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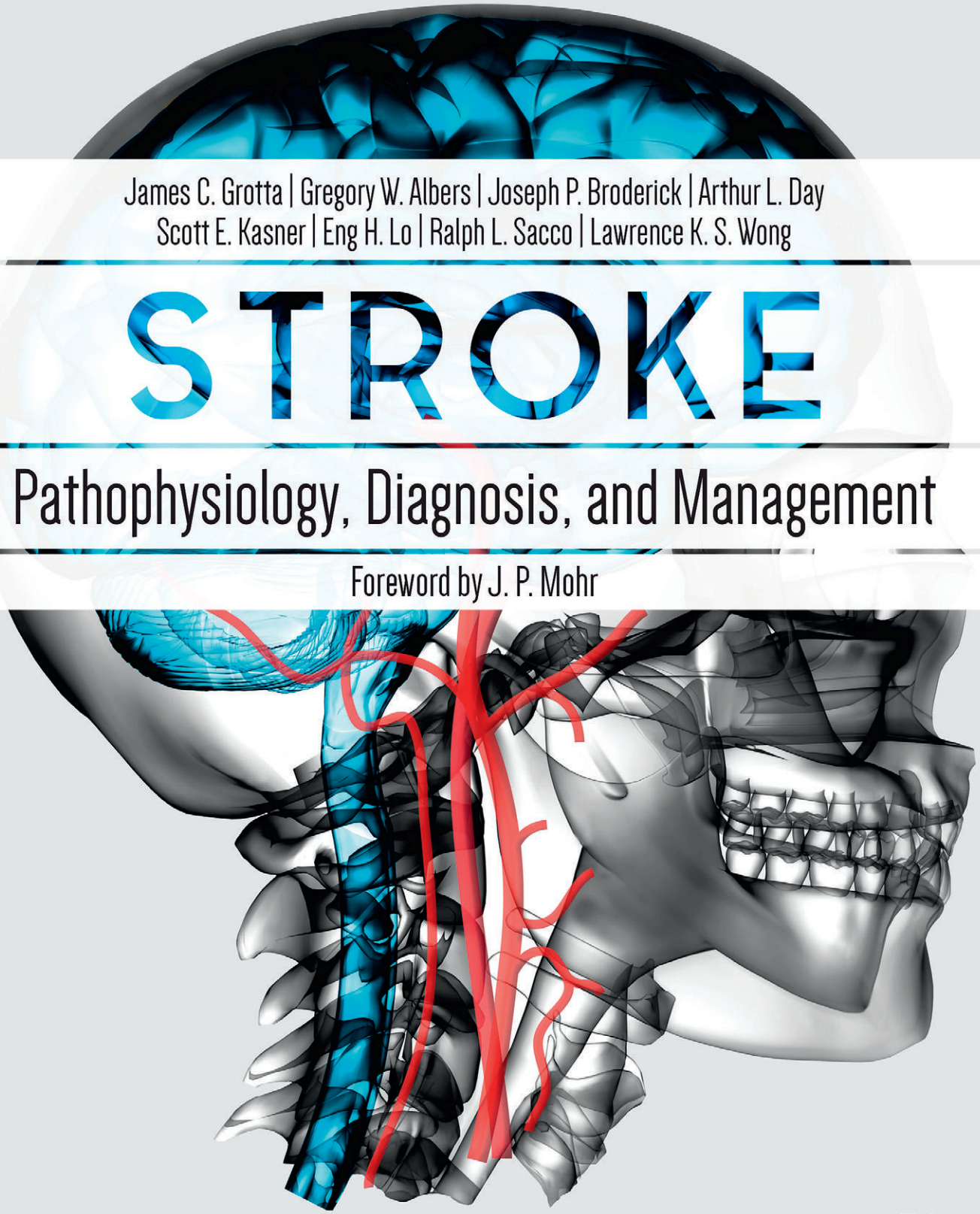
STROKE

Pathophysiology, Diagnosis, and Management

Foreword by J. P. Mohr



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Stroke

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Pathophysiology, Diagnosis, and Management

SEVENTH EDITION

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Foreword to the Seventh Edition

This original editor continues to marvel at the advances in the field of stroke, justifying the seventh edition of this book. Among other topics, the first edition had a mere 15 chapters, and in 347 pages covered "Stroke Therapy." The subjects ranged from management of risk factors to rehabilitation. This edition has no less than 28 such chapters, clustered in stand-alone sections for medical and interventional therapy. The page length for all of subjects has steadily expanded by editions: proof, if needed, of progress. Gone—and good riddance!—are the days when those interested in stroke were considered clinically irrelevant for lack of definitive therapies. Instead, far from an arcane subspecialty, stroke prevention and management now has an impact on the clinical practice of many medical and surgical fields whose training not long ago scarcely touched on the subject.

Stroke clinicians now find their clinical judgment tested—sometimes to their vexation—by the application of hyperacute management algorithms driven mainly by scoring systems, meta-analyses, and outcomes from the wave of clinical trials. Few can argue with the positive effect of rapid assessment and intervention, especially for acute ischemic strokes. Insights spanning genetics, basic biology, computer-driven population studies, web-based meta-analyses, and increasingly common longitudinal outcome reports are welcome signs of progress. Only novelists should designate their published work free from revision. The current contributors can expect further changes to justify an eighth edition in the foreseeable future.

The growing participation in stroke management by those in allied fields has done nothing to displace the role of neurovascular clinicians, whose commitment includes studying how the brain works. Insights from modern basic biology, increasingly sophisticated imaging, prospective clinically detailed databases, and even access to video Zoom follow-ups are providing windows into what was formerly called semiology. Decades ago, the neurology literature was dotted with titles beginning with "The Neurology of…" by which the author(s) implied how a clinical syndrome allowed insight into diagnosis or prognosis. Today, a surprising number of outcomes for acute focal syndromes formerly considered static, prevented from their full development, or deemed modified favorably by acute interventions, are yielding insights into the mysteries of functional reorganization. The increasing opportunities to understand this effect offers literature-oriented neurovascular clinicians the chance to be links in an unbroken chain of inquiry dating back to antiquity.

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Preface

The seventh edition of the text has a number of important changes. First, this edition has even more on-line features, making it easier to access its content in a digital-friendly format. The eBook includes the entire book plus full reference lists (as opposed to the Key References that appear in the chapters) and a larger number of videos than the previous edition. Access to the Expert Consult eBook version is included with print purchase. This enhanced eBook experience allows you to search all of the text, figures, and references on a variety of devices. The content can also be downloaded to tablets and smart phones for offline use.

Another important change includes our new Surgical Therapy section editor, Arthur Day, MD. Dr. Day is an international authority on the surgical management of cerebral aneurysms, intracranial hemorrhage, and extracranial vascular disease. He is the recipient of numerous neurosurgical leadership awards, and from first-hand experience I can attest to his passion for teaching and the wisdom that has grown out of decades of skillfully managing the complexities of the entire array of neurovascular surgical cases. Of particular value for his role as editor, Dr. Day has been an important leader of the neurosurgical field as it has emerged from open to endovascular approaches and as it has partnered with vascular neurology in the conduct of clinical trials. As a result of his intimate knowledge of the entire neurovascular landscape and its leaders, you will see that the authors of almost all of the chapters in the Surgical Therapy section have changed and the chapters have all been updated. I think that the readers will be impressed by the combined experience, fresh perspective, and new information in every chapter in the section.

Other notable changes in this edition justified enlarging attention given to several underappreciated and yet unresolved problems in the field. In line with the increasing evidence of vascular disease as the most important modifiable contributor to dementia and much-needed attention to the biology underlying small vessel disease, a new chapter on this topic has been added to the Pathophysiology section, which has been overseen by the senior editor, Dr. Lo. In addition, the chapters on the clinical aspects of vascular dementia and small vessel disease have been updated by new authors (Drs. Rundek, Seshadri, and Caunca) in the Epidemiology and Risk Factors section, and important new information is found in the chapters on genetics and CADASIL. Somewhat linked to this topic and also reflecting a maturing interest in non-imaging stroke biomarkers in general is an entirely new chapter on "OMICs," written by Drs. Jickling and Sharp.

Disparities in stroke incidence and outcomes has become a hot topic, accentuated recently by the spotlight cast on this issue during the COVID-19 pandemic and the racial unrest in the United States. The already outstanding chapter on stroke disparities by Drs. Howard, Howard, and McCullough has been updated, and this topic has also been woven through other chapters where relevant.

Other unsolved areas that receive substantial updating include intracerebral hemorrhage by new author Dr. Anderson and arteriovenous malformations by Drs. Samaniego, Roa, Ortega-Gutierrez, and Derdeyn. In addition, the chapters on the surgical management of different types of brain hemorrhage have all been updated by new authors.

The previous edition appeared just as the trials demonstrating the benefit of endovascular thrombectomy were published, so the coverage of this revolution in treatment was incomplete. In this edition, Dr. Broderick's section, Interventional Therapy, and in particular the chapter by Drs. Saver and Jahn on the endovascular treatment of acute ischemic stroke, have been substantially updated to include the results of all those pivotal clinical trials, as well as the myriad studies that followed.

The final unresolved topic receiving increased coverage in this edition is how best to deliver these effective new treatments (e.g., stroke systems of care). We have added a new chapter on this topic, written by Drs. Czap, Harmel, Audebert, and myself, that explores different models and approaches to reorganizing our stroke centers, resources, and staffing. and In addition, first-time contributors to this title, Drs. Kircher and Adeoye, have expanded the chapter on prehospital and emergency care.

While I have focused my editorial spotlight on a few of the major unresolved topics that are receiving substantial and well-deserved increased attention, I want to emphasize that each and every chapter has been updated with new information. There is a new chapter on posterior reversible encephalopathy, which replaces the old chapter on hypertensive encephalopathy with new authors (Drs. Balu and Fischer); the imaging chapters on CT and MRI have been updated, with expanded discussion of the important role of imaging in patient selection for acute therapy; the chapters on cardiac disease, cryptogenic stroke, and secondary prevention provide more information on atrial fibrillation detection, other possible causes of embolic-appearing stroke without known source, and their long term management; the antiplatelet therapy chapter includes updated data from recent trials of dual antiplatelet therapy; and the design of stroke clinical trials chapter has been rewritten by new authors (Drs. Perez, Elm, and Saver) and includes emerging novel approaches to figuring out if new treatments work.

All in all, I hope that the exciting relevant new data that fill the pages of our journals and make stroke such a dynamic and interesting field are distilled into these pages in a readable and authoritative format that will help the reader understand their patients and their underlying disease, which they see every day, and also provide the foundation for new knowledge that will be the substrate for the next edition.

