted to CPB 150 minutes + NO inhalation (with ventilation, NO 40 ppm) + 60 minutes reperfusion versus the control group.

There were no statistically significant differences between the 6 groups for endothelium-dependent relaxations to BK and endothelium-independent relaxations to SNP. CPB without reperfusion did not induce a pulmonary endothelial dysfunction. CPB with reperfusion induced a statistically significant decrease in endothelium-dependent relaxations to ACh compared to groups 1, 2 and 3. Normal volume ventilation and NO inhalation prevented the reduction of endothelium-dependent relaxations to ACh.

Conclusion. CPB without reperfusion did not cause endothelial dysfunction of the pulmonary artery. Reperfusion after CPB caused an alteration of the endothelial cell vasorelaxations, most likely through the production of activated oxygen radicals. Mechanical ventilation and NO inhalation during CPB prevented the endothelial dysfunction due to reperfusion of the pulmonary artery after extracorporeal circulation.

KEY WORDS: Endothelium, pulmonary arteries, cardiopulmonary bypass, ventilation and NO inhalation.

Introduction

Cardiopulmonary bypass (CPB) replaces the functions of the heart and lungs during most cardiac operations and causes a significant amount of stress and injury to the organism as the blood elements come in contact with the nonbiocompatible material of the bypass circuit resulting in the pathological activation of numerous inflammatory and coagulatory cascades leading to the phenomenon of systemic inflammatory response syndrome (SIRS). SIRS causes the activation of the alternate complement pathway, leukocytes, and endothelial cells releasing cytokines, proteases, leukotrienes, arachidonic acid metabolites, oxygen free radicals and neutrophil clumping with obstruction of capillary blood flow [90]. Leukocyte adhesion to the microvascular endothelium, leukocyte extravasation, and tissue damage are the final steps [90] leading to major organ dysfunction.

Lung injury remains an important cause of postoperative morbidity, despite continuing improvements in CPB techniques and postoperative care. Reperfusion injury is also observed after lung transplant. A significant consequence of reperfusion injury is the dysfunction of the pulmonary vascular endothelium, which may manifest itself as pulmonary hypertension and increased vascular permeability, resulting in pulmonary edema and impaired gas exchange [94]. Pulmonary hypertension occurs in 20% of cases and its reversibility depends on the underlying endothelial dysfunction and on the presence of intimal proliferation and medial hypertrophy.

The normal endothelium plays a central role in local vasoregulation by releasing a variety of vasoactive substances and inhibiting the contraction of vascular smooth muscle cells, the proliferation of smooth muscle cells and

fibrocytes, providing a nonthrombogenic surface and a selective barrier to circulating proteins and cellular components [4]. Furchgott, and Zawadzki [9], and Ignarro et al. [10] proposed NO, a labile diffusible, nonprostanoid substance that mediates the endothelium-dependent relaxation to acetylcholine (ACh), as the most logical candidate for the endothelium derived relaxing factor (EDRF). NO is formed from L-arginine, by the enzyme endothelial NO synthase (eNOS), which is constitutive in normal endothelial cells [95]. The degree of activation of eNOS depends on the intracellular concentration of calcium ions present in endothelial cells and is calmodulin-dependent [19]. When NO diffuses to the vascular smooth muscle cells, it stimulates the cytosolic enzyme, soluble guanylate cyclase that leads to an increase in cyclic GMP (cGMP) and causes vasodilatation [12]. The impairment of endothelial function may result in abnormal pulmonary vasodilator and vasoconstrictor responses.

Nyhan et al. observed that CPB induced a selective pulmonary artery endothelial dysfunction to acetylcholine at 4 days post CPB with recuperation of the function at 14 days after-CBP in a chronic canine model [5]. This study focuses on the mechanisms of pulmonary endothelial dysfunction in the short term/acute phase, rather than in the chronic phase, since it is often immediately after weaning from CPB that severe pulmonary problems are encountered.

The objectives of the present study were to identify the mechanism of pulmonary endothelial dysfunction, specifically the role of reperfusion after CPB in the acute pathogenesis of endothelial dysfunction, to characterize the alterations in signal transduction pathway of endothelial cells in second degree pulmonary

arteries, and to determine whether ventilation with and without additional NO inhalation during CPB could prevent the pulmonary endothelial dysfunction.

Material and Methods

Experimental Preparation for all Groups (Anesthesia)

All experiments were performed using Landrace white swine of either gender, aged 8 weeks and weighing 23.2 ± 0.4 kg. Animals were treated in compliance with the recommendations of the Guidelines on the Care and Use of Laboratory Animals issued by the Canadian Council on Animal and the Guidelines of the Animal Care and were approved by a local committee. The piglets were fasted for 12 hours before the surgery, sedated with an intramuscular injection of ketamine (20 mg/kg; Rogarsetic, Quebec, Canada) and xylazine (2 mg/kg; Rompun, Ontario, Canada), and induction was achieved using mask ventilation with 2% isoflurane (Ohmeda, Ontario, Canada). Subsequently, the animals were intubated and general anesthesia was maintained by inhalation of isoflurane with normal ventilation of an O_2/air mixture at 20 breaths/minute with a tidal volume of 12 ml/kg. Arterial and venous blood gases were measured at regular intervals during the experiment and maintained within the physiological range by adjusting the inspired oxygen fraction (FiO_2), ventilation rate, tidal volume, and acidosis were balanced with 8.4% sodium bicarbonate (Abbott Laboratories, Ville St-Laurent, Quebec, Canada).

Experimental Groups

Six groups were compared: group 1 = control, group 2 = sham without CPB, group 3 = CPB 150 minutes + no reperfusion, group 4 = CPB 150 minutes + reperfusion 60 minutes, group 5= CPB 150 minutes + ventilation (tidal volume 12 ml/kg) + reperfusion 60 minutes, group 6 = CPB 150 minutes + NO inhalation (with ventilation, NO 40 ppm + 60 minutes of reperfusion) (Table 1).

Group 1: Control (n = 6)

After induction of general anesthesia, a median sternotomy was performed, the animal was exsanguinated, and the heart and lungs were harvested *en bloc*.

Group 2: Sham Without CPB (n = 6)

With the piglets in a supine position, the EKG probes and the rectal thermometer probe were installed. Then, the swine was shaved, disinfected and drapped with sterile fields. The jugular vein and the carotid artery were canulated to obtain a central venous line and for monitoring of the systemic arterial pressure respectively. The femoral vessels were isolated on both sides and a median sternotomy was performed. The pericardium was suspended using silk 5-0 and a double purse string was made on the right atrial appendage using a prolene 4-0.

Three minutes after heparin administration (3 mg/kg), a blood sample was drawn from the right atrium and the level of anticoagulation was assessed using an activated coagulation time (ACT) with Hemochron 801 (Technidyne, NJ, USA). When the ACT was superior to 200 seconds, the atrial appendage and the right femoral artery were canulated with a dlp 30-Fr double-staged (Medtronics,

Mississauga, Ontario, Canada) and a Bardic 12-Fr (USCI, Division of BARD, NY, USA) respectively. After 60 minutes, the heart and lungs were harvested *en bloc*.

Group 3: CPB 150 Minutes no Reperfusion (n = 4)

In group 3: CPB 150 minutes no reperfusion, the same procedure was followed as in group 2 with the use of CPB. The jugular vein was canulated and a bolus of fentanyl (Abbott Laboratories, Ville St-Laurent, Quebec, Canada) 15 μg/kg was given in 10 minutes. Anesthesia was maintained using a continuous infusion of fentanyl (2,500 μg) and midazolam (Sabex, Boucherville, Québec, Canada) (100 mg) given at a rate of 15 ml/hour. The carotid artery was canulated to monitor the systemic arterial pressure. A cystostomy was performed to monitor the urine output per-CPB.

CPB was initiated when the ACT was superior to 400 seconds. The bypass circuit (figure1) consisted of a filtered hardshell venous reservoir (Minimax 1316, Medtronics, Mississauga, Ontario, Canada), a hollow fiber membrane oxygenator (Minimax Plus 3381 without Carmeda Bioactive Surface, Medtronics, Mississauga, Ontario, Canada), a heater-cooler, and a roller pump (Sarns 7000, Ann Arbor, Michigan, USA). No arterial filter was used. The circuit was primed with 15 mEq of bicarbonate, 25 ml of mannitol, 5,000 units of heparin, 300 ml of lactated ringer and 300 ml of pentaspan. After initial stabilization, the pump flow was adjusted to obtain an index of 2.4 L/min/m^2 and the mean systemic arterial pressure maintained between 50 and 75 mm Hg using phenylephrine, as needed. After a steady state under CPB was obtained, ventilation was stopped and the body temperature of the animal drifted to 34°C. The heart was not arrested during CPB. Approaching the

150 minutes of CPB, the animal was rewarmed to 37°C. After 150 minutes of CPB, the pump was stopped, the animal was immediately sacrificed, and the heart and lungs were harvested *en bloc*.

