8TH INTERNATIONAL SYMPOSIUM ON RESISTANCE ARTERIES

New Developments in Resistance Arteries Research: From Molecular Biology to Bedside

Meeting report: highlights of the 8th International Symposium on Resistance Arteries

The Eighth International Symposium on Resistance Arteries (ISRA) was held in Angers, in the Loire Valley of France, between June 20 and 23, 2004. The meeting was a celebration of the 20th anniversary of this series of symposia and comprised excellent summative review lectures by plenary speakers, oral presentations, and poster-based discussions of current original research. The meeting boasted 158 attendees from 22 countries and was also valued for the participation by 12 on-site exhibitors of state-of-the-art equipment used in resistance artery experimentation. In this brief summary of the meeting, we summarize selected highlights from various components of the meeting.

The opening presentation by Harry Struijker-Boudier (Maastricht, The Netherlands) was an enlightening overview on “Resistance Artery Research: Future Directions,” suggesting that major advances could be anticipated in the areas of improved technology (for in vivo experimentation in small animals, using intravital microscopy, contrast and laser scanning microscopy, MRI, angiography, etc.), arterial plasticity (vascular remodeling), and signaling events in target organ disease. The second lecture (Axel Pries; Berlin, Germany) on the mathematical modeling of vascular structure dealt with the regulation of blood flow focusing on long-term structural changes (6). It was hypothesized that vascular networks possess a high degree of plasticity and vessels continuously adapt to local stimuli, mainly related to the local hemodynamic situation and the metabolic state of the tissue supplied. Consequently, structural and functional properties of vascular beds rely on the nature and balance of adaptive responses (6). Ernesto Schiffrin (Montreal, Quebec, Canada) summarized the current views on the role of peroxisome proliferator-activated receptor (PPAR) activation in vascular remodeling in cardiovascular disease. The discussion included a possible role for PPARs in vascular reactivity (7). Ulrich Pohl (Munich, Germany) reviewed calcium-sensitizing and -desensitizing mechanisms in the control of microvascular tone. The presentation first briefly reviewed the role of the three small GTPases RhoA, Rac, and Cdc42 in the control of cellular processes such as migration, proliferation, and regulation of cytoskeletal function. The primary focus of the presentation considered the potential role of GTPases in controlling vascular tone in resistance arteries. All three GTPases influenced vascular tone by modulating the sensitivity of the contractile apparatus to Ca\(^{2+}\). RhoA increased Ca\(^{2+}\) sensitivity through inhibition of myosin light chain phosphatase by a Rho kinase-dependent mechanism. Cdc42 reduced Ca\(^{2+}\) sensitivity by inhibiting myosin light chain kinase in a p21 kinase-dependent manner. The talk by Michael Hill (Sydney, Australia) considered the contribution of myogenic contraction of arterioles in the regulation of basal vascular tone and local control of hemodynamics. Importantly, this intravascular pressure-mediated contraction provides a level of resistance that can be accessed by neurohumoral and metabolic vasodilator stimuli to increase blood flow when metabolically required. Despite its importance, there are significant gaps in our knowledge regarding the detailed mechanisms of myogenic vasoconstriction. For example, whereas an increase in intraluminal pressure leads to depolarization and Ca\(^{2+}\) entry via voltage-gated Ca\(^{2+}\) channels, it is uncertain how this membrane potential is linked to the initial mechanical stimulus. One important possibility considered was that the membrane events are linked through the extracellular matrix via cellular integrins. The lecture by Bernard Levy explored the cellular basis of the proangiogenic role of angiotensin II (ANG II). Its angiogenic effect is associated with increases in the expression of VEGF, endothelial growth factor, endothelial nitric oxide (NO) synthase (eNOS), and cyclooxygenase (COX)-2 protein levels. The proangiogenic effects of angiotensin-converting enzyme (ACE) inhibition are mediated by B2 receptors and are associated with upregulation of eNOS, and not VEGF, levels.