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AHA Evidence-Based Classifications

TABLE 1 Applying Classification of Recommendations and Level of Evidence

ESTIMATE OF CERTAINTY (PRECISION) OF TREATMENT EFFECT	SIZE OF TREATMENT EFFECT →				
	CLASS I	**CLASS IIa**	**CLASS IIb**	**CLASS III *No Benefit* or CLASS III *Harm***	
	Benefit* > > > *Risk **Procedure/ Treatment SHOULD be performed/ administered**	***Benefit* > > *Risk* *Additional studies with focused objectives needed*** **IT IS REASONABLE** to perform procedure/ administer treatment	***Benefit* ≥ *Risk* *Additional studies with broad objectives needed; additional registry data would be helpful*** Procedure/Treatment **MAY BE CONSIDERED**	**Procedure/ Test**	**Treatment**
				COR III: No benefit — **Not Helpful**	**No Proves Benefit**
				COR III: Harm — **Excess Cost w/o Benefit or Harmful**	**Harmful to Patients**
LEVEL A Multiple populations evaluated* Data derived from multiple randomized clinical trials or meta-analyses	• Recommendation that procedure or treatment is useful/effective • Sufficient evidence from multiple randomized trails or meta-analyses	• Recommendation in favor of treatment or procedure being useful/effective • Some conflicting evidence from multiple randomized trials or meta-analyses	• Recommendation's usefulness/efficacy less well established • Greater conflicting evidence from multiple randomized trials or meta-analyses	• Recommendation that procedure or treatment is not useful/effective and may be harmful • Sufficient evidence from multiple randomized trials or meta-analyses	
LEVEL B Limited populations evaluated* Data derived from a single randomized trial or nonrandomized studies	• Recommendation that procedure or treatment is useful/effective • Evidence from single randomized trial or nonrandomized studies	• Recommendation in favor of treatment or procedure being useful/effective • Some conflicting evidence from single randomized trial or nonrandomized studies	• Recommendation's usefulness/efficacy less well established • Greater conflicting evidence from single randomized trial or nonrandomized studies	• Recommendation that procedure or treatment is not useful/effective and may be harmful • Evidence from single randomized trial or nonrandomized studies	
LEVEL C Very limited populations evaluated* Only consensus opinion of experts, case studies, or standard of care	• Recommendation that procedure or treatment is useful/effective • Only expert opinion, case studies, or standard of care	• Recommendation in favor of treatment or procedure being useful/effective • Only diverging expert opinion, case studies, or standard of care	• Recommendation's usefulness/efficacy less well established • Only diverging expert opinion, case studies, or standard of care	• Recommendation that procedure or treatment is not useful/effective and may be harmful • Only expert opinion, case studies, or standard of care	
Suggested phrases for writing recommendations†	should is recommended is indicated is useful/effective/ beneficial	is reasonable can be useful/effective/ beneficial is probably recommended or indicated	may/might be considered may/might be reasonable usefulness/effectiveness is unknown/unclear/ uncertain or not well established	COR III No Benefit: is not recommended is not indicated should not be done is not useful/ beneficial/effective	COR III Harm: potentially harmful causes harm associated with excess morbidity/ mortality should not be done
Comparative effectiveness phrases†	treatment/strategy A is recommended/ indicated in preference to treatment B treatment A should be chosen over treatment B	treatment/strategy A is probably recommended/ indicated in preference to treatment B it is reasonable to choose treatment A over treatment B			

BOX 1 Evidence Classifications

1. Size of treatment effect
   - Class I: Benefit >>> Risk. Procedure/treatment SHOULD be performed/administered.
   - Class IIa: Benefit >> Risk. IT IS REASONABLE to perform procedure/administer treatment.
   - Class IIb: Benefit ≥ Risk. Procedure/treatment MAY BE CONSIDERED.
   - Class III: No Benefit/Harm. Procedure/treatment is not useful/effective and may be harmful.
2. Certainty of treatment effect
   - Level A: Data derived from multiple randomized clinical trials or meta-analyses.
   - Level B: Data derived from a single randomized trial or nonrandomized studies.
   - Level C: Only consensus opinion of experts, case studies, or standard of care.

Adapted from Sacco RL, Adams R, Albers G, et al. Guidelines for prevention of stroke in patients with ischemic stroke or transient ischemic attack: a statement for healthcare professionals from the American Heart Association/American Stroke Association Council on Stroke. *Stroke.* 2006;37:577–617.

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SECTION I Pathophysiology

Eng H. Lo

The first section in this new edition of *Stroke* provides an updated and comprehensive survey of the molecular, cellular, and pathophysiologic mechanisms that underlie the brain's reaction to ischemia and hemorrhage. At the cellular level, stroke affects pathways of hemostasis and perturbs interactions between circulating blood elements, the blood vessel itself, and brain parenchyma. At the functional level, the regulation and dysregulation of hemodynamics and metabolism mediates an integrated neurologic response. At the organ level, stroke induces histopathologic reactions in all neural, glial, and vascular cells. Hence this section begins with three chapters that define basic principles of vascular biology, cerebral blood flow and metabolism, and brain tissue injury. Updates include new information on hemodynamic responses to thrombectomy and reperfusion, as well as new sections that discuss correlations between experimental animal models and clinical pathology.

Building on these fundamental principles, the next few chapters then explore the molecular mechanisms of cell death and survival. Genes and pathways underlying necrosis and programmed cell death are balanced against an expanding family of endogenous neuroprotection mediators. The neurovascular unit chapter remains a centerpiece for the overall concept of cell-cell signaling. However, beyond the brain itself, interactions with other organ systems are also discussed in terms of crosstalk with neuroinflammatory cascades and immune cells. New sections describe emerging opportunities in tolerance and preconditioning, as well as interactions between the immune system and the microbiome. The chapter on stroke recovery reviews a complex spectrum of compensatory response in resident precursor and circulating progenitor cells. New insights have been added to explore the role of exosomes and micro-RNA that may transfer and coordinate signals between all cell types in the remodeling neurovascular unit. The chapter on white matter has also been expanded, with added material that links exercise to oligodendrocyte homeostasis and resilience. The chapter on cerebral hemorrhage surveys advances in molecular and cellular phenomena with new ideas that may link ferroptosis to translational opportunities and clinical trials. The chapter on vascular malformations has been updated to link signaling cascades in advanced zebrafish and mouse models with genes that are implicated in clinical disease. Finally, this section ends with the addition of a new chapter that defines novel pathways in the neurovascular unit that mediate vascular contributions to cognitive impairment and dementia.

Optimal translation for cerebrovascular disease cannot occur without a rigorous dissection of the molecular and cellular fundamentals in neurovascular and gliovascular biology. The basic principles established in this section should provide not only mechanistic foundations but also a rational basis for pursuing therapeutics and diagnostics in stroke.

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SECTION I

Pathophysiology

1 Cerebral Vascular Biology in Health and Disease

T. Michael De Silva, Christopher G. Sobey

KEY POINTS

- Cerebral artery tone is substantially modulated under physiologic conditions by endothelium-derived nitric oxide, by reactive oxygen species, and through hyperpolarization mediated by several types of K^+ channels.
- Cerebral vascular function is very sensitive to endothelial dysfunction that occurs during chronic disease, resulting in impairment of vasodilator mechanisms.
- Oxidative stress and inflammation occur in the cerebral circulation in response to cardiovascular risk factors present during atherosclerosis and chronic hypertension, such as elevated plasma levels of cholesterol and angiotensin II, respectively.

INTRODUCTION

The brain has a limited supply of nutrients; thus normal brain function relies on adequate perfusion by the cerebral circulation for the delivery of oxygen and nutrients, as well as the removal of waste products. It is for this reason that cerebral vascular tone is tightly regulated, and why any alterations in mechanisms that modulate cerebral vessel function can predispose to cerebrovascular disease and stroke. Atherosclerosis is the underlying pathologic process for both coronary and cerebral artery disease, which are the two most common forms of cardiovascular disease.[1]

The purpose of this chapter is thus to provide insight into major mechanisms that regulate cerebral artery function, and alterations in these mechanisms in two major clinical conditions that have a significant negative impact on health worldwide—hypertension and atherosclerosis. The scope is mostly limited to discussion of cerebral blood vessels and mechanisms that regulate their tone, either under basal conditions or in response to physiologically relevant agonists.

ORGANIZATION OF THE CEREBRAL CIRCULATION

The brain is predominantly perfused by three pairs of intracranial arteries: the anterior, middle, and posterior cerebral arteries (ACA, MCA, and PCA, respectively). These arise from the circle of Willis, a ring of arteries formed by the anterior and posterior communicating arteries that connect the terminal ends of the basilar and internal carotid arteries. The ACA, MCA, and PCA travel along the pial surface of the brain, branching into smaller arterioles. Importantly, anastomoses exist between the smaller arterioles of these three major arterial trees, and collateral flow is thought to be important when blood flow in one region is compromised.[2] The pial arterioles then dive into the brain to give rise to parenchymal arterioles. Parenchymal arterioles are long, relatively unbranched arterioles that perfuse a distinct area of brain tissue.[3] The capillary network arises from the parenchymal arterioles, which is where the majority of nutrient and gas exchange occurs. Although much less is known about their function during health or disease, cerebral venules and veins are also important components of the cerebral circulation. For example, major disruption to blood-brain barrier function during acute hypertension occurs in the pial venules.[4]

PHYSIOLOGIC REGULATION OF CEREBRAL VASCULAR TONE

Numerous mechanisms regulate cerebral artery function. Most of the recent experimental evidence regarding such mechanisms has come from pharmacologic studies and the use of genetically modified mice. Major mechanisms include the release of nitric oxide (NO) from the endothelium to underlying smooth muscle cells (discussed in the Nitric Oxide and Cyclic Guanosine Monophosphate section); potassium ion (K^+) channels (see K^+ Channels), which includes a discussion of the newly described two-pore domain (K_{2P}) channels, Rho/Rho-kinase activity (see RhoA/Rho-Kinase); reactive oxygen species (ROS), which are discussed in the Reactive Oxygen Species section; and the recently described transient receptor potential (TRP) channels (discussed in the Transient Receptor Potential Channels section).

Nitric Oxide and Cyclic Guanosine Monophosphate

A major mechanism for maintenance of vascular tone by the endothelium involves the production of endothelium-derived NO. In endothelium, NO is synthesized from endothelial nitric oxide synthase (eNOS); it then diffuses to the underlying smooth muscle, where it activates soluble guanylate cyclase, which in turn leads to increased intracellular cyclic guanosine monophosphate levels and subsequent relaxation of the smooth muscle.[5] Experimental evidence for modulation of cerebral vascular tone by endothelium-derived NO has been obtained by applying inhibitors of NOS to cerebral blood vessels from several different species, both in vivo and in vitro, and has involved such inhibitors causing vasoconstriction (reviewed extensively in Faraci and Heistad[6]).

NO release from the endothelium can also be stimulated in response to receptor- (e.g., acetylcholine, bradykinin) or non-receptor-mediated agonists, or in response to shear stress. Endothelium-dependent, NO-mediated cerebral vascular relaxation in response to such agonists is often used to determine the functional integrity of the endothelium.