Group 4: CPB 150 Minutes + Reperfusion 60 Minutes (n = 6)

In group 4: CPB 150 minutes + reperfusion 60 minutes, the same procedure was followed as in group 3. After 150 minutes on pump, mechanical ventilation with isoflurane was reinstituted. Phenylepherine was started at the completion of the CPB to maintain the hemodynamics within normal range when needed. After 60 minutes of reperfusion the heart and lungs were harvested *en bloc*.

Group 5: CPB 150 Minutes + Ventilation (Tidal Volume 12ml/kg) + Reperfusion 60 Minutes (n = 4)

In group 5: CPB 150 minutes + ventilation (tidal volume 12 ml/kg) + reperfusion 60 minutes, the same procedure was followed as in group 4, however ventilation without isoflurane was maintained during the whole experiment.

Group 6: CPB 150 Minutes + Ventilation (Tidal Volume 12ml/kg) + NO Inhalation (NO 40 ppm) + 60 Minutes of Reperfusion (n = 4)

In group 6: CPB 150 minutes + Ventilation (Tidal Volume 12ml/kg) + NO inhalation (NO 40 ppm + 60 minutes of reperfusion), the same procedure was followed as in group 4. Ventilation was maintained during the whole experiment and NO was administered at a rate of 40 ppm during the 150 minutes on pump.

NO was administered using a NO apparatus developed by the department of anaesthesiology in conjunction with the department of biomedical physical physics at

the pavillon Notre-Dame (CHUM), Quebec, Canada. This apparatus was calibrated prior to each experiment and injected NO cyclically with a precision flowmeter during the inspiratory phase. NO was measured using a NO/NO2 electrode.

Vascular Reactivity Studies

After harvest, the heart and lungs were rapidly placed in a modified Krebs-bicarbonate solution (composition in mM: NaCl 118.3, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 11.1, $CaCl_2$ 2.5, $NaHCO_3$ 25 and ethylenediaminotetraacetic acid 0.026). Oxygenation was ensured using a carbogen mixture (95% O_2 and 5% CO_2). The heart was removed and the primary pulmonary artery was dissected. From the primary pulmonary artery, branches of second degree pulmonary arteries were isolated and dissected free of connective and adventitial tissue and divided into rings (4 mm wide; 16 rings per animal). In some rings, the endothelium was removed by inserting the tip of a pair or forceps in the lumen and gently rubbing the ring back and forth on a paper towel wetted with the Krebs-bicarbonate solution.

The vascular reactivity of rings of second degree pulmonary arteries from the 6 groups was studied in organ chambers filled with the modified Krebs-bicarbonate solution (20 ml at 37°C, see above for composition) and oxygenated with a 95% O_2 and 5% CO_2 mixture. The rings were suspended between 2 metal stirrups, one of which was connected to an isometric force transducer. Data were collected with a data acquisition software (IOS3, Emka Inc., Paris, France).

Each preparation was stretched to its active length curve (usually 3.5 g), as determined by measuring the contraction to potassium chloride (30 mM) at different levels of stretch, and then stabilized for 30 minutes. The maximal contraction was

determined with potassium chloride (60 mM) and rings were excluded if they failed to contract to potassium chloride (exclusion rate less than 5%). The baths were then washed and indomethacin (10^{-5} M) was added to exclude the production of endogenous prostanoids. After 60 minutes of stabilization, phenylephrine (range 2×10^{-7} M to 3×10^{-6} M) was added to achieve a contraction averaging 50% of the maximal contraction to KCl (60 mM).

The NO-mediated relaxation pathway was studied by constructing concentration-response curves to acetylcholine (ACh, 10^{-9} M to 10^{-3} M; an agonist binding to muscarinic receptors coupled to Gi proteins), and Bradykinin (BK, 10^{-12} M to 10^{-6} M; an agonist binding to B_2 receptors coupled to Gq proteins) (Figure 2). Endothelium-independent relaxations were studied in rings with and without endothelium using sodium nitroprusside (SNP, 10^{-10} M to 10^{-5} M; an exogenous NO donor). A bolus of SNP (10^{-5} M) was also given at the end of the experiment in every organ chamber to assess the integrity of the vascular smooth muscle cells. No rings were submitted to more than one endothelium-dependent agonists.

Drugs

All solutions were prepared daily. Potassium chloride, phenylephrine, acetylcholine, bradykinin, indomethacin, and sodium nitroprusside were purchased from Sigma Chemical Co (Oakville, Ontario, Canada).

Cyclic GMP measurement

Basal levels of cGMP present in the second degree pulmonary arteries of all groups were measured. Segments were frozen in liquid nitrogen and stored at -70°C until the measurement of cGMP. The samples were subsequently pulverized and resuspended in trichloroacetic solution (TCA; 6.25% w/v) to precipitate the proteins of the tissue. After centrifugation, the supernatant was washed with diethylether to preserve the cGMP and eliminate the TCA. Finally, the samples were heat dried by nitrogen gaz to obtain purified cGMP. The cGMP was measured using enzymeimmunoassay (EIA) system with acetylation based on rabbit anti-cGMP antibody (Amersham). cGMP levels were adjusted to the quantity of proteins measured in the tissue using the Bradford microassay technique (Bio-Rad).

Endothelial coverage studies using silver nitrate staining

The endothelial coverage was studied by staining using silver nitrate segments of pulmonary arteries of the group 4 = CPB 150 min + reperfusion 60 minutes. The rings were fixed first for 10 minutes with buffer paraformaldehyde (4%). They were then washed for 1 minute with Hepes sucrose buffer solution. Silver nitrate 0.25% was applied for 1 minute. Washing was performed for 1 minute before a second fixation for 2 minute. The rings were exposed to ultraviolet light for 2 to 4 hours in cacodylate buffer solution. Preparation were read by a blinded investigator and representative photomicrograph were taken.

Statistical Analysis

Contractions to phenylephrine (PE) are expressed as a percentage of the maximal contraction to potassium chloride (60 mM) for each group and expressed as

mean ± standard error of the mean (SEM); "n" refers to the number of animals studied. Relaxations are expressed as percentage of the maximal contraction to PE for each ring. ANOVA studies with repeated measures were performed to compare contraction-response curves and to compare cGMP measurements. Differences were considered to be statistically significant when $p < 0.05$.

Results

Vascular Reactivity

Contraction

There was a statistically significant decrease in the amplitude of the contraction to KCl (60 mM) between group 3: CPB 150 minutes no reperfusion and group 1: control and also between group 6: CPB 150 minutes + NO inhalation (with ventilation, NO 40 ppm) + 60 minutes reperfusion and group 1: control. For all the other groups, there were no significant differences in the amplitude of the contraction to KCl (60 mM) (Table 2).

There was a statistically significant decrease in the amplitude of the contraction to PE between group 3: CPB 150 minutes no reperfusion and group 1: control and also between group 6: CPB 150 minutes + NO inhalation (with ventilation, NO 40 ppm) + 60 minutes reperfusion and group 1: control. For all the other groups, there were no significant differences in the amplitude of the contraction to PE (Table 2).

Endothelium-dependent Relaxations

CPB with reperfusion (group 4) induced a statistically significant decrease in endothelium-dependent relaxations to ACh compared to groups 1, 2 and 3 (Figure 3a). CPB with reperfusion (group 4) induced a statistically significant decrease in endothelium-dependent relaxations to ACh compared to groups 1, 5 and 6 (Figure 3b). There were no statistically significant differences between groups 1,2,3,5,6 for endothelium-dependent relaxations to ACh.

There were no statistically significant differences between the 6 groups for endothelium-dependent relaxations to BK. The concentration-response curves to BK of the 6 groups are depicted on 2 separate graphs for clarity (Figure 4 a & b).

Endothelium-independent Relaxations

There were no statistically significant differences between the six groups for endothelium-independent relaxations to SNP with and without endothelium. The concentration-response curves to SNP of the 6 groups are depicted on 2 separate graphs for clarity (Figure 5 a & b).

Cyclic GMP measurement

There was a statistically significant decrease in the levels of cGMP in the sham (group2), CPB without reperfusion (group 3), CPB with reperfusion (group 4), ventilation (group 5) and NO inhalation (group 6) when compared to group 1: control (Figure 6).

There was a statistically significant decrease in the levels of cGMP in the CPB without reperfusion (group 3), CPB with reperfusion (group 4), ventilation (group 5) and NO inhalation (group 6) when compared to group 2: sham (Figure 6).

Endothelium coverage studies using silver nitrate staining

In the present study, there is no endothelial denudation immediately after CPB with reperfusion since staining with silver nitrate of the second degree pulmonary arteries show preservation of the normal cobblestone patterns of the endothelial surface (Figure 7).