The opening session of oral presentations dealt with the ionic control of vascular tone and intercellular communication. There were two presentations on resistance artery vasomotion from Aalkjaer and Nilsson’s laboratory (Aarhus, Denmark). In the first presentation, Matchkof discussed the possibility that cGMP can depolarize smooth muscle cells by activation of a novel Ca\(^{2+}\)-activated Cl\(^{-}\) channel that is regulated by cGMP. This channel was present in rat mesenteric, cerebral, and tail artery cells. Briggs, from the same group, evaluated the link between the cGMP-dependent, Ca\(^{2+}\)-activated Cl\(^{-}\) channel and vasomotion and concluded that this channel most likely mediated oscillations in rat mesenteric arterioles. A related paper by Beny (Lausanne, Switzerland) showed that Ca\(^{2+}\) permeability communication was important for the occurrence of intercellular Ca\(^{2+}\) oscillations. The role of Rho kinase in modulating agonist constriction was discussed by Morel (Bruxelles, Belgium). Experiments by Rasmussen (Aarhus, Denmark) demonstrated a selective effect of nimodipine and nifedipine in rat cerebral compared with mesenteric artery responses. Schubert (Restock, Germany) discussed the role of Kv3 channels in resistance arteries.

In the second session, Lynch (Manchester, UK) discussed the effect of hypoxia on myogenic tone developed by isolated pressurized human coronary arteries. Hypoxic vasodilation was insensitive to glibenclamide, suggesting that ATP-sensitive K\(^{+}\) channels are not involved. Hughes (London, UK) next considered the role of Src tyrosine kinase in hypoxosmotic contraction. The evidence supporting an activation of Src tyrosine kinases was derived through the use of relatively selective inhibitors of Src (herbimycin, protein phosphatase 1, and SU-6656) as well as by Western blot analysis. Matz (Illkirch, France) showed that aging decreases contractile pro-
tein sensitivity in addition to alterations in the sarcoplasmic reticulum (SR) Ca\(^{2+}\) content. The presentation by Kaley’s group (New York, NY) showed that in leptin receptor deficient \(db^{-/db}\) mice, hypertension resulted from both an increase in myogenic tone as well as augmented COX-2-mediated generation of thromboxane \(A_2/PGH_2\). Two presentations from Nishizawa’s group (Hamamatsu, Japan) discussed in detail the biochemical and histopathological changes in a canine model of cerebral vasospasm. Whereas decreases in diameter were initiated by PKC-\(\alpha\), maintenance of vasospasm was associated with a translocation of PKC-\(\alpha\) to the membrane. Importantly, prolonged vasospasm was related to a shift from PKC-\(\alpha\) to tyrosine kinase-mediated constriction, with a reduced role for myosin light chain regulation of contractile function. Coats (Glasgow, UK) evaluated the inhibition of human smooth muscle cell proliferation by a Ras-farnesyl transferase inhibitor, FTPIII. The data presented demonstrated that both short-term (15 min) and long-term (18 h) exposure to FTPIII inhibited proliferation. De Mey (Maastricht, The Netherlands) showed that in VEGF-deficient mice, capillary density was unaltered, whereas there were reductions in body, heart, kidney, and brain weights. A poster by Vila and Salaices (8) presented in this session provided details on the important roles of cytokines such as TNF-\(\alpha\), IL-1\(\beta\), and IL-6 on the reactivity of resistance arteries. Cytokines are important in a number of pathological conditions such as septic shock, where they cause pronounced vasodilation, whereas levels levels of TNF-\(\alpha\) and IL-6 are elevated in preeclampsia, where it is proposed that they can decrease bioavailability of endogenous vasodilator substances and also directly release constrictor substances (8).