Endothelial dysfunction, manifested as diminished NO bioavailability experimentally by impaired endothelium-dependent vasodilation, or reduced vasoconstriction in response to a NOS inhibitor, is a common feature of many cerebrovascular-related diseases (discussed in the Alterations in Cerebral Vascular Function During Hypertension and Atherosclerosis section). Such exogenously applied agonists are often useful in this way experimentally, and they may also be important endogenously. For example, neurovascular coupling in some brain regions is mediated by neuronally released acetylcholine acting on the endothelium to stimulate eNOS.[7]

K^+ Channels

The activity of K^+ channels is a major regulator of smooth muscle cell membrane potential and, as such, is an important regulator of vascular tone. This is because vessel diameter is in large part dependent on cytosolic Ca^{2+} concentration, which in turn is dependent on membrane potential. There are five major types of K^+ channels known to be expressed in cerebral blood vessels: calcium (Ca^{2+})-activated (K_{Ca}) K^+ channels, ATP sensitive K^+ (K_{ATP}) channels, voltage-sensitive K^+ (K_V) channels, inwardly rectifying K^+ (K_{IR}) channels, and tandem-pore (TREK-1) channels, and all are regulators of vascular tone. This is supported by the wealth of information using both pharmacologic inhibitors and gene-targeted mice to study the regulation of membrane potential and vascular function. Potassium channels are also important mediators of vasodilator responses to several vasodilators that regulate vascular tone, and this will be also be discussed.

K_{Ca}-Activated K^+ Channels

There are three subtypes of K_{Ca} channels present in the vasculature: large-conductance K_{Ca} (BK_{Ca}) channels, intermediate-conductance (IK_{Ca}) channels, and small-conductance (SK_{Ca}) channels. Most research regarding the functional importance of this channel, especially in cerebral arteries, has centered around the BK_{Ca} channel.

As the name suggests, these channels are activated in response to increases in intracellular Ca^{2+}. Membrane depolarization, myogenic responses (i.e., pressure-induced vasoconstriction, important in development and maintenance of basal vascular tone), and elevations in arterial pressure are associated with elevations in intracellular Ca^{2+} concentration in cells of the vasculature.[8] Thus an important function of these channels appears to be to act as a negative feedback mechanism during increases in Ca^{2+} to limit vasoconstriction. A major mechanism of elevations in intracellular Ca^{2+} appears to be via Ca^{2+} sparks, which are localized elevations in cytosolic Ca^{2+}, due to the opening of ryanodine-sensitive Ca^{2+} release channels in the sarcoplasmic reticulum to K_{Ca} channels located on the plasma membrane.

These channels are important in modulating the basal tone of cerebral arteries, as selective inhibition of BK_{Ca} channels with tetraethylammonium ion (TEA) produces vasoconstriction.[8–10] In mice deficient in the β1 subunit of BK_{Ca} channels, increased intracellular Ca^{2+} concentration in response to ryanodine (which at low concentrations depletes Ca^{2+} stores from the sarcoplasmic reticulum so that intracellular Ca^{2+} concentration increases) and cerebral vascular constriction to iberiotoxin (selective inhibitor of BK_{Ca} channels) was reduced, suggesting that Ca^{2+} spark activity modulates myogenic tone through BK_{Ca} channel activation.[11] These channels may be more important in the modulation of basal tone in larger cerebral arteries.[8]

Recent evidence of the importance of Ca^{2+} spark activity and BK_{Ca} channels as mediators of vasodilators has emerged, as TEA and iberiotoxin inhibit vasodilator responses in response to vasodilators that activate adenylate cyclase and guanylate cyclase.[12] Acidosis markedly increased Ca^{2+} spark activity and caused dilatation of brain parenchymal arterioles. Dilatation was inhibited by inhibitors of ryanodine receptors (ryanodine) and BK_{Ca} channels (paxilline), as well as in mice lacking the BK_{Ca} channel.[13] Hydrogen sulfide (an important signaling molecule in the regulation of vascular tone and blood pressure) also increased Ca^{2+} spark and BK_{Ca} current frequency, as well as causing dilatation in cerebral arterioles—the vasodilatation was inhibited by ryanodine and iberiotoxin, suggesting Ca^{2+} spark activity is important in the response.[14] Intermittent hypoxia increased myogenic tone through loss of hydrogen sulfide activation of K_{Ca} channels.[15] Hypoxia had no effect on Ca^{2+} spark frequency but reduced K_{Ca} channel activity.[16] Protein expression of K_{Ca}2.2, 2.3, and 3.1,[16] as well as α- and β1-subunits of BK_{Ca} channels[17] in cerebral arteries, have been reported.

K_{ATP} Channels

K_{ATP} channels are defined by their sensitivity to intracellular ATP, with their activity being inhibited by intracellular ATP.[18] Generally, the intracellular concentration of ATP is normally sufficient that these channels have a low open probability in most vascular smooth muscle cells under normal conditions,[19] and this appears to also be the case in the cerebral circulation, where glibenclamide, a selective inhibitor of K_{ATP} channels, has no effect on cerebral vascular tone.[20] However, K_{ATP} channels appear to be present and functional in cerebral vessels based on direct evidence for their expression (discussed as follows) and a wealth of evidence reporting glibenclamide-sensitive relaxation of cerebral arteries in response to K_{ATP} channel activators.[18]

Several more recent studies have investigated the expression of K_{ATP} in cerebral vessels. K_{ATP} channels are thought to be a hetero-multimeric complex of two subunits: one is a pore-forming inward-rectifying K^+ channel type 6 (i.e., 6.1 or 6.2), and the other is a sulfonylurea receptor (SUR), either SUR1 and SUR2, with the SUR2 gene generating the two splice variants SUR2A and SUR2B.[21] Messenger RNA (mRNA) expression for both the pore-forming subunits (K_{IR}6.1 and 6.2) and SUR1, 2A, and 2B has been demonstrated in cerebral arteries,[21,22] although another study investigating SUR expression found no expression of SUR1 and reported only SUR2B expression.[23] Protein expression of K_{IR}6.1 and 6.2, as well as SUR1 and 2B, was also reported.[22] Cerebral arterioles were found to express K_{IR}6.1 and SUR2B,[24] with human cerebral arteries found to express SUR2B.[23]

Acidosis and reductions in intracellular pO_2 are known to produce cerebral vasodilatation. K_{ATP} channels have been shown to be involved in cerebral vasodilatation in response to acidosis,[25,26] as well as in vasodilatation to NMDA, which may be important in the coupling of cerebral metabolism and blood flow.[27] More direct evidence for a role of K_{ATP} channels in mediating vasodilatation in response to oxygen/glucose deprivation was reported in that vasodilatation was impaired in SUR-deficient compared with wild-type mice.[23] Myogenic tone, and vasodilatation in response to hypoxia, are not dependent on SUR2 expression,[23] although relaxation to hypoxia is inhibited by glibenclamide,[18,28] suggesting a role for K_{ATP} channels in hypoxia-induced vasodilatation where the K_{ATP} subunit composition does not involve SUR2. Hydrogen sulfide also dilates cerebral arteries, an effect that is inhibited by glibenclamide and in SUR2-deficient mice.[24]

K_V Channels

K_V channels are activated in response to increases in pressure in cerebral arteries and modulate cerebral vascular tone, in that pharmacologic inhibition of K_V channels with 4-aminopyridine causes cerebral artery depolarization and constriction.[29,30] K_V channels are also known to mediate cerebral artery dilations, including in response to NO.[29,31] K_V channel subunits are expressed in cerebral vessels (e.g., K_V1.2 and 1.5,[32–34] and K_V2.1 and 2.2[35,36])—including in humans.[37] K_V2-mediated current is proposed to underlie K_V-dependent modulation of cerebral artery tone in that inhibition of the K_V2 channel with stromatoxin-caused cerebral artery constriction.[36]

K_{IR} Channels

This channel is so named since it conducts K^+ current more readily into than out of the cell over a wide range of membrane potentials. However, at membrane potentials within the physiologic range, these channels actually conduct a small outward current. Consequently, when this channel is inhibited with the pharmacologic blocker, barium ion (Ba^{2+}), depolarization and constriction of cerebral arteries are observed.[38–44] Furthermore, in mice lacking the K_{IR}2.1 subunit—the subunit thought to be important in mediating vascular K_{IR} current—cerebral artery K_{IR} channel currents are absent.[45]

In the cerebral circulation, K^+ is released during neuronal activity and may be siphoned to cerebral vessels directly by astrocytes after neuronal activation.[46] Basal concentration of K^+ in cerebrospinal fluid is ~3 mM and may increase to between 4 and 7 mM during neuronal activity. In this concentration range (i.e., from 3 to 10 mM), K^+ causes dilatation of cerebral arteries[38,40–42,47,48] and arterioles.[39,43,44,49–56] Moreover, K^+-induced hyperpolarization and vasodilatation in this concentration range are inhibited by Ba^{2+},[38–42,48,53–55,57–59] suggesting K_{IR}-mediated K^+-induced vasodilation may be an important mechanism in the coupling of cerebral metabolism and blood flow (neurovascular coupling). Furthermore, cerebral vascular relaxation responses to K^+ are absent in mice lacking the K_{IR}2.1 subunit.[45] There have been reports of K_{IR}2.1 channel expression in cerebral arteries.[38,58] Regarding the role for K_{IR}2.1 channels in neurovascular coupling, recent work identified K_{IR}2.1 channel on capillaries as critical for sensing neuronal activity (via K+ release) and initiating a retrograde signal to dilate upstream arterioles, thereby increasing local blood flow.[60]

K_{2P} Channels

A new family of channels—two pore domain K^+ (K_{2P}) channels—have recently been characterized.[61] These channels require two protein subunits, each contributing two pore domains, to form a functional channel. There are several members of the K_{2P} family expressed in the vasculature, with some reported to be functionally important in the cerebral vasculature. Expression of TREK-1, TREK-2, TASK-1, TWIK-2, TRAAK, and THIK-1 has been reported in cerebral arteries, with TREK-1 being the most abundant.[62,63] Protein and mRNA expression of TREK-1 in the basilar artery was associated with vasodilatation induced by polyunsaturated fatty acids (which are important, as they improve brain resistance against cerebral ischemia), such as α-linolenic acid in wild-type mice; vasodilatation in response to linolenic acid was absent in mice deficient in TREK-1.[64] Nevertheless, another study reported similar vasodilator responses of the basilar artery to α-linolenic acid in wild-type and TREK-1-deficient mice.[65] Cerebral artery expression of TRAAK was associated with an important role in mediating endothelium-independent vasodilatation.[66]