Discussion

The major findings of the present study are that: (1) CPB without reperfusion does not cause endothelial dysfunction of the pulmonary artery tree as evidenced by the absence of significant differences between the control, the sham and the CPB without reperfusion group; (2) CPB with reperfusion induced a selective decrease in endothelium-dependent relaxation to ACh which is coupled to Gi proteins; (3) normal ventilation as well as ventilation with NO inhalation during CPB prevented the reduction of relaxation after reperfusion following CPB; and (4) there are no significant differences between the normal ventilation and NO inhalation groups compared to the control, the sham and the CPB without reperfusion group.

Vascular Reactivity

Contraction

The decrease in contraction to KCl and PE for the CPB 150 minutes + no reperfusion group may be due to the release of vasodilating factors during CPB via the release of cytokines resulting in an increase of inducible nitric oxide synthase (iNOS), or to a decrease in vasoconstrictive factors or a combination of both in the absence of reperfusion.

The decrease in contractions to KCl and PE in the CPB 150 minutes + ventilation (Tidal Volume 12 ml/kg) + NO inhalation (NO 40 ppm) + 60 minutes reperfusion is possibly mediated via a reversible negative feedback loop. Several studies demonstrated that NO inhalation causes a downregulation of eNOS to stop the endogenous endothelial release of NO [96-99]. The eNOS downregulation is reversible and followed by an upregulation of eNOS when inhaled NO is stopped to increase to synthesis of NO to pre-NO inhalation value [96-98]. Thus, inhaled NO can possibly mediate a sustained vasodilatation of the pulmonary artery after the inhalation has been stopped.

Endothelium-dependent Relaxations to ACh

ACh binds to muscarinic receptors (M_2) on endothelial cells which are coupled to Gi proteins, sensitive to the pertussis toxin, releasing NO and thus causing endothelium-dependent relaxation in human pulmonary arteries.

The Effect of Reperfusion on Endothelium-dependent Relaxations to ACh

During total CPB, as used clinically, pulmonary blood flow is completely shut off and the lungs are perfused by bronchial flow alone. This state of reduced pulmonary perfusion followed by restoration of normal antegrade perfusion causes an ischemia-reperfusion mediated injury to the lungs [71]. Reperfusion of the pulmonary arterial tree after CPB (group 4) induced a significant decrease in endothelium-dependent relaxations to ACh which suggests that ischemia followed by reperfusion causes endothelial dysfunction in second degree pulmonary arteries.

In support of this finding, Shafique and colleagues also demonstrated that reperfusion of the lungs markedly altered pulmonary microvascular responses to ACh which was primarily due to pulmonary vascular ischemia and reperfusion rather than to the extracorporeal circulation [71]. Serraf and colleagues also observed a decrease in endothelium-dependent relaxations to acetylcholine in pulmonary artery rings of piglets undergoing CPB with lung reperfusion when compared to the pulmonary artery rings of sham piglets [7]. Furthermore, gas exchanges worsened after reperfusion with pulmonary hypertension, myeloperoxidase increased in the lungs, viability of pulmonary endothelial cells were reduced by 50%, endothelial cell growths were faster in pulmonary arteries and leukocyte-pulmonary endothelial cell adhesion and cytotoxicity increased [7]. In Nyhan et al., acetylcholine evoked concentration-dependent relaxation in rings with endothelium that was significantly depressed four days post-CPB compared to control [5]. Chai and colleagues also demonstrated that cessation of pulmonary arterial flow is an important factor in post-CPB lung injury [72]. Richter and colleagues have used the Drew-Anderson technique, where the patient's own lungs are used as the oxygenator, and observed that perfusion of the lungs during CPB causes a

significant decrease of inflammation and pulmonary dysfunction as assessed by the decrease in pro-inflammatory interleukin-6 and interleukin-8, and by the shorter intubation time and reduced blood loss [100].

Reperfusion after CPB is followed by a massive production of oxygen free radicals which most likely mediate the decrease in endothelium-dependent relaxations to ACh. Oxygen free radicals, which are neutral molecular species with an unpaired electron in their outer electron shell [101], are generated by pulmonary endothelial cells during reoxygenation after hypoxia [102]. Seccombe and Schaff have demonstrated that the oxidative injury may selectively injure the receptor/G-protein complex specific to the NO signal transduction pathway rather than causing global receptor/G-protein dysfunction [103]. After reperfusion of the pulmonary tree, endothelium-dependent relaxations to acetylcholine are significantly impaired and involve selectively the pertussis toxin sensitive Gi-protein [104]. This concurs with the observations in this model of CPB with reperfusion in which only the endothelium-dependent relaxations to ACh and not those to BK were affected by reperfusion of the pulmonary tree. In vascular beds other than the pulmonary artery, ischemia followed by reperfusion also results in a selective endothelial dysfunction associated with a decrease in endothelium-dependent relaxations to ACh suggesting that Gi proteins are more sensitive to injury. In the present study, the selective decrease in endothelium-dependent relaxation to ACh (Gi protein) is not mediated by endothelial denudation for two reasons: 1) endothelium-dependent relaxation to BK are preserved and 2) silver nitrate staining shows no denudation.

The Effect of Ventilation on Endothelium-dependent Relaxations to ACh

Maintenance of mechanical ventilation during CPB prevented the occurrence of the impairment of endothelium-dependent relaxations as suggested by the absence of significant difference between the normal ventilation group compared to the control, the sham and the CPB without reperfusion groups. Using a ferret lungs model, Becker and Sylvester showed that pulmonary ischemia is not synonymous with hypoxia if ventilation is maintained while blood flow is maintained at low levels. Furthermore, they observed that maintenance of intravascular pressures at physiological levels in the pulmonary arterial tree during ventilated ischemia attenuated lung injury by maintaining basal levels of NO production, increasing the degree of static and circumferential hoop stretch of the endothelium, preserving a rhythmic movement of fluid between alveolar and extra-alveolar vessels during ventilation generating shear forces on the endothelium, increasing intravascular volume and diluting the effect of toxic mediators released into the vasculature during the ischemic period [105,106]. The protective effect of ventilation during CPB may be mediated by the preservation of the blood flow through the pulmonary artery hence preventing the endothelial dysfunction seen in the CPB with reperfusion group. Loeckinger et al. have found that ventilation (7 ml/kg tidal volume at 15 breaths/min respiratory rate with positive end-expiratory pressure (PEEP) of 5 cm H_2O and with a continuous positive airway pressure (CPAP) of 10 cm H_2O) during CPB resulted in improved postoperative gas exchange when compared to deflated lungs open to the atmosphere [107].

However, Serraf et al. observed in a neonatal piglet model of CPB with maintenance of ventilation (40 breaths/min, tidal volume 15 ml/kg) impaired pulmonary artery endothelium-dependent relaxations to ACh [6]. This contradictory result could

possibly be explained by 2 reasons. First, Serraf et al. used a neonatal piglet model. The neonatal pulmonary endothelium responds differently than the adult pulmonary endothelium. Second, a tidal volume of 15 ml/kg may cause barotrauma and volumetrauma which could possibly result in impaired endothelium-dependent relaxations to ACh.

The Effect of NO Inhalation on Endothelium-dependent Relaxations to ACh

NO inhalation during CPB prevented the alteration of endothelium-dependent relaxations compared to the CPB with reperfusion group since no significant differences between the NO inhalation group were found compared to the control, the sham, the CPB without reperfusion groups and the CPB with ventilation group. McMullan and colleagues have demonstrated that the endogenous production of NO is decreased after hypothermic CPB with a 30% decrease in the lung tissue concentrations of NO metabolites and cGMP post-CPB independent of changes in eNOS activity or gene expression [108]. Morita and colleagues have also found, in a piglet model undergoing 120 min of CPB followed by 60 min of reperfusion, that CPB impairs pulmonary NO production, resulting in pulmonary vasoconstriction and right ventricular dysfunction [109].

Inhaled nitric oxide provides a selective pulmonary vasodilatation with maintenance of systemic blood pressure and coronary perfusion pressure [110]. In the presence of right ventricular failure with increased pulmonary vascular resistance, inhaled nitric oxide in the range of 2-40 ppm is effective in reducing elevated pulmonary vascular resistance and does not increase cardiac output [110].

The effect of NO has been compared to the effect of intravenous nitropruside and sublingual nifedipine. Nitroprusside caused a similar degree of pulmonary vasodilation, although it caused increased heart rate and contractility, but overall systemic hypotension was observed [110]. Sublingual nifedipine causes pulmonary vasodilation, but right ventricular diastolic pressure increased and right ventricle contractility decreased [110]. Hence, the use of vasodilatory agents by inhalation such as NO have an advantage over intravenous agents because they cause the same decrease in the pulmonary pressure without the systemic hypotension.

Many studies have showed the efficacy of NO inhalation therapy for postoperative pulmonary hypertension as a supplementation of endogenous EDRF. However, 2 studies have demonstrated that NO inhalation at the start of reperfusion failed to provide protection of the pulmonary endothelium and was associated with the highest rate of lung neutrophil sequestration [94]. Indeed, as oxygen becomes available at the start of reperfusion, xanthine oxidase creates a burst of superoxide anion production with resultant tissue injury [94,111].