The third oral presentation session focused on arterial remodeling. Bakker (Amsterdam, The Netherlands) showed the critical role of transglutaminase in inward remodeling of cultured coronary resistance arteries. Transglutaminase serves to link lysine and glutamine residues in extracellular matrix proteins. Inward remodeling in these vessels was augmented by retinoic acid and inhibited by the transglutaminase blocker cadaverine. The second presentation from this group by Bakker (Amsterdam, The Netherlands) discussed the structure and function of subepicardial porcine coronary arteries maintained for 3 days at 60 mmHg. Pressurized arteries maintained in organ culture under conditions of flow were able to readjust their active diameters to a specific value of shear stress, and inward remodeling was inhibited by flow. An important role of transglutaminase in inward remodeling was also provided by Bramsen (Aarhus, Denmark) using endothelin (ET)-1 (10 nM for 1 day) to induce contraction and so prime arteries for structural changes. The inward remodeling that ensued was blocked by cystamine. Related to aspects of resistance artery remodeling were the experiments of Giradot (Montreal, Quebec, Canada), who presented data on the time course of expression of structural and regulatory proteins of focal adhesions during eutrophic adaptation. Eutrophic remodeling requires cellular reorganization of cytoskeletal proteins such that changes in contractile protein structure and function can adapt appropriately. The role of arterial wall ACE (t-ACE) activity in flow-related arterial remodeling was addressed by Hilgers (Maastricht, The Netherlands) (3). Four-week ligation of carotid arteries in mice lacking t-ACE led to reduced inward remodeling. The lack of t-ACE did not alter the outward hypertrophic remodeling of uterine arteries that occurred during pregnancy. It was concluded that bradykinin protects against excessive inward remodeling in response to a chronic stoppage in blood flow and that ANG II induced medial hyperplasia. On the other hand, neither bradykinin nor tissue angiotensin were necessary for pregnancy-induced remodeling of uterine arteries (3). Related to ANG II and remodeling was the presentation by De Ciuceis (Montreal, Quebec, Canada), who investigated the role endothelial regulation of vascular remodeling using rats mice lacking in macrophage-stimulating factor (osteopetrotic mice). The effects of a 14-day ANG II infusion on mesenteric artery structure and function were recorded, and osteopetrotic mice were more resistant to endothelial dysfunction, vascular remodeling, and oxidative stress induced by the pressor agent. Carlier and Bertoldi (1) applied a technique that combined arterial spin labeling with NMR imaging to create precise hemodynamic measurements such as absolute blood flow, cell oxygenation, and ATP metabolism, allowing for detailed examination of microcirculatory control of organ perfusion. Of importance is the added ability to precisely record functional aspects of microcirculatory vaso- motion and perfusion heterogeneity using these noninvasive methods of functional imaging (1).
master arterioles, indomethacin and N-nitro-l-arginine methyl ester were competitive inhibitors of ACh-induced dilations, but maximal responses to ACh could still be achieved. This non-NO, non-PG component was abolished by combined treatment with apamin (1 μM) and charybdotoxin (0.1 μM), suggesting important roles for intermediate- and small-conductance Ca2+-activated K+ channels.

In a continuation of the mechanistic study of arterial vaso-motion, Rahman (Aarhus, Denmark) investigated whether the oscillations in artery vasomotion were also associated with endothelial Ca2+ oscillations. Confocal microscopy with calcium green as a Ca2+ reporter dye was used to track patterns of endothelial Ca2+ changes induced by ACh. Treatment of arteries with norepinephrine (after inhibiting SR Ca2+ uptake with cyclopiazonic acid) produced oscillations in tension and smooth muscle Ca2+, both of which occurred in phase with low frequency and high amplitude. There were also large oscillations in endothelial Ca2+ associated with norepinephrine-induced tone, but, however, these oscillations were in antiphase with the responses in smooth muscle cells. Oscillations in Ca2+ after cyclopiazonic acid in both smooth muscle and endothelium were sensitive to a combination of apamin and charybdotoxin.

Gopalakrishnan (Saskatoon, Saskatchewan, Canada) provided a detailed exploration of the vasodilator effects of ghrelin and des-acyl ghrelin, peptides that are released from the stomach to regulate growth and food intake. Ghrelin dilated the isolated perfused mesenteric bed by endothelial-dependent机制, and apamin or ouabain and Ba2+ abolished vasodilation.