RhoA/Rho-Kinase

Smooth muscle cell contractility is ultimately governed by the phosphorylation state of myosin light chain (MLC), vascular smooth muscle tone occurring in association with increasing levels of MLC phosphorylation. MLC is phosphorylated by MLC-kinase—a Ca^{2+}-calmodulin-dependent enzyme—and is dephosphorylated by MLC phosphatase (MLCP). MLC phosphorylation and smooth muscle contractility are not always directly proportional to intracellular Ca^{2+} concentration. Other mechanisms can regulate smooth muscle contractility independent of changes in intracellular Ca^{2+} concentration, a phenomenon known as Ca^{2+}-sensitization. Ca^{2+}-sensitization can occur through several pathways and ultimately results in inhibition of MLCP. One such pathway is the RhoA/Rho-kinase (ROCK) pathway. When ROCK is activated, it phosphorylates the myosin-binding (i.e., regulatory) subunit of MLCP, and thus inhibits MLCP activity, which ultimately leads to smooth muscle (and thus vascular) contractility.[67,68]

In vascular muscle, RhoA can be activated by stretch. This is important since myogenic tone is characterized by pressure-induced vasoconstriction, making it important for the development of basal vascular tone. The contribution of ROCK activity to the cerebral artery myogenic response has been studied through the use of Y-27632 and fasudil (HA-1077), pharmacologic inhibitors of Rho-kinase.[69] For example, Y-27632 relaxes cerebral artery segments following pressure-induced constriction,[70] and pressure-induced cerebral artery constriction is inhibited by Y-27632 and fasudil.[71–73] In vivo, where myogenic tone is present, several studies have reported that Y-27632 and fasudil cause the dilatation of cerebral arteries[74–78] and arterioles.[79] Recent work has begun to define the role of ROCK isoforms in the cerebral vasculature. The use of the selective ROCK2 inhibitor SLX-2119 (also known as KD025) has revealed that myogenic tone in brain parenchymal arterioles is ROCK2-dependent.[80] In addition, SLX-2119 dilates pial arterioles in vivo.[80]

ROCK is also important in the regulation of endothelial cell function via effects on NO signaling. ROCK has been shown to reduce NO bioavailability, which occurs via reducing NO production via reducing phosphorylation of the stimulatory Ser,[11, 77] direct phosphorylation of the inhibitory Thr^{495} residue on endothelial NOS, and/or reducing eNOS mRNA stability. These findings, in combination with the role of ROCK in vascular muscle, provide good evidence that the RhoA/Rho-kinase pathway is a major mechanism contributing to cerebral vascular tone.

Reactive Oxygen Species

ROS are known to influence cerebral vascular tone, and this is reviewed extensively elsewhere.[81] These ROS include the parent molecule superoxide (O_2^-), as well as hydroxyl radical (OH) and hydrogen peroxide (H_2O_2). The closely related reactive nitrogen species (RNS)—peroxynitrite—is also commonly involved in such effects.

Superoxide, a negatively charged anion, can elicit either dilatation[82–85] or constriction[82,86] of cerebral arteries. Superoxide reacts extremely efficiently with NO. As has been discussed, NO is a major regulator of cerebral vascular tone; thus reduced NO bioavailability following increased superoxide levels will likely result in vasoconstriction, with vasoconstriction being reported in response to higher concentrations of superoxide[82,83] and vasorelaxation at low concentrations.[82]

H_2O_2 is a chemically more stable species than superoxide, and it diffuses much more readily across cell membranes, thus

potentially being important as a signaling molecule. Many studies have reported that H_2O_2 acts as a cerebral vasodilator, both in vivo and in vitro,[85,87–94] although vasoconstriction has also been reported.[95]

Peroxynitrite, formed from the rapid chemical reaction of superoxide with NO, can also affect cerebral vascular tone, with both dilatation[96,97] and constriction[97–99] of cerebral arteries reported. Lower concentrations of peroxynitrite appear to cause cerebral vasoconstriction, with higher concentrations typically causing vasodilatation.[97,100]

Transient Receptor Potential Channels

TRP channels are a superfamily of cation channels comprising at least 28 members and are assigned to 6 subfamilies based on their sequence homology.[101] These are TRPC (classical), TRPV (vanilloid), TRPM (melastatin), TRPA (ankyrin), TRPP (polycystin), and TRPML (mucolipin).[102] The structure, expression profile, and function of TRP channels have been reviewed in detail.[103]

Depending on the specific TRP channel in question, activation can result in constriction or dilation of cerebral arteries. TRPC1 channels have been shown to mediate constriction of cerebral arteries via facilitating receptor-operated calcium entry in response to endothelin-1.[104] TRPC3 channels also facilitates vasoconstriction to endothelin-1,[105] but this does not occur via receptor-operated calcium entry. TRPC3 has also been shown to mediate constriction to the nucleotide, uridine triphosphate.[106] Myogenic tone in cerebral arteries isolated from hypertensive mice was inhibited by treatment with SKF93635 (a specific inhibitor of TRPC6 channels at the concentration used in that study). SKF93635 was without effect in arteries from aged mice, suggesting TRP channel function is disrupted in cerebral arteries from aged mice.[107] Some TRP channels, such as the vanilloid TRP channel (TRPV3), are chemosensitive. The TRPV3 channel is expressed in the endothelium of cerebral arteries, and the dietary agonist carvacrol, which may be cardioprotective, mediates endothelium-dependent cerebral vasodilatation that is inhibited by a pharmacologic inhibitor of TRPV1-4 channels.[108] TRPV4 channels are expressed in endothelium and vascular muscle cells and appear to mediate vasodilation. While activation of TRPV4 channels results in calcium entry in vascular muscle cells, the resulting calcium sparks activate BK channels and thus hyperpolarization and dilation of the artery.[109] Endothelial TRPV4 channels are activated (resulting in calcium influx) and mediate dilation in response to shear stress[110] and uridine triphosphate.[111] TRPV1, TRPV5, and TRPV6 channels do not appear to be expressed in cerebral arteries.[112] The melastatin TRP channel 4 (TRPM 4) is activated by high levels of intracellular Ca^{2+} and is known to be expressed in cerebral arteries.[113] Expression in smooth muscle cells is consistent with a role in the myogenic response, in that myogenic vasoconstriction was attenuated in cerebral arteries administered TRPM4 antisense.[114] Pharmacologic inhibition of the TRPM4 channel with 9-phenanthrol was able to cause hyperpolarization and prevent the development and maintenance of myogenic tone, further underlining its importance in the maintenance of myogenic tone in the cerebral circulation.[115] Another study also reported cerebral vascular expression of TRPM4 protein, which, once inactivated, results in reduced myogenic vasoconstriction in response to a PKC activator.[116] TRPA1 channels are known to be expressed in cerebral vessels, specifically in endothelium, and mediate endothelium-dependent vasodilatation.[117] Finally, TRPP2 channels have been shown to contribute to myogenic tone generation in cerebral arteries.[118] The role, if any, of other TRP channels in the cerebral vasculature is presently unknown.

ALTERATIONS IN CEREBRAL VASCULAR FUNCTION DURING HYPERTENSION AND ATHEROSCLEROSIS

Atherosclerosis

Atherosclerosis is the underlying pathologic process for both coronary and cerebral artery disease.[1] However, atherosclerotic lesions progress at a slower rate in intracranial arteries compared with extracranial arteries in both animal models and humans.[119] Atherosclerosis is thought to be initiated by trapping of lipids in the subendothelial layer, leading to the generation of biologically active oxidized species (i.e., oxidized low-density lipoprotein [LDL]), ultimately leading to recruitment of leukocytes to the artery wall.[120] Oxidative modification of LDL present in the intima by ROS may thus be a key initiating step in atherosclerosis.[121] Endothelial dysfunction is an early step in the development of atherosclerosis, and traditional cardiovascular risk factors (e.g., dyslipidemia, hypertension) are associated with endothelial dysfunction.[122] Furthermore, atherosclerosis is characterized by chronic inflammation of the vasculature; thus these three key processes characteristic of atherosclerosis—oxidative stress, endothelial dysfunction, and inflammation—will be discussed here (also summarized in Fig. 1.1), with much of the discussion referring to data from the apolipoprotein E-deficient ($ApoE^{-/-}$)

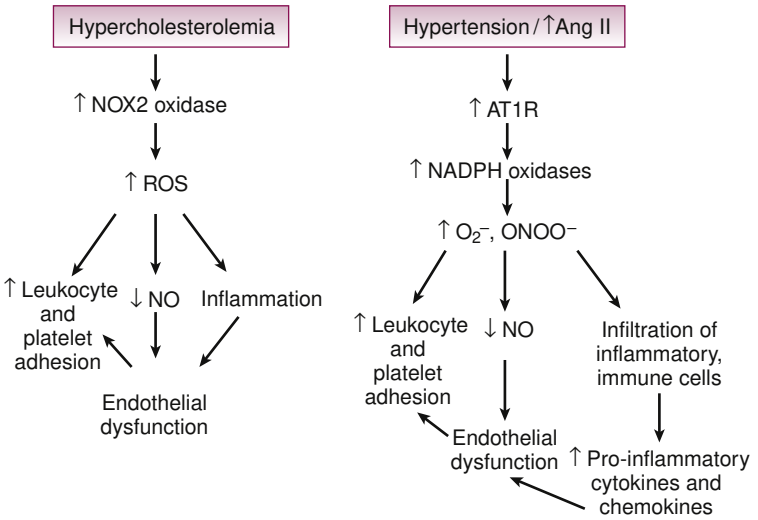

Fig. 1.1. Schematic diagram summarizing cerebrovascular effects of hypercholesterolemia, and elevated Ang II and hypertension. Hypercholesterolemia induces oxidative stress, and ultimately inflammation—comprising leukocyte and platelet adhesion, and endothelial dysfunction. These effects are all attenuated in NOX2 oxidase-deficient mice. Ang II increases leukocyte and platelet adhesion, infiltration of inflammatory/immune cells, and causes endothelial dysfunction due to reduced nitric oxide *(NO)* bioavailability. These effects are largely inhibited by AT1 receptor *(AT1R)* inhibitors and in AT1R-deficient mice; reactive oxygen species *(ROS)* scavengers and NOX2 oxidase-deficiency, as well as in lymphocyte-deficiency ($RAG^{-/-}$) mice, implicating AT1R; and nicotinamide adenine dinucleotide phosphate *(NADPH)* oxidase-derived ROS and the adaptive immune system in the detrimental effects of chronic hypertension in the cerebral circulation.

mouse. The ApoE$^{-/-}$ mouse, characterized by high levels of plasma cholesterol due to deletion of the APOE gene (important in cholesterol metabolism), provides a very useful experimental model for understanding the mechanisms of disease initiation.[1]

Cerebral Vascular Oxidative Stress in Models of Atherosclerosis

Some evidence suggests the prevalence of oxidative stress in cerebral vessels during hypercholesterolemia or atherosclerosis. For example, in wild-type mice placed on a high cholesterol diet for 2 weeks,[123] and ApoE$^{-/-}$ mice on high-fat diet for 7 weeks,[124] oxidative stress was found to be present in cerebral arteries. The study by Miller et al.[124] went on to suggest that NOX2 oxidase was the source of the oxidative stress, as the oxidative stress present in ApoE$^{-/-}$ was abolished in mice deficient in both ApoE and NOX2 (i.e., ApoE$^{-/-}$/NOX2$^{-/y}$; Fig. 1.2).