Serraf et al, observed in a neonatal piglet model, receiving nitric oxide ventilation (30 ppm) after CPB, an impairement of pulmonary artery endothelium-dependent relaxation to ACh when compared to the control group without CPB. Nontheless, they concluded that nitric oxide ventilation can prevent hemodynamic alterations after CPB but failed to prevent the biochemical disturbances. The contradictory results between the Serraf study and this study can possibly be explained by the difference in the model: neonatal versus adult and by the fact that NO inhalation was used after the completion of CPB in the Serraf study.

In this study, NO inhalation during CPB prevented the selective endothelial dysfunction as seen in the CPB with reperfusion group. NO inhalation during CPB group showed preservation of endothelium-dependent relaxation to ACh whereas the CPB with reperfusion group had impaired endothelium-dependent relaxation to ACh. However, this protective effect could possibly be obtained by ventilation alone because the CPB with ventilation group also showed preservation of endothelium-dependent relaxation to ACh. Hence, it is possible that the protective effect is not generated by NO inhalation per se but rather by normal ventilation during CPB.

A number of investigators have reported severe rebound pulmonary hypertension on inhaled nitric oxide withdrawal [112]. Gradual weaning is suggested in order to avoid the adverse rebound effects [113]. The easiest method is to increase the FiO_2 and to withdraw inhaled NO after the patient has significantly improved [114].

Endothelium-dependent Relaxations to BK

No statistically significant differences in endothelium-dependent relaxations to BK was observed in the concentration-response curves between the six groups. Hence, the endothelial dysfunction observed in this study is a selective pulmonary endothelial dysfunction that involves only Gi proteins and not Gq proteins.

Nyhan et al., found that in canine pulmonary arteries the endothelium-dependent relaxation to BK were not impaired 4 days after CPB in the CPB with reperfusion group [5]. In fact, in the Nyhan study the endothelium-dependent relaxation to BK were similar in control and post-CPB arteries [5].

Endothelium-independent Relaxations

At the end of each experiment using ACh and BK, a bolus of SNP was given and all rings relaxed appropriately confirming the integrity of the vascular smooth muscle cells. This also confirms that the endothelial dysfunction is not due to a functional disturbance of the vascular smooth muscle cell.

No statistically significant differences in endothelium-independent relaxations to NO donor, SNP was observed in the concentration-response curves between the 6 groups with and without endothelium. In two studies, Serraf et al. observed that the response to SNP were not altered after CPB termination [6,7].

Cyclic GMP measurement

In the present study, CPB without reperfusion (group 3), CPB with reperfusion (group 4), ventilation (group 5) and NO inhalation (group 6) have statistically significant decrease in the levels of cGMP when compared to the group 1: control. This finding is supported by McMullan et al. who have found, in 1 month-old lambs with normal or preexisting increased pulmonary blood flow undergoing CPB, that lung tissue concentration of cGMP decreased after CPB to ≈70% of pre-CPB value [108]. A decrease in nitrate and nitrate has also been found post CPB and appears to be independent of changes in eNOS, iNOS activity of gene expression [108]. In addition, Western blot analysis detected no changes in iNOS protein levels [108]. A decrease in NOS substrate or cofactor availability after CPB are possible mechanisms which warrants further studies [108]. From all this data, yet another mechanism could be inferred, CPB causes neutrophil activation leading to the

formation of free radicals which could bind NO forming peroxynitrites thus causing a decrease in the bioavailability of NO which in turn could lead to a decrease in cGMP.

However, it is unclear why there is a decrease in cGMP in shams versus the controls. The sham group was subjected to surgery (without CPB) which causes activation of the alternate complement pathway and could possibly explain the discrepancy in the cGMP result between the control and the sham.

The fact that CPB was not used in the sham group could possibly explain the statistically significant decrease in levels of cGMP in the CPB without reperfusion (group 3), CPB with reperfusion (group 4), ventilation (group 5) and NO inhalation (group 6) when compared to the group 2: sham.

Endothelium coverage studies using silver nitrate staining

In the present study there is no endothelial denudation immediately after CPB with reperfusion since silver nitrate staining of the second degree pulmonary arteries show preservation of the endothelial surface. This supports the finding that there is a selective Gi mediated dysfunction of endothelium dependent relaxation of the second degree pulmonary arteries

Clinical Relevance

The results of the present study are of significant clinical relevance because pulmonary complications remain an important cause of mortality and morbidity after CPB. In the present study, ventilation and NO inhalation during CPB prevented endothelial dysfunction post-CPB occurring during reperfusion of the pulmonary arterial tree after completion of the CPB run. Possibly all patients could

benefit from ventilation while being on pump at no additional cost. With the advent of beating heart surgery or of off-pump coronary artery bypass (OPCAB) surgeons are becoming used to operating while the lungs are ventilated and should be less reluctant to use mechanical ventilation during CPB. Furthermore, since lung injury is also observed in lung tranplantation, the current observations could be used in a broad platform of research leading to the development of preventive stategies.

Although, NO inhalation per CPB did not confer an added benefit in the current study over CPB with ventilation, the use of NO should not be undermined since it also prevented lung injury. The prophylactic use of inhaled NO could be beneficial in some subsets of patients with a severe underlying pulmonary hypertension preoperatively who may be more susceptible to pulmonary injury associated with a longer pump run. Inhaled NO has proved to be a very potent vasodilator and its clinical use against pulmonary hypertension is increasing. Finally, the use of ventilation and inhaled NO could be combined with the use of scavenger of free radicals, catalase, antioxidants, heparin-coated bypass circuits, and monoclonal antibodies to counterattack individual inflammatory mediators and hence minimize lung damage.

Morita et al. have shown that CPB impairs pulmonary NO production, resulting in pulmonary vasoconstriction which is reduced by antioxidants such as N-mercaptopropionylglycine and catalase [109]. Normandin et al. demonstrated that the addition of L-arginine or pentoxifylline during reperfusion prevented the pulmonary endothelial alteration resulting from warm reperfusion [65]. Turkoz et al. observed that pentoxifylline inhibits the postoperative increase in pulmonary vascular resistance and greatly minimize leukocyte sequestration in the lung due to CPB [115].

Redmond et al. demonstrated that heparin-coated bypass circuits reduce pulmonary injury [116]. Mayers et al. confirmed that blocking neutrophil adherence using anti-CD18 antibodies resulted in improved heart and lung function following cardiopulmonary bypass in dogs [117].

Limitation

The current study has several limitations. Young healthy swines were used in this model to examine pulmonary endothelial function after CPB. This model might not adequately represent the subsets of patients who present themselves for coronary artery bypass surgery, valvular surgery or transplantation and suffer from various associated clinical pathologies such as atherosclerosis, chronic hypertension, and ventricular dysfunction. Also, the experimental model did not assess the function of G proteins, nor the role of increased vasoconstrictor production such as endothelin or thromboxane A_2 on the pathogenesis of the endothelial dysfunction. Cardioplegia was not used in this model resulting in an empty but beating heart on pump. Protamine was not used at the end of the experiment to reverse the anticoagulant effect of heparin. The heparin-protamine complex has been shown to provoke catastrophic pulmonary vasoconstriction in some patients [118] which could have increased the injury observed in this model. On the other hand, protamine is a polycationic protein rich in the amino acide L-arginine, can also mediate pulmonary vasodilation [118]. The pulmonary resistance, pulmonary blood flow, pulmonary compliance, alveolo-arterial gradient and other parameters of pulmonary hemodynamics were not measured so the exact repercussions of pulmonary endothelial dysfunction on oxygenation is unknown. The time course and reversibility of these pathological findings remain unknown as

well as their relationship to the length of bypass time. Longer pump runs increase pulmonary complications and results in mortality and morbidity [69]. Finally, only one method of ventilation and one dose of inhaled NO (40ppm) were evaluated. Further investigations are needed to evaluate the effects of different ventilation techniques and different doses of NO.

Conclusion

CPB without reperfusion does not cause pulmonary arterial dysfunction. Reperfusion of the pulmonary arterial tree after CPB causes an alteration of the endothelial cell vasorelaxations. Normal mechanical ventilation with and without additional NO inhalation during CPB prevented the endothelial dysfunction due to reperfusion of the pulmonary tree after extracorporeal circulation and could potentially be used to minimize lung injury and associated morbidity in cardiac surgery using CPB.

Acknowledgement

We wish to thank Hugues Jeanmart, MD, Éric Dumont, MD, and Simon Fortier, MD for their technical and scientific contributions.

Figure Legends

Table 1 Experimental groups

Table 2 Contractions to KCl and PE of porcine pulmonary arteries.

Figure 1 CPB Setup

Figure 2 Agonists acting on the receptors on the endothelium with the subsequent response on the smooth muscle cells. (From:[1])

Figure 3a Concentration-response curve in porcine pulmonary arteries of endothelium-dependent relaxations to acetylcholine in four groups (group 1 = control, group 2 = sham without CPB, group 3 = CPB 150 min no reperfusion, group 4 = CPB 150 min + reperfusion 60 min). Relaxations are expressed as % contraction to phenylephrine and are presented as mean ± SEM.