Flow-mediated dilation and myogenic tone in estrogen receptor-β knockout mouse was discussed by Douglas (London, UK). Incubation of isolated mesenteric arteries with estradiol enhanced flow-mediated dilation in both wild-type females and males and in females from the estrogen receptor-β knockout group. After incubation with [4,4′,4″-(4-propyl-[1H]-pyrazole-1,3,5-triyltris-phenol], a selective agonist for estrogen receptor-α, flow-mediated dilation was increased in arteries from wild-type females and not males, suggesting that estrogens (through estrogen receptor-β) mediate flow-induced dilation in male mice, whereas estrogen receptor-α may have a greater role in mediating this response in females. The extent of pressure-induced myogenic tone was similar in both groups, although incubation with estrogen decreased myogenic tone selectively in equally in wild-type and estrogen receptor-β knockout females. Huang (Hong Kong, China) next considered the dilation produced by raloxifene, a selective estrogen receptor modulator, in rat mesenteric arteries. Disruption of endothelial function reduced the vasodilator response to raloxifene, whereas the addition of charybdotoxin and apamin was without effect.

The role of plasma microparticles on endothelial function was introduced by Tesse (Illkirch, France) (5). These proagulant and proinflammatory microparticles are membrane proteins released from the plasma membrane of various cell types during activation by agonists, shear stress, or apoptosis. Incubation of mouse aortic rings with microparticles resulted in time-related impairment of ACh and flow-mediated dilation. These effects were correlated with decreased expression of eNOS and overexpression of caveolin-1. In addition, lymphocyte-derived microparticles from diabetic or human immunodeficiency virus-infected patients also reduced eNOS expression (5).

The properties of isolated cerebral arteries from hypercholesterolemic mice were studied by Thorin’s group (Montreal, Quebec, Canada). Myogenic tone in cerebral arteries from hypercholesterolemic mice did not develop adequately at higher pressures. Moreover, the maximal vasodilation produced by ACh was significantly reduced in hypercholesterolemic mice.

Endothelial regulation of mesenteric arterioles from streptozotocin-induced diabetic rats was discussed by Nakayama (Shizuoka, Japan), where intravital videomicroscopy was utilized to study arterioles having diameters of ~20 μm. Arterioles from diabetic rats had increased constrictor responses to phenylephrine [before and after N-nitro-l-arginine (L-NNA) treatment]. Endothelium-dependent dilation to ACh was blunted in the diabetic group, whereas constriction to L-NNA was similar in both groups. Inhibition of inducible NO synthase (iNOS) caused a significantly greater constriction in arteries from diabetic animals, and immunostaining revealed iNOS activity in macrophages and smooth muscle of diabetic arterioles, whereas iNOS immunoreactivity was present only in macrophages in control arterioles. These data indicated that iNOS, instead of eNOS, contributed to the regulation of basal tone and reactivity to phenylephrine in diabetic arterioles.

In the session “Reactive Oxygen Species and Inflammation,” Tabernero (Illkirch, France) examined the role of COX-2 and iNOS in modulating vascular tone during liver disease. The expression of the inducible enzymes COX-2 and iNOS was investigated in small omental arteries harvested from patients undergoing surgery. Tissues were divided into three groups by liver disease: fulminant hepatic failure (FHF), cirrhogenic viral hepatitis (CH), and limited hepatocarcinoma (controls). Western blot analysis and immunostaining revealed significant upregulation of COX-2 and iNOS in FHF and CH patients. A study from Koller’s group, delivered by Toth, examined the role of the sorbitol pathway in the development of oxidative stress-induced vascular dysfunction in a rat model of diabetes mellitus. Sorbitol synthesis via the polyol pathway is a minor (<2%) fate of glucose normally; however, during diabetes, this could be increased to ~35% by the aldose reductase pathway. It was reasoned that higher concentrations of sorbitol, due to increased oxidative stress, cause endothelial dysfunction. In keeping with this, extraluminally applied sorbitol produced constriction of pressurized skeletal muscle arterioles, an effect that was partly inhibited by either superoxide dismutase or catalase and abolished by combined application of these agents. Incubation of arteries with sorbitol (0.01 μM, 40 min) significantly reduced ACh dilations and converted flow-induced dilations to constrictions. These results led to the proposal that higher intracellular levels of sorbitol (via oxidative stress) promote increased vascular resistance and disturbed regulation of blood flow in diabetes mellitus.