Cerebral Vascular Endothelial Dysfunction in Models of Atherosclerosis

Several lines of evidence suggest that atherosclerosis is associated with reduced NO bioavailability and endothelial dysfunction. In earlier reports, relaxation responses of the basilar artery to acetylcholine were impaired in hypercholesterolemic versus normal rabbits,[125] although cerebral vascular responses to acetylcholine were reportedly preserved[126,127] or even augmented[128] during atherosclerosis. In atherosclerotic monkeys, contraction of basilar arteries in response to inhibition of soluble guanylate cyclase was reduced compared with that in normal monkeys, suggesting the basal influence of soluble guanylate cyclase on basal tone of cerebral arteries is diminished during atherosclerosis, perhaps reflecting a reduced production/activity of NO during atherosclerosis.[129] Similarly, cerebral artery contractions in response to the application of L-NAME (a NOS inhibitor) were reduced in vessels from ApoE$^{-/-}$ compared to normal mice,[124] suggesting reduced NO bioavailability was present during atherosclerosis. Reduced cerebral vascular relaxation to acetylcholine in ApoE$^{-/}$ versus normal mice further suggests that reduced NO bioavailability is associated with endothelial dysfunction in the cerebral circulation during atherosclerosis.[130,131] Interestingly, magnetic resonance imaging of cerebral arteries in rabbits fed a diet high in cholesterol were narrower compared with their control counterparts,[132] which may suggest increased vascular tone or potentially structural alterations.

Further experiments were conducted to provide a link between oxidative stress and vascular dysfunction. Impaired NO-dependent responses of cerebral vessels from ApoE$^{-/-}$ mice were reversed in vessels from ApoE$^{-/-}$ mice treated with a scavenger of ROS (tempol),[124,130] the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase inhibitor apocynin,[130] or in ApoE$^{-/-}$/NOX2$^{-/y}$ mice,[124] strongly suggesting that NOX2 oxidase-derived superoxide is a major mediator of cerebral vascular dysfunction during atherosclerosis (see Fig. 1.2). Oxidative stress and endothelial dysfunction is present despite the apparent absence of lesions in cerebral blood vessels.[124,130]

Cerebral Vascular Inflammation in Models of Atherosclerosis

Atherosclerosis is characterized by chronic inflammation of the vasculature. Platelet endothelial cell adhesion molecule-1 (PECAM-1) is involved in the inflammatory process and in leukocyte-endothelial interactions, and its expression is increased in cerebral arterioles of ApoE$^{-/-}$ mice.[133] Leukocyte and platelet adhesion, as well as oxidative stress, were elevated in cerebral vessels of hypercholesterolemic mice—leukocyte and platelet adhesion was prevented by immunoneutralization of P-selectin and in NOX2-deficient mice, suggesting that P-selectin and NOX2-dependent oxidative stress are important mechanisms in hypercholesterolemia-induced inflammation in the brain.[123] Arginase type 1 expression was also increased in cerebral vessels from ApoE$^{-/-}$ mice,[134] which is relevant since oxidized LDL increases arginase activity and decreases endothelial NO levels, ultimately leading to impaired NO function in the vascular endothelium.[135] Vascular cell adhesion molecule-1 (VCAM-1) expression was not altered in brain microvessels of ApoE$^{-/-}$ mice.[136]

Hypertension

Hypertension profoundly and negatively impacts the cerebral circulation and brain, and is a major risk factor for stroke and a leading cause of cognitive decline and dementia.[137] Hypertension may promote the formation of atherosclerotic plaques in cerebral arteries and arterioles,[137] and there is a wealth of experimental evidence demonstrating detrimental functional consequences of hypertension on the cerebral circulation. Many initial studies focused on the spontaneously hypertensive rat (SHR), where augmented NADPH oxidase-derived superoxide production[91] and impaired endothelium-dependent responses have been reported.[58,75,138–141] What follows is a discussion of more recent data regarding the influence of hypertension on the cerebral circulation—specifically, hypertension in response to elevated angiotensin II (Ang II) levels (also summarized in Fig. 1.1). Ang II is of major importance because it is involved in many of the functional and structural changes occurring in the cerebral circulation during chronic hypertension.[5,119,137]

Oxidative Stress in Hypertension Involving Elevated Ang II

Ang II increases ROS production in the cerebral circulation. Work from Iadecola's group has found that acute intravenous infusion of mice with Ang II increases both blood pressure and ROS production by cerebral blood vessels.[142–146] Increased ROS levels were prevented by treatment with the ROS scavenger MnTBAP.[146] This treatment also reportedly increases 3-nitrotyrosine immunoreactivity (indicative of nitrosative stress) in mouse cerebral vascular endothelial cells, an effect that was prevented by a peroxynitrite scavenger and a NOS inhibitor, and was also absent in NOX2 oxidase-deficient mice.[143] Thus these findings suggest that Ang II increases peroxynitrite formation in the cerebral vasculature largely via the reaction of NOX2 oxidase-derived superoxide with NO (see Fig. 1.2).

Endothelial Dysfunction in Hypertension Involving Elevated Ang II

Acute intravenous administration of Ang II has been reported to impair NO-dependent increases in cerebral blood flow(CBF),[145,146] an effect that was reversed by MnTBAP and the angiotensin type 1 (AT_1) receptor antagonist losartan.[146] Topical application of Ang II to cerebral arterioles in vivo causes impaired NO-dependent responses that can be prevented by the superoxide scavenger tiron.[147] Similarly, Ang II-induced endothelial dysfunction in cerebral arterioles of ECSOD-deficient mice in vivo was reversed by tempol.[148] In a more chronic model of Ang II-dependent hypertension, Ang II increased blood pressure and caused endothelial dysfunction of the basilar artery. This effect of Ang II was absent in NOX2 oxidase-deficient mice, and partially attenuated in NOX1 oxidase-deficient mice, suggesting Ang II-induced endothelial dysfunction is dependent on NOX2 oxidase and, perhaps to

some, extent NOX1 oxidase.[149] In spite of these findings, Ang II increases blood pressure in both NOX2 and NOX1 oxidase deficient mice, suggesting that the cerebral vascular and pressor actions of Ang II are independent of one another.[149] To further confirm this point, previous studies have reported that systemic administration of a nonpressor dose of Ang II caused endothelial dysfunction in the cerebral circulation.[150] In addition, endothelial dysfunction precedes the development of hypertension in response to a slow-pressor dose of Ang II.[151] Endothelial dysfunction in response to Ang II was reversed by ROS scavengers.[150,152] It has recently been reported that inhibition of the mineralocorticoid receptor (MR) improves endothelial dysfunction in Ang II hypertension via a mechanism involving TRPV4.[153] Thus there is potential cross-talk between AT1R and MR. In a genetic model of hypertension (mice overexpressing human renin and angiotensinogen), endothelial dysfunction of the basilar artery was completely reversed by the administration of polyethylene glycol superoxide dismutase (PEG-SOD).[154] Taken together, these data suggest that Ang II causes endothelial dysfunction in the cerebral circulation by activating AT1R expressed in the vessel wall, leading to an increase in superoxide production, and subsequent oxidative inactivation of NO (see Fig. 1.2). Recent evidence suggests that MR may also be involved in the dysfunction caused by Ang II.