Figure 3b Concentration-response curve in porcine pulmonary arteries of endothelium-dependent relaxations to acetylcholine in four groups (group 1 = control, group 4 = CPB 150 min + reperfusion 60 min, group 5 = CPB 150 min + ventilation (tidal volume 12 ml/kg) + reperfusion 60 min, group 6 = CPB 150 min + nitric oxide inhalation (with ventilation, NO 40 ppm) + 60 min of reperfusion). Relaxations are expressed as % contraction to phenylephrine and are presented as mean ± SEM.

Figure 4a Concentration-response curve in porcine pulmonary arteries of endothelium-dependent relaxations to bradykinin in four groups (group 1 = control, group 2 = sham without CPB, group 3 = CPB 150 min no reperfusion, group 4 = CPB 150 min + reperfusion 60 min). Relaxations are expressed as % contraction to phenylephrine and are presented as mean ± SEM.

Figure 4b Concentration-response curve in porcine pulmonary arteries of endothelium-dependent relaxations to bradykinin in four groups (group 1 = control, group 4 = CPB 150 min + reperfusion 60 min, group 5 = CPB 150 min + ventilation (tidal volume 12 ml/kg) + reperfusion 60 min, group 6 = CPB 150 min + nitric oxide inhalation (with ventilation, NO 40 ppm) + 60 min of reperfusion). Relaxations are expressed as % contraction to phenylephrine and are presented as mean ± SEM.

Figure 5a Concentration-response curve in porcine pulmonary arteries of endothelium-independent relaxations to sodium nitroprusside in three groups (group 1 = control, group 2 = sham without CPB, group 3 = CPB 150 min no reperfusion) with and without endothelium. Relaxations are expressed as % contraction to phenylephrine and are presented as mean ± SEM.

Figure 5b Concentration-response curve in porcine pulmonary arteries of endothelium-independent relaxations to sodium nitroprusside in three groups (group 4 = CPB 150 min + reperfusion 60 min, group 5 = CPB 150 min + ventilation (tidal volume 12 ml/kg) + reperfusion 60 min, group 6 = CPB 150 min + nitric oxide inhalation (with ventilation, NO 40 ppm) + 60 min of reperfusion) with and without endothelium. Relaxations are expressed as % contraction to phenylephrine and are presented as mean ± SEM.

Figure 6 Measurments of cGMP level in the 2nd degree pulmonary arteries of the 6 groups

Figure 7 Endothelial coverage studies using silver nitrate staining in group 4 = CPB 150 min + reperfusion 60 min.

Table 1

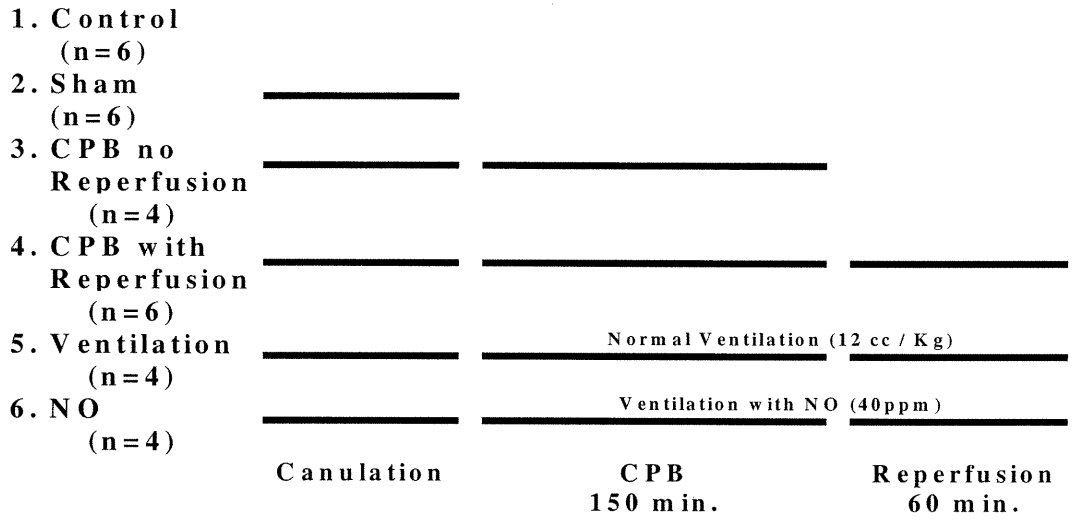


Table 2

Contraction to KCl and PE of porcine pulmonary arteries						
Group	**Control**	**Sham**	**CPB no reperfusion**	**CPB reperfusion**	**Ventilation**	**NO and Ventilation**
n	6	6	4	6	4	4
KCl (60 mM) (g)	4.97±0.22	4.20±0.20	3.64±0.20 *	4.94±0.23	4.98±0.38	3.28±0.23 *
PE (g)	2.92±0.12	2.50±0.12	2.11±0.10 *	2.49±0.13	3.07±0.23	2.06±0.40 *
Dosage of PE (10^{-7} M)[1]	5.30±0.70	5.10±0.67	10.33±1.93	9.10±1.19	12.15±1.40	6.14±.095

[1] For achieving target level of contraction Data are shown as means ± SEM

KCl : Potassium chloride, PE : Phenylepherine, CPB : Cardiopulmonary bypass, NO : Nitric oxide