The regulation of myogenic tone in critical limb ischemia (CLI) was discussed by Coats (2). There is inadequate regulation of blood volume within the capillary beds during CLI.
This study investigated the roles of reactive oxygen species and G/F actin-based mechanisms of regulation of myogenic tone in CL1 using arteries taken from skeletal muscle biopsies. There was a significant reduction in the myogenic tone in arteries from ischemic vascular beds compared with nonischemic beds. Incubation with antioxidants or inhibitors of NAD(P)/H oxidase or actin polymerization reduced the extent of myogenic tone only in arteries from healthy subjects. The data suggested roles for NAD(P)/H oxidase, elevations of reactive oxygen species, and actin polymerization as mediators of myogenic tone, with a proposal for a downregulation of this pathway in the generation of myogenic tone in patients with CL1 (5).

The mechanisms of postischemic coronary endothelial dysfunction were discussed by Favre (Rouen, France). Toll-like receptors (TLR) are activated by endogenous ligands (e.g., heat shock proteins, oxygen free radicals) during cardiac ischemia-reperfusion, playing an important role in the inflammatory response. In addition, neutrophil-endothelium interactions are regulated by TLR-2 and TLR-4 in endothelial and myocardial cells. Thus coronary artery ligation was performed in TLR-2 or TLR-4 knockout mice followed by reperfusion. In coronary arteries, isometric contraction was then studied. Endothelium-dependent dilation was reduced in sites distal to the ligation in wild-type mice. Interestingly, this reduced endothelial response to ACh after ischemia-reperfusion was either not evident (TLR-4 knockout) or paradoxically reversed (TLR-2 knockout) in TLR knockout mice. Likewise, the extent of neutrophil accumulation during ischemia-reperfusion was either reduced (TLR-4 knockout) or absent (TLR-2 knockout) in the TLR knockout mice, indicating important roles for these receptors in mediating ischemia induced vascular dysfunction that includes neutrophil-mediated coronary artery endothelial injury.

The final oral presentation session was devoted to studies on hypertension. The effects of reduced uteroplacental perfusion on blood pressure and renal function was examined by Sanders from De Mey’s group. Intraterine stress (IUS) was produced in rats by bilateral ligation of uterine arteries at day 17 of pregnancy. Some animals underwent unilateral nephrectomy. Litter size and birth weight was reduced by IUS, whereas there was increased hematocrit. In adult offspring, there was increased glomerular size and reduced number of glomeruli accompanied by increased renal perfusion and filtration fraction. The data indicated that IUS impaired renal development to the extent of causing persistent reductions in renal reserves of the offspring. Gilbert (Montreal, Quebec, Canada) examined the roles of the antiadhesive matricellular proteins thrombospondine (TSP) and tenasin-c (TN-C) in remodeling of the rat aorta and mesenteric arteries. Increases in TSP levels in the aorta were transient, whereas TN-C levels generally remained elevated. On the other hand, TSP overexpression occurred only at later stages, whereas TN-C content decreased progressively. Thus hypertrophic remodeling was possibly due to a decrease in cell adhesion, whereas eutrophic remodeling was accompanied by an initial increase of adhesion followed by TSP overexpression. Resistance artery remodeling in hypertension was reviewed by Loirand (4), who presented evidence for an important role of RhoA in this process. In addition to its well-described role in modulating Ca\(^{2+}\) sensitivity in vascular smooth muscle, RhoA also mediates smooth muscle differentiation, proliferation, and migration. Thus inhibitors of Rho kinase reduce medial hypertrophy and are antihypertensive (4).

The role of ET\(_B\) receptors in ET-1-induced pulmonary vasconstriction (Sauvageau; Montreal, Quebec, Canada) was examined in rat lungs. Changes in perfusion pressure were recorded to infusion of ET (10–300 nM) before and after bosentan (0.1 mM). The vasoconstrictor effects of ET and sarafotoxin were associated with severe pulmonary oedema, with both the vascular and fluid changes being bosentan sensitive. Removal of the endothelium had no effect. Thus ET-1-induced vasoconstriction was mediated mainly by ET\(_B\) receptors with some concurrent release of vasoconstrictor COX products because meclofenamic acid partially inhibited ET responses.

The meeting culminated with a hearty appreciation of the excellence of both the scientific and social components of the 8th ISRA Symposium. By unanimous acclamation, the site for the 9th ISRA Symposium in 2007 was declared to be Sydney (Australia), where Michael Hill and colleagues will organize the meeting.

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