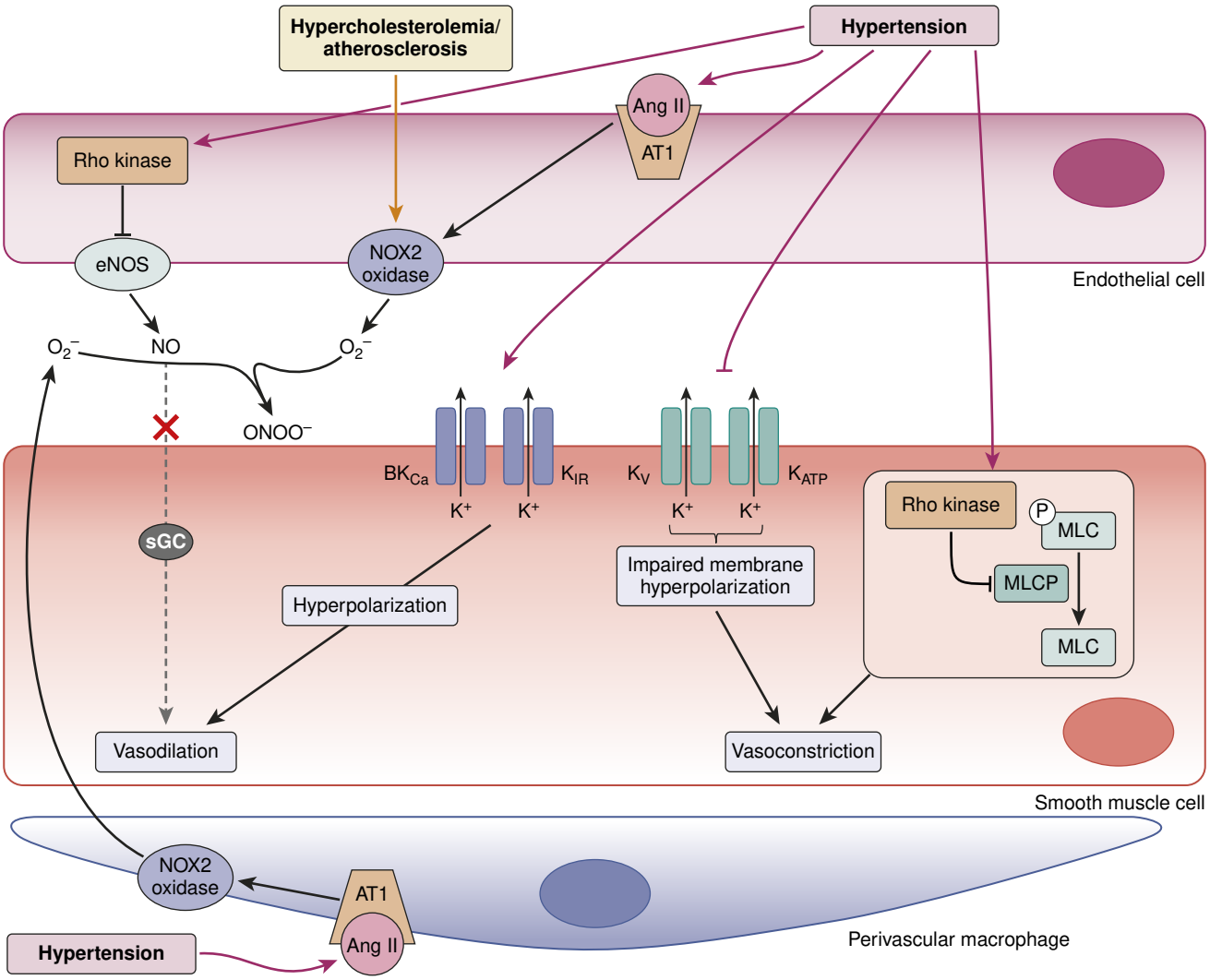

Fig. 1.2. Atherosclerosis/hypercholesterolemia and hypertension profoundly alter key mechanisms that modulate cerebral artery tone. Atherosclerosis/hypercholesterolemia and hypertension increase oxidative stress via activation of NOX2 oxidase. The increased superoxide *(O_2^-)* levels scavenges endothelial nitric oxide synthase *(eNOS)*-derived nitric oxide *(NO)*, resulting in reduced NO bioavailability and hence reduced NO-mediated vasodilation and peroxynitrite formation *($ONOO^-$)*. $ONOO^-$ can directly influence cerebrovascular tone (see text). K^+ channel activity modulates vascular tone. Most studies have investigated the effect of hypertension on BK_{Ca} channel function, with the outcome (i.e., increased or decreased channel function) depending on the model of hypertension studied (see text). Baseline K_V channel function is impaired, as is K_{ATP}-mediated vasodilatation compared with normotensive conditions. Baseline K_{IR} channel function is augmented, whereas K_{IR}-mediated K^+-induced vasodilatation is impaired. In endothelial cells, Rho kinase can reduce eNOS activity (see text for details). In vascular muscle, Rho kinase phosphorylates (and inactivates) myosin light chain phosphatase *(MLCP)*, leading to enhanced phosphorylation of myosin light chain *(MLC)* and increased contractility. During hypertension, Rho kinase activity is increased, impairing normal cerebrovascular regulation.

Cerebrovascular Inflammation in Hypertension Involving Elevated Ang II

Hypertension induces inflammation in the cerebral circulation. This includes models that involve Ang II. Ang II results in elevated leukocyte and platelet adhesion in cerebral vessels, an effect prevented by the AT1 receptor antagonists candesartan and losartan, as well as diphenyleneiodonium, an inhibitor of flavoproteins such as NADPH oxidase.[155] In studies performed to extend these findings, Ang II-induced hypertension was associated with a marked increase in leukocyte and platelet adhesion in cerebral vessels, which was attenuated in $RAG^{-/-}$ mice (i.e., deficient in T and B lymphocytes), $AT1R^{-/-}$ mice, and by treatment with losartan,[156] suggesting the involvement of immune cells and AT1 receptors in this effect. Leukocyte adhesion in response to Ang II in pial vessels in vivo was also prevented by tempol,[157] further confirming that cerebrovascular inflammation involves ROS production and oxidative stress. Interestingly, although leukocyte and platelet adhesion in cerebral vessels was enhanced in models of Ang II and deoxycorticosterone acetate (DOCA)-salt hypertension, these effects were prevented in the presence of mild hypercholesterolemia, possibly due to the involvement of high-density lipoprotein (HDL), suggesting that mild elevations in certain types of cholesterol may be beneficial in the setting of hypertension.[158]

Other models of hypertension have also implicated a role for Ang II in cerebrovascular inflammation. For example, in a DOCA-salt model of hypertension, leukocyte and platelet adhesion was prevented by losartan and in $AT1R^{-/-}$ mice.[159] Furthermore, not only were these effects inhibited by tempol; they were also inhibited by mito-tempol,[159] implicating mitochondria-derived ROS in the cerebrovascular inflammatory response. These anti-inflammatory effects occurred in the absence of any depressor action, suggesting that blood pressure is not necessarily a key mediator of cerebrovascular inflammation where Ang II is involved. In the SHR, increased expression of intracellular adhesion molecule-1 (ICAM-1), as well as an increased number of infiltrating and adherent macrophages in brain microvessels, were inhibited by candesartan.[160] Widespread inflammation in many brain regions of the SHR was also inhibited by candesartan,[161] further implicating a role for activation of AT1R by Ang II and demonstrating a beneficial use for AT1R inhibitors in preventing inflammation associated with cerebrovascular disease.

Perivascular macrophages appear to play a central role in cerebrovascular dysfunction in response to Ang II.[162] Faraco et al. showed that Ang II acts on AT1R on perivascular macrophages, resulting in NOX2 oxidase-dependent ROS production and endothelial dysfunction.[162] Thus inflammation appears to be a key mechanism, leading to both oxidative stress and subsequent endothelial dysfunction.

K^+ Channel Function in Chronic Hypertension

Expression of K^+ channels in the cerebral vasculature and the importance of their role in modulating arterial tone, including mediation of vasodilator responses, has been described. The deleterious actions of chronic hypertension in the cerebral vasculature are also well known;[137] it is thus unsurprising that K^+ channel function is altered in association with chronic hypertension (see Fig. 1.2).

BK_{Ca} Channels. Basal activity of BK_{Ca} channels may be greater in cerebral arteries during chronic hypertension, in that pharmacologic inhibition of these channels (with TEA and iberiotoxin) elicits greater contraction of cerebral arteries from hypertensive vs normotensive rats.[10] Consistent with this, iberiotoxin elicited enhanced contraction of cerebral arterioles from hypertensive versus normotensive rats, an effect associated with enhanced cerebral vascular expression of the K_{Ca} channel α subunit.[163] Inhibition of BK_{Ca} channels with TEA and charybdotoxin cause cerebral vascular contraction in hypertensive but not normotensive rats.[164]

By contrast, in a model of Ang II-dependent hypertension, contraction of cerebral arteries in response to iberiotoxin was reduced in hypertensive vs normotensive rats, and this was associated with reduced coupling efficiency between Ca^{2+} sparks and BK_{Ca} channels, as well as reduced β1 subunit expression, although α subunit expression was unaltered during chronic hypertension.[165] The mechanism may involve calcineurin/NFATc3 signaling, as Ang II-induced reduction in β1 subunit expression was absent in calcineurin/NFATc3-deficient mice. Calcineurin/NFATc3 is also important in the development of Ang II-dependent hypertension.[166] Furthermore, although iberiotoxin caused myogenic constriction in normal mice, in a model of Ang II-dependent hypertension, it had no effect on myogenic constriction in cerebral arteries.[107] In a model of diet-induced obesity where blood pressure was elevated, cerebral vascular BK_{Ca}β1 subunit expression was increased, although myogenic tone was not altered.[17] Thus functional alterations in K_{Ca} channels appear to be dependent on the model of experimental hypertension studied.

K_{ATP} Channels. To our knowledge, there is little information regarding K_{ATP} channel function in hypertension. Vasodilator responses to the K_{ATP} channel activator aprikalim were significantly impaired in cerebral arteries from hypertensive versus normotensive rats, suggesting impaired K_{ATP} channel function during hypertension.[138] Although SUR2B expression appears to be increased in small cerebral arteries from hypertensive rats compared with their normotensive controls,[167] the functional significance of this finding is unknown.

K_V Channels. Experimental hypertension may be associated with cerebral artery depolarization and increased myogenic response, perhaps indicating impaired K_V channel function. Pharmacologic inhibition of K_V channels with correolide and psora-4 constricted cerebral arteries from normotensive rats, but was without effect in cerebral arteries from two models of hypertension, suggesting a reduced contribution of K_V channels to the modulation of basal tone. This was associated with reduced expression of the pore-forming $\alpha_{1.2}$ and $\alpha_{1.5}$ subunits that compose K_V channels in hypertensive versus normotensive rats.[168] This is in agreeance with the impaired K_V2 channel function of cerebral arteries reported in a model of Ang II-dependent hypertension, in that stromatoxin-induced contraction of cerebral arteries was decreased in arteries from hypertensive vs normotensive rats.[36] In Dahl salt-sensitive rats, K_V channel current density was decreased in cerebral artery myocytes from hypertensive vs normotensive rats.[169] Lower K_V current density was reported in cerebral vascular smooth muscle cells from SHR compared with WKY.[170]

K_{IR} Channels. The first evidence for impaired K_{IR} channel function during chronic hypertension was the finding that Ba^{2+}-sensitive cerebral vascular relaxant responses to K^+ in hypertensive rats were impaired when compared with normotensive controls.[171] A subsequent study reported altered K_{IR} channel function during chronic hypertension, whereby K_{IR} channels were not the predominant mediator of cerebral vasodilator responses to K^+, unlike in normal animals. This was despite responses to K^+ being preserved (or even enhanced), $K_{IR}2.1$ expression being preserved, and an enhanced role for

K_{IR} channels in modulating arterial tone during chronic hypertension.[58] In the cerebral microvasculature, preserved (or even enhanced) vasorelaxation to K^+ during chronic hypertension was mediated by K_{IR} channels.[55] K_{IR} channel function may thus be preserved in smaller arterioles[55] during chronic hypertension, as opposed to impaired function in larger arteries,[58,171] at least in mediating responses to K^+.

Rho-Kinase in Hypertension

Enhanced dilator responses of the basilar artery to Y-27632 in models of chronic hypertension suggest an increase in Rho-kinase function in hypertension (see Fig. 1.2).[75,77,78] Furthermore, pressure-dependent development of myogenic tone of cerebral arteries is inhibited by Y-27632 to a greater extent in hypertensive vs normotensive rats,[172] thus supporting an important role for Rho-kinase in increased myogenic tone of cerebral arteries. A recent study demonstrated that acute systemic elevations in endothelin-1 levels impaired cerebral vascular endothelial function, an effect that was reversed by Y-27632.[173] Interestingly, the role of Rho-kinase as a key mediator of cerebral vascular dysfunction during chronic hypertension may be dependent on the cause of the hypertension, as Ang II-induced cerebral endothelial dysfunction was not reversed by Y-27632.[173]

CONCLUSION

Experimental evidence for some major mechanisms regulating cerebral vascular function has been presented, together with how many of these mechanisms are altered during hypertension and atherosclerosis—two disease states that predispose to clinical stroke. Consequently, molecular targets that may be of benefit in cerebrovascular disease, and perhaps the prevention and/or treatment of ischemic stroke, are being identified. However, clearly further work is needed to ultimately identify effective therapies, as these diseases are currently poorly controlled.

The complete reference list is available at www.expertconsult.inkling.com.