Figure 1

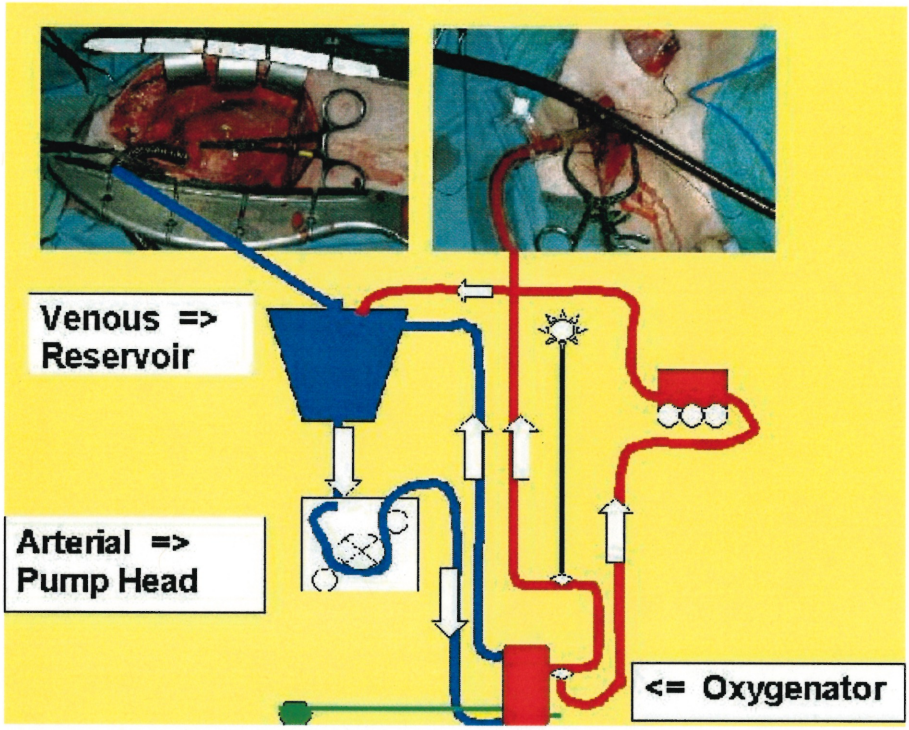


Figure 2

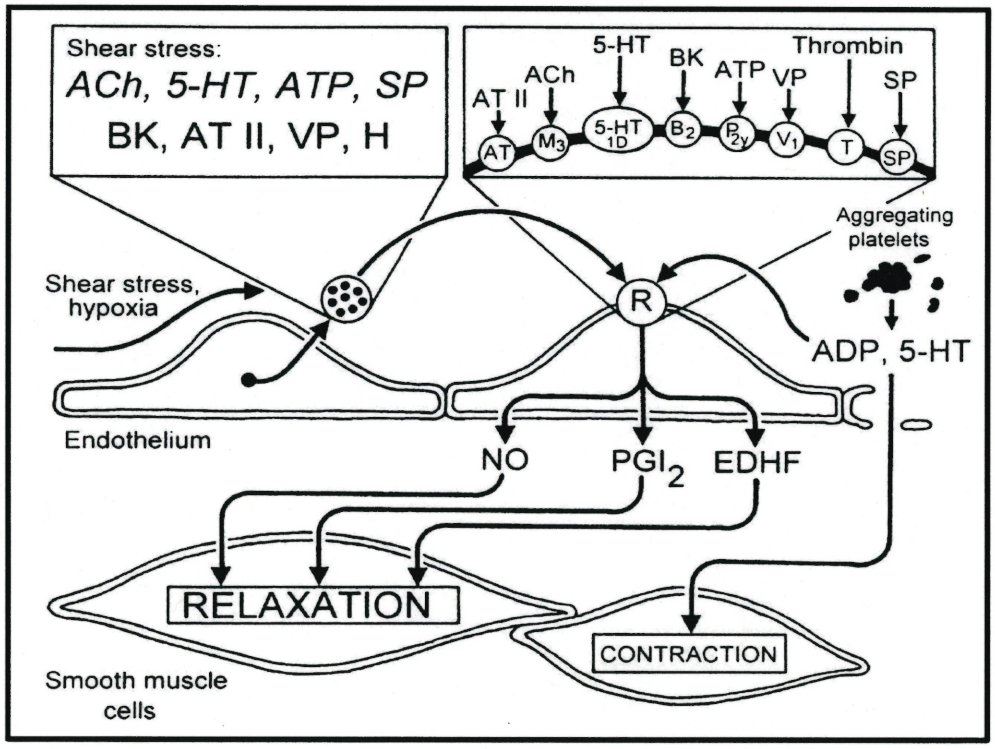


Figure 3a

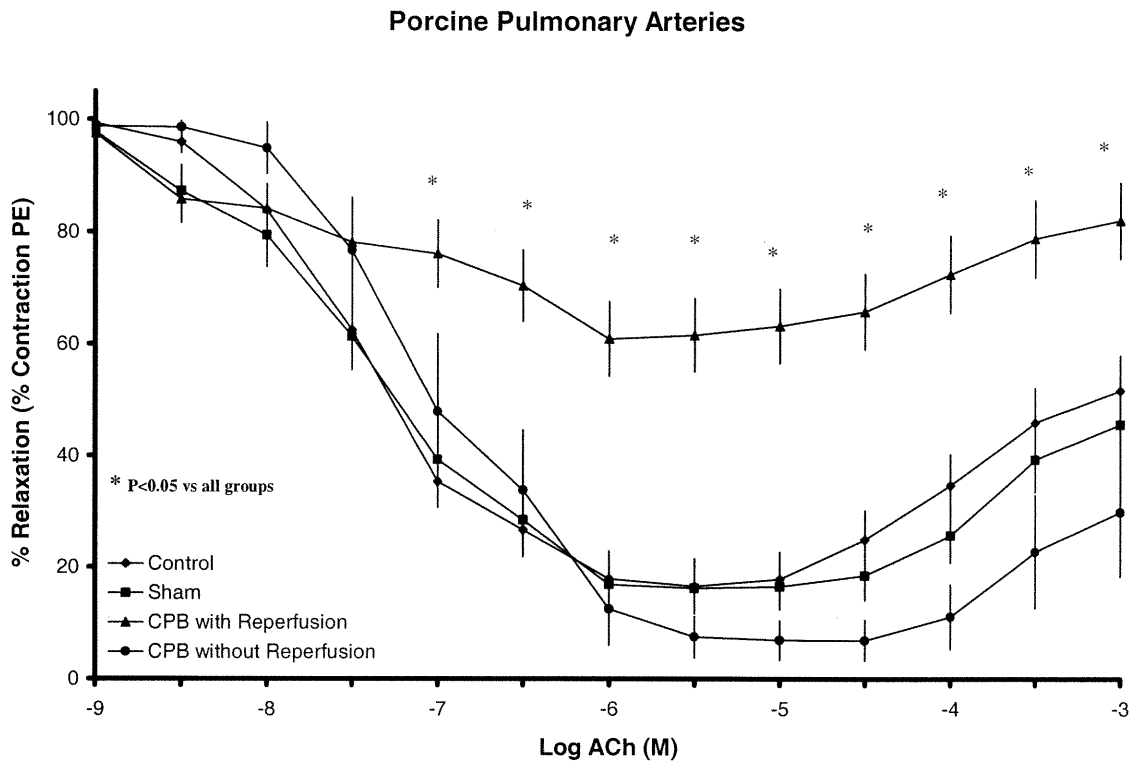


Figure 3b

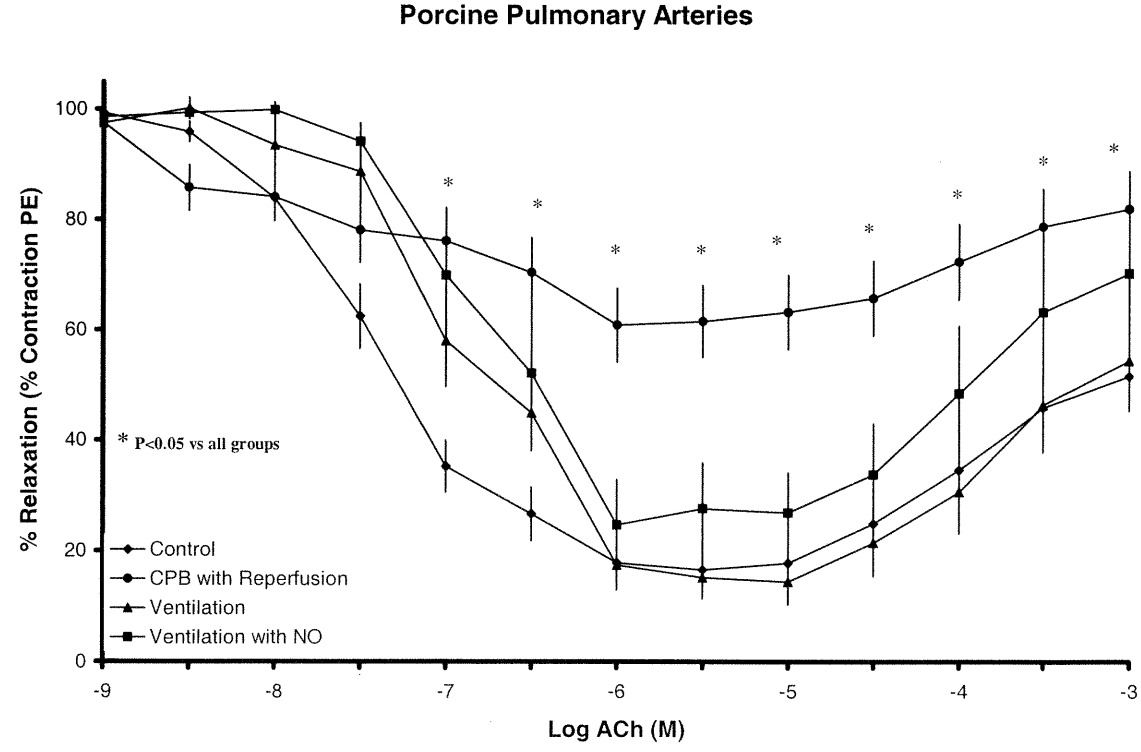


Figure 4a

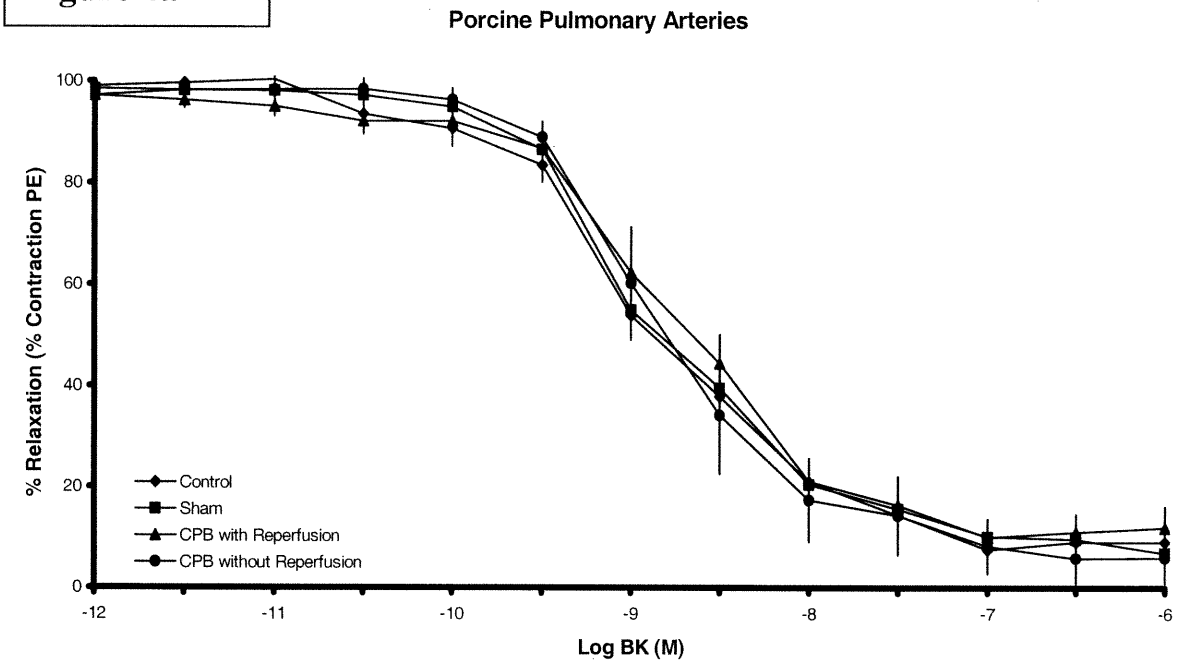


Figure 4b

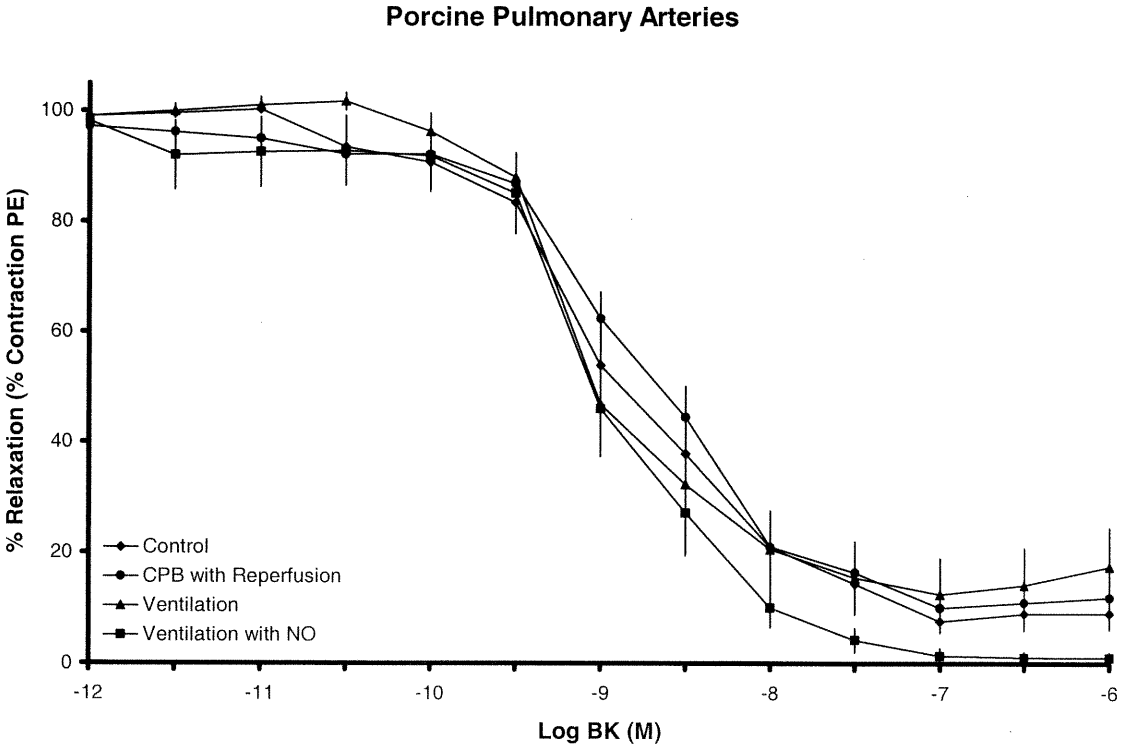


Figure 5a

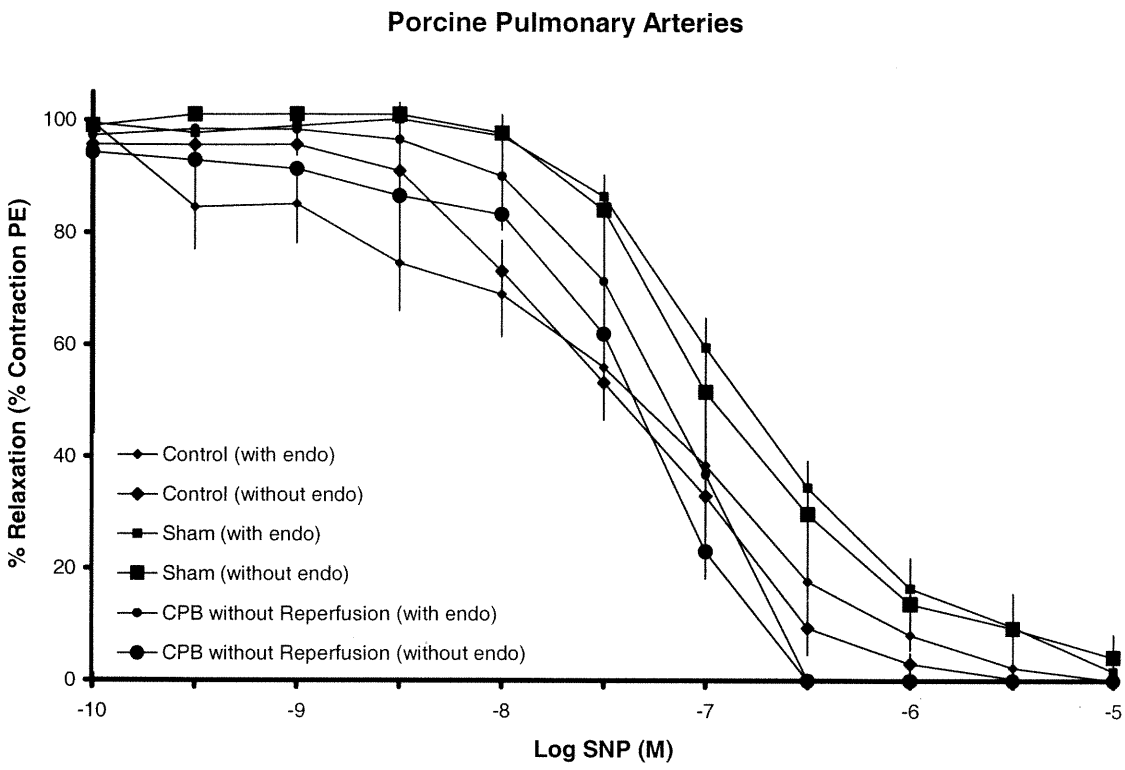


Figure 5b

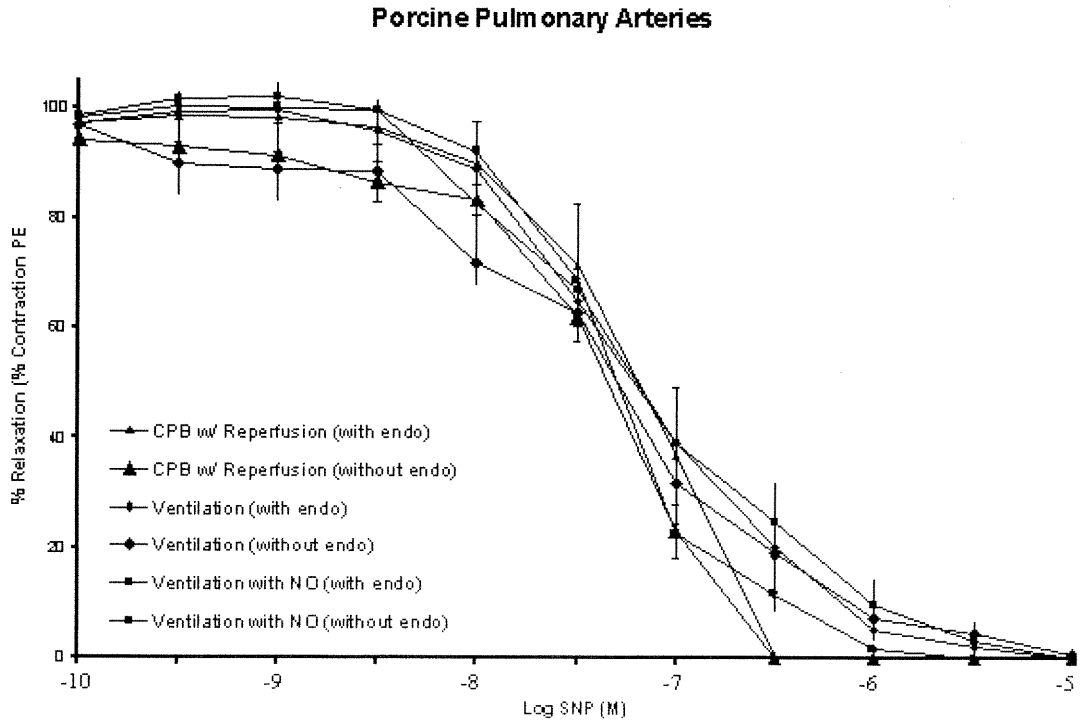


Figure 6

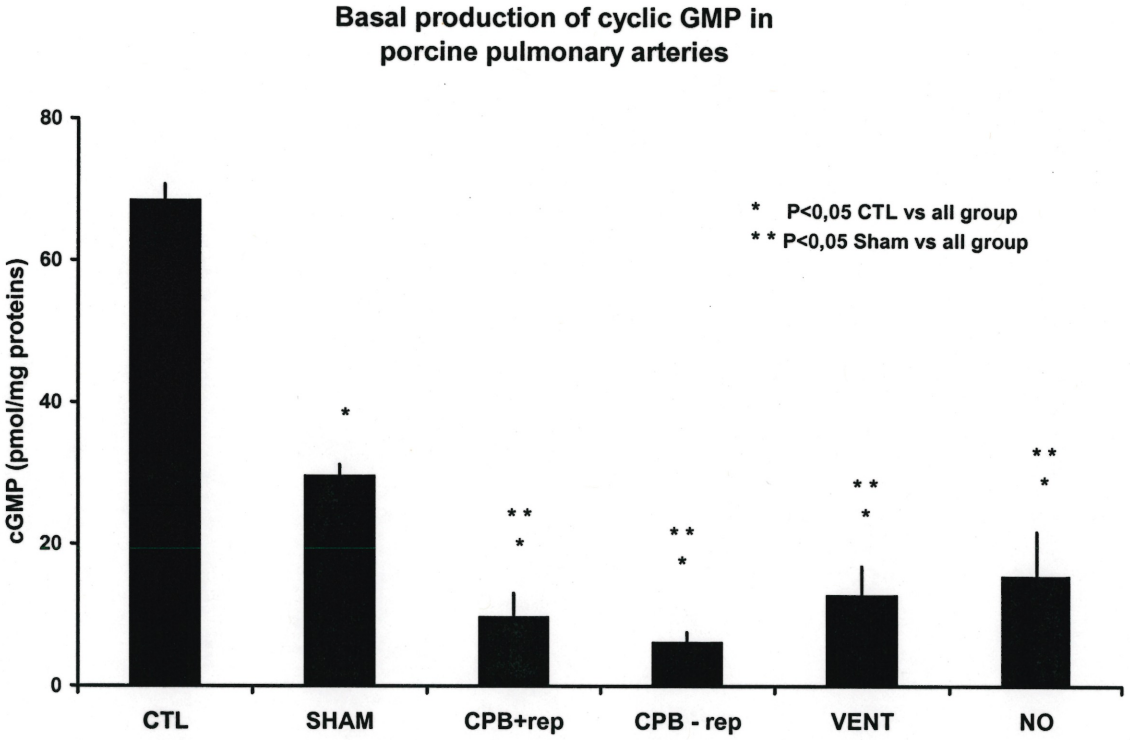
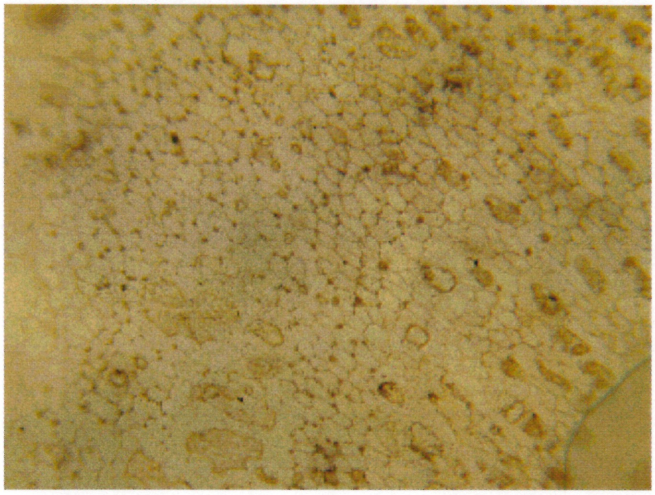


Figure 7



CORPS DE LA THÈSE : CHAPITRE IV

DISCUSSION DES RÉSULTATS

The major findings of the present study are that: (1) CPB without reperfusion does not cause endothelial dysfunction of the pulmonary artery tree as evidenced by the absence of significant differences between the control, the sham and the CPB without reperfusion group; (2) CPB with reperfusion induced a selective decrease in endothelium-dependent relaxation to ACh which is coupled to Gi proteins; (3) normal ventilation as well as ventilation with NO inhalation during CPB prevented the reduction of relaxation after reperfusion following CPB; (4) there are no significant differences between the normal ventilation and NO inhalation groups compared to the control, the sham and the CPB without reperfusion group; (5) all groups have decreased cGMP levels compared to the control group; and (6) all groups submitted to CPB have decreased cGMP levels compared to the sham group.

Endothelium-dependent relaxation to acetylcholine of second degree pulmonary artery are decreased in this porcine model of CPB with reperfusion. Reperfusion of the pulmonary artery tree releases oxygen free radical causing an alteration of Gi-protein mediated endothelium-dependent relaxations associated with a decrease of NO bioavailibility demonstrated by the decrease in cGMP levels. In the present study there is no endothelial denudation immediately after CPB since staining with silver nitrate of the second degree pulmonary arteries show preservation of the endothelial surface. This supports the finding that there is a selective Gi protein mediated dysfunction of endothelium-dependent relaxation in second degree pulmonary arteries

In the present study, ventilation and NO inhalation during CPB prevented the endothelial dysfunction occurring during reperfusion of the pulmonary arterial tree after completion of the CPB run. Possibly all patients could benefit from ventilation while being on pump at no additional cost and with little morbidity.