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2 Mechanisms of Thrombosis and Thrombolysis

Gregory J. del Zoppo

KEY POINTS

- The fundamental processes involved in thrombus formation, thrombus dissolution, and thrombus stability and their relevance to the central nervous system (CNS) are described.
- The role(s) of endogenous plasminogen activators (PAs, including tissue-type plasminogen activator, urokinase-type plasminogen activator) in thrombus dissolution are presented, together with considerations of their regulation in vivo. Their relevance to derived therapeutics is emphasized.
- Fibrinolytic agents tested or used as pharmaceuticals including recombinant and purified endogenous PAs and exogenous PAs (including streptokinase, staphylokinase, PAs derived from *Desmodus* species, and novel plasminogen activators) are presented.
- The molecular basis for PA inhibition and modulation of vascular fibrinolysis is made.
- These considerations form a basis for exploration of current information about the impact of PAs and of plasmin generation on CNS vessel and microvessel integrity.
- Exploration of the role(s) of endogenous PAs in CNS development, CNS integrity, and on neuronal function in the CNS is presented, and the potential effects of therapeutic PAs on the CNS.
- The pioneering use of therapeutic plasminogen activation in the acute setting in ischemic/thrombotic stroke, acute cerebral arterial recanalization, and its consequences are described.
- The use of PAs in experimental cerebral ischemia, recanalization and tissue injury reduction, and their limitations and relevance to the clinical setting are discussed.
- The risks of PAs in the acute intervention in ischemic stroke and the quantitative effects on intracerebral hemorrhage are presented. Limitations to the clinical use of fibrinolytic agents in ischemic stroke are considered.

Thrombosis, and thrombus growth, dissolution, and migration are inextricably connected. Thrombus formation involves activation of platelets, activation of the coagulation system, and the processes of fibrin dissolution. The central feature of each of these processes is the generation of thrombin from prothrombin. Thrombin, in turn, generates the thrombus fibrin network by the cleavage of circulating fibrinogen with formation of the fibrin network. Excess local vascular fibrin deposition can contribute to thrombus growth, while vascular injury and excess degradation of fibrin in "hemostatic plugs" at sites of vascular injury can lead to hemorrhage. Plasmin can degrade fibrin and fibrinogen. Plasminogen activators (PAs), which convert plasminogen to plasmin, have been exploited to dissolve clinically significant vascular thrombi acutely. Notably, all substances that promote plasmin formation have the potential to increase the risk of hemorrhage.

The acute use of PAs has been associated with detectable clinical improvement in selected patients with symptoms of focal cerebral ischemia.[1-9] Acute thrombolysis has thus attained pride of place in the treatment of ischemic stroke so far. Currently, recombinant tissue plasminogen activator (rt-PA) is licensed in the United States, Japan, Europe, and many other countries for the treatment of ischemic stroke within 3 hours of symptom onset, and up to 4.5 hours in some jurisdictions.[6,9] Early studies, a phase III prospective trial, and more recent experience suggest that extension of the treatment window is possible with strict limitations to patient selection.[3-5,9] Early on, few studies of acute rt-PA delivery correlated improvement in patient outcome with imaging evidence of recanalization of an occluded brain-supplying artery, however.[4]

The development of agents that promote fibrin degradation in the clinical setting stems from observations in the 19th century of the spontaneous liquefaction of clotted blood and the dissolution of fibrin thrombi. A growing understanding of plasma proteolytic digestion of fibrin paralleled enquiry into the mechanisms of streptococcal fibrinolysis. Streptokinase (SK) was the first PA employed to dissolve closed space (intrapleural) fibrin clots, but purified preparations were required for lysis of intravascular thrombi. The development of PAs for therapeutic lysis of vascular thrombi has progressed along with insights into the mechanisms of thrombus formation and degradation. It should be remembered that the concentrations of PAs used to degrade fibrin thrombi clinically far exceed those required to perform the same task endogenously.

THROMBUS FORMATION

The relative platelet-fibrin composition of a specific thrombus depends on the vascular bed, the local development of fibrin, platelet activation, and regional blood flow or shear stress. Even in the same arterial territory there may be considerable variability and local heterogeneity in thrombus composition as evidenced by thrombi removed in situ.[10-13] Pharmacologic inhibition of the platelet activation/aggregation and coagulation processes can also alter thrombus composition and volume. At arterial flow rates thrombi are predominantly platelet rich, whereas at lower shear rates characteristic of venous flow, activation of coagulation seems to predominate. It has been suggested that the efficacy of pharmacologic thrombus lysis depends on (i) the relative fibrin content and (ii) the extent of fibrin cross-linking of the thrombus that may reflect thrombus age and thrombus remodeling. The latter may vary with location within a vascular bed (e.g., arterial, capillary, or venular).

Thrombin (factor IIa) is the central player in clot formation (Fig. 2.1). Thrombin, a serine protease, cleaves fibrinogen to generate fibrin, which forms the scaffolding for the growing thrombus. Inter-fibrin strand cross-linking requires active factor XIII, a transglutaminase bound to fibrinogen that is itself activated by thrombin. Factor XIIIa stabilizes the fibrin network (Fig. 2.2).[14,15] Thrombin-mediated fibrin polymerization leads to the generation of fibrin I and fibrin

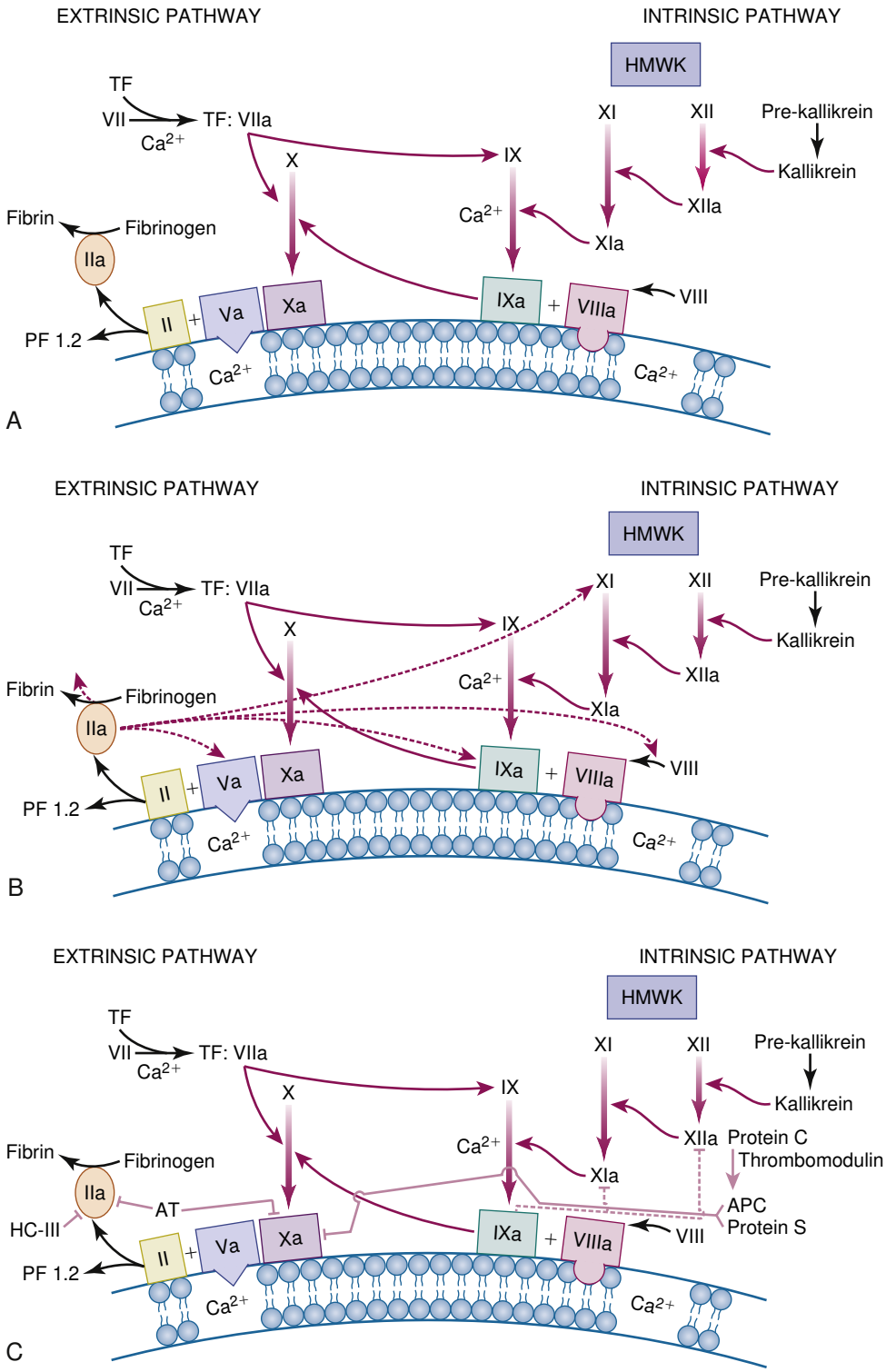

Fig. 2.1. Intrinsic and extrinsic coagulation pathways (see text). Phospholipid-containing membranes (e.g., platelets) provide the scaffold for accelerating coagulation pathway activation. Both intrinsic and extrinsic pathways lead to prothrombin (factor II) activation, with fibrin generation from circulating fibrinogen. The *extrinsic* pathway initiates coagulation through the interaction of factor VII with tissue factor *(TF)* in the vascular adventitia, brain perivascular parenchyma, and activated monocytes. The TF:VIIa complex catalyzes activation of factor X and acceleration of thrombin generation. The *intrinsic* system involves activation of components within the vascular lumen. Initiation of coagulation through this pathway involves pre-kallikrein, kallikrein, high-molecular-weight kininogen *(HMWK)*, and factors XI and XII. (A) Thrombin generation**.** The intrinsic system activates factor X through the "tenase" complex (factors VIIIa and IXa, and Ca^{2+} on phospholipid). Both intrinsic and extrinsic pathways activate prothrombin through the common "prothrombinase" complex (factors Xa and Va, and Ca^{2+}). The platelet surface has receptors for factors Va and VIIIa. Cleavage of prothrombin generates the prothrombin fragment 1.2 (PF 1.2) and thrombin (factor IIa). (B) Thrombin has multiple stimulatory positive feedback effects. It catalyzes activation of factors XI and VIII as well as the activities of the tenase and prothrombinase complexes. Thrombin also stimulates activation of platelets and granule secretion via specific thrombin receptors on their surface. (C) Coagulation activation is regulated by interleaving inhibitor pathways. The effects of factors Va, Xa, and VIIIa are modulated by the protein C pathway. Activated protein C *(APC)*, generated by the action of the endothelial cell receptor thrombomodulin on protein C, with its cofactor protein S, inhibits the action of factor V. *AT*, Antithrombin; *HC-III*, heparin cofactor-III.

II monomers and to the release of fibrinopeptide A (FPA) and fibrinopeptide B (FPB).