Although, NO inhalation per CPB did not confer an added benefit in the current study, the use of NO should not be undermined since it also prevented lung injury. The prophylactic use of inhaled NO could be beneficial to subsets of patients with an underlying pulmonary hypertension preoperatively who are more susceptible to pulmonary injury or those with a longer pump run. Inhaled NO is a very potent vasodilator and its clinical use against pulmonary hypertension is increasing. The use of ventilation and inhaled NO could be combined with the use of scavenger of free radicals, catalase, antioxidants, heparin-coated bypass circuits, and monoclonal antibodies to counterattack individual inflammatory mediator and hence minimize lung damage.

All groups submitted to CPB have decreased cGMP compared to the control group. McMullan et al. who have also found a decrease in cGMP post CPB [108]. Hence, CPB causes a decrease in cGMP.

Sham operated animals exhibit a decrease in cGMP levels compared to the control group. It is unclear why there is a decrease in cGMP in the sham versus the control. The sham was subjected to surgery (without CPB) which is known to cause the activation of the alternate complement pathway and could possibly explain the discrepancy in the cGMP result between the control and the sham groups.

The pulmonary vascular endothelium represents the largest vascular bed in the body and is a natural target for inflammatory processes which can disrupt vascular function. Lung injury post CPB resulting in pulmonary hypertension is an important area of research. There is sufficient circumstantial evidence to suggest that injury is mediated by an inflammatory response attacking the endothelium of the pulmonary vasculature. During total CPB, the pulmonary bed is subjected to

ischemia-reperfusion injury. Many strategies have been used in previous studies in the prevention of lung injury. Serraf et al. have shown that lung protection by continuous perfusion, pneumoplegia or nitric oxide ventilation can minimize hemodynamic alterations after CPB [6]. Morita et al. have shown that CPB impairs pulmonary NO production, resulting in pulmonary vasoconstriction which is reduced by antioxidants such as N-mercaptopropionylglycine and catalase [109]. Normandin et al. have demonstrated that the addition of L-arginine or pentoxifyline during reperfusion prevented the pulmonary endothelial alteration resulting from warm reperfusion [65].

It is possible that, as a result of endothelial injury, the production of vasodilatory factors is reduced as well as the production of vasoconstrictor factors is increased from activated platelets potentiating the vascular response. The restoration of the balance between vasoconstrictor and vasodilator using various treatments could decrease pulmonary morbidity and improve survival. Different forms of pulmonary hypertension involve separate mechanisms and distinct patterns of endothelial dysfunction. Hence, a better understanding of the pathological changes in the pulmonary vasculature and mechanisms producing these changes will lead to improved treatment of endothelial dysfunction.

CORPS DE LA THÈSE : CHAPITRE V

CONCLUSION

There is considerable evidence that CPB leads to activation of complement, neutrophils, monocytes, macrophages, platelets, and endothelial cells resulting in the deterioration of pulmonary function. A better understanding of some of the mechanisms that regulate the inflammatory response to CPB has already allowed the development of several therapeutic strategies aiming the inhibition of the adverse effect of inflammation. This study identified the role of reperfusion in the acute pathogenesis of pulmonary endothelial dysfunction and characterized some of the alterations in signal transduction pathway of endothelial cells of second degree pulmonary artery. In fact some of the most significant findings of this study are that: CPB without reperfusion does not cause a pulmonary arterial dysfunction, reperfusion of the pulmonary arterial tree after CPB causes an alteration of the endothelial cell vasorelaxations, and mechanical ventilation and NO inhalation during CPB prevent the endothelial dysfunction of the pulmonary tree. Definite clinical benefit has yet to be demonstrated but the results from this study are promising. Hopefully, a deeper comprehension of the mechanisms involved in endothelial dysfunction associated with CPB will result in improved management and treatment of patients undergoing surgery using extracorporeal circulation.

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