Platelet activation is required for thrombus formation under arterial flow conditions and accompanies thrombin-mediated fibrin formation. Platelet membrane receptors and phospholipids form a workbench for the generation of thrombin through both the intrinsic and extrinsic coagulation pathways.[16] Platelets promote activation of the early stages of intrinsic coagulation by a process that involves the factor XI receptor and high-molecular-weight kininogen (HMWK) (see Fig. 2.1).[17] Also, factors V and VIII interact with specific platelet membrane phospholipids (receptors) to facilitate the activation of factor X to Xa (the "tenase complex") and the conversion of prothrombin to thrombin (the "prothrombinase complex") on the platelet surface.[18] Platelet-bound thrombin-modified factor V (factor Va) serves as a high-affinity platelet receptor for factor Xa.[19] These mechanisms accelerate the rate of thrombin generation, further catalyzing fibrin formation and the fibrin network.

This process also leads to the conversion of plasminogen to plasmin and to the activation of *endogenous* fibrinolysis. Thrombin provides one direct connection between thrombus formation and plasmin generation, through the localized release of tissue plasminogen activator (t-PA) and single chain urokinase (scu-PA) from endothelial cells. Thrombin has been shown in vitro and in vivo to markedly stimulate t-PA release from endothelial stores.[19,20] In one experiment, infusion of factor Xa and phospholipid into non-human primates resulted in a pronounced increase in circulating t-PA activity, suggesting that significant vascular stores of this PA can be released by active components of coagulation. Other vascular and cellular stimuli also augment PA release, thereby pushing the hemostatic balance toward thrombolysis (see below).

The development of arterial or venous thrombi requires loss of the constitutive antithrombotic characteristics of endothelial cells. In addition to both the antithrombotic properties of endothelial cells and the circulating anticoagulants and their cofactors (i.e., activated protein C [APC], protein S), thrombus growth is limited by the *endogenous* thrombolytic system. Thrombus dissolution or remodeling results from the preferential conversion of plasminogen to plasmin on the thrombus surface. There, fibrin binds t-PA in proximity to its substrate (fibrin-bound) plasminogen, thereby accelerating local plasmin formation, in concert with local shear stress.[21] The parallel role of scu-PA is discussed below.

These processes may also promote embolization into the downstream cerebral vasculature. However, little is known about the endogenous generation and secretion of PAs within cerebral vessels.[22] *Exogenous* application of pharmacologic doses of PAs can accelerate conversion of plasminogen to plasmin and thereby prevent thrombus formation and promote thrombus dissolution, as discussed later.

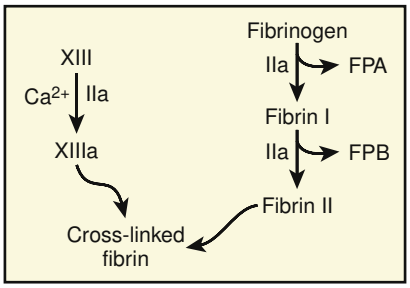

Fig. 2.2. Generation of cross-linked fibrin. Fibrinogen is cleaved successively to form fibrin I and fibrin II by thrombin (factor IIa) with the release of fibrinopeptides A and B *(FPA and FPB)*. Thrombin activates factor XIII to the active transglutaminase, which promotes cross-linking of fibrin and stabilization of the growing thrombus.

FIBRINOLYSIS

Plasmin formation is central to the lysis of vascular thrombi. The endogenous fibrinolytic system comprises plasminogen, scu-PA, urokinase (u-PA), and t-PA, and their inhibitors. Hence, plasmin degrades fibrin (and fibrinogen). Plasminogen, its activators, and their inhibitors contribute to the balance between vascular thrombosis and hemorrhage (Fig. 2.3; Tables 2.1 and 2.2).

Plasmin formation occurs (i) in the plasma, where it can cleave circulating fibrinogen and fibrin into soluble products,[23]

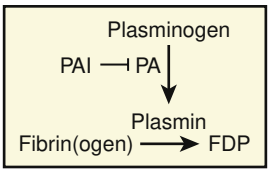

Fig. 2.3. Plasminogen activation and fibrin(ogen)olysis. Degradation of fibrinogen and fibrin is catalyzed by plasmin. Plasminogen activators *(PAs)*, including tissue PA, urokinase PA, and novel constructs, cleave plasminogen to the active plasmin. Characteristic products of fibrin and fibrinogen degradation *(FDP)* are generated (see text). *PAI*, Plasminogen activator inhibitor.

TABLE 2.1 Plasminogen Activators.

Plasminogen Activators	Molecular Weight (kDa)	Chains	Plasma Concentration (mg/dL)	Plasma Concentration Half-Life ($t_{1/2}$)	Substrates
ENDOGENOUS					
Plasminogen	92	2	20	2.2 days	(Fibrin)
Tissue PA (t-PA)	68 (59)	1→2	5×10^{-4}	5–8 min	Fibrin/plasminogen
Single-chain urokinase PA (scu-PA)	54 (46)	1→2	$2–20 \times 10^{-4}$	8 min	Fibrin/plasmin(ogen)
Urokinase PA (u-PA)	54 (46)	2	8×10^{-4}	9–12 min	Plasminogen
EXOGENOUS					
Streptokinase	47	1	0	41 and 30 min	Plasminogen, fibrin(ogen)
Anisoylated plasminogen-streptokinase activator complex (APSAC)	131	Complex	0	70–90 min	Fibrin(ogen)
Staphylokinase	16.5		0		Plasminogen
Desmoteplase	52	1	0	138 min	Plasminogen

TABLE 2.2 Plasminogen Activator Inhibitors.

Inhibitor	Molecular Weight (kDa)	Chains	Plasma Concentration (mg/dL^{-1})	Plasma Concentration Half-Life ($t_{1/2}$)	Inhibitor Substrates
PLASMIN INHIBITORS					
α_2-antiplasmin	65	1	7	3.3 min	Plasmin
α_2-macroglobulin	740	4	250		Plasmin (excess)
PLASMINOGEN ACTIVATOR INHIBITORS					
PAI-1	48–52	1	5×10^{-2}	7 min	t-PA, u-PA
PAI-2	47, 70	1	$<5 \times 10^{-4}$	24 h	t-PA, u-PA
PAI-3	50				u-PA, t-PA

PAI, Plasminogen activator inhibitor; $t_{1/2}$, half-life; *t-PA*, tissue plasminogen activator; *u-PA*, urokinase plasminogen activator.

and (ii) on reactive surfaces (e.g., thrombi or cells). The fibrin network provides the scaffold for plasminogen activation, whereas various cells, including polymorphonuclear (PMN) leukocytes, platelets, and endothelial cells, express receptors for plasminogen to bind to.[23] Specific cellular receptors concentrate plasminogen and specific activators (e.g., urokinase plasminogen activator [u-PA]) on the cell surface, thereby enhancing local plasmin production. Similar receptors on tumor cells (e.g., the urokinase plasminogen activator receptor [u-PAR], which concentrates u-PA) also facilitate dissolution of basement membranes and matrix, promoting metastases. u-PA and u-PAR are both expressed by microvessels and neurons in the ischemic bed.[24,25] Plasmin can also cleave various extracellular matrix (ECM) glycoprotein components (e.g., laminins, collagen IV, perlecan) found in the basal lamina of microvessels of the central nervous system, and in other organs.[26-28]

Plasminogen

The naturally circulating PAs, single-chain t-PA and single-chain u-PA (scu-PA or pro-UK), catalyze plasmin formation.[29,30] Plasmin derives from the zymogen plasminogen, a glycosylated single-chain 92-kDa serine protease.[31,32] Structurally, plasminogen contains five kringles and a protease domain, two of which (K1 and K5) mediate the binding of plasminogen to fibrin through characteristic lysine-binding sites (Fig. 2.4).[31,33,34] Glu-plasminogen has an NH_2-terminal glutamic acid, and lys-plasminogen, which lacks an 8-kDa peptide, has an NH_2-terminal lysine. Plasmin cleavage of the NH_2-terminal fragment of glu-plasminogen generates lys-plasminogen. Glu-plasminogen has a plasma clearance half-life ($t_{1/2}$) of ~2.2 days, whereas the $t_{1/2}$ of lys-plasminogen is 0.8 days. Both t-PA and u-PA catalyze the conversion of glu-plasminogen to lys-plasmin through either of two intermediates, glu-plasmin or lys-plasminogen.[35] The lysine-binding sites of plasminogen mediate the binding of plasminogen to α_2-antiplasmin, thrombospondin, components of the vascular ECM, and histidine-rich glycoprotein (HRG).[32] α_2-Antiplasmin prevents binding of plasminogen to fibrin by this mechanism.[35] Partial degradation of the fibrin network enhances the binding of glu-plasminogen to fibrin, promoting further local fibrinolysis.

Plasminogen Activation

Plasminogen activation is tied to activation of the coagulation system and can involve secretion of physiologic PAs ("extrinsic activation"). It has been suggested that kallikrein, factor XIa, and factor XIIa, in the presence of HMWK, can directly activate plasminogen.[35,36] Several lines of evidence suggest that scu-PA activates plasminogen under physiologic conditions. Tissue-type PA, which is secreted from the endothelium and other cellular sources, appears to be the primary PA in the vasculature. Thrombin, generated by either intrinsic or extrinsic coagulation, stimulates secretion of t-PA from endothelial stores.[19,37]

Several serine proteases can convert plasminogen to plasmin by cleaving the arg^{560}-val^{561} bond.[31] Serine proteases have common structural features, including an NH_2-terminal "A" chain with substrate-binding affinity, a COOH-terminal "B" chain with the active site, and intra-chain disulfide bridges. Plasminogen-cleaving serine proteases include the coagulation proteins factor IX, factor X, and prothrombin (factor II), protein C, chymotrypsin and trypsin, various leukocyte elastases, the plasminogen activators u-PA and t-PA, and plasmin itself.[31]

Activation of plasminogen by t-PA is accelerated by a ternary complex with fibrin. In the circulation, plasmin binds rapidly to the inhibitor α_2-antiplasmin and is thereby inactivated. Activation of thrombus-bound plasminogen also protects plasmin from the inhibitors α_2-antiplasmin and α_2-macroglobulin.[31] Here, the lysine-binding sites and the catalytic site of plasmin are occupied by fibrin, thereby blocking its interaction with α_2-antiplasmin.[31] Furthermore, fibrin and fibrin-bound plasminogen render t-PA relatively inaccessible to inhibition by other circulating plasma inhibitors.

Thrombus Dissolution

Fibrinolysis occurs predominantly at the surface, and so may be augmented by increased local blood flow, but also by flow within the thrombus.[38,39] During thrombus consolidation, plasminogen bound to fibrin and to platelets allows local release of plasmin. In the *circulation*, plasmin cleaves the fibrinogen Aα chain appendage, generating fragment X (DED), Aα fragments, and Bβ. Further cleavage of fragment X leads to the generation of fragments DE, D, and E. By contrast, degradation of the fibrin network generates YY/DXD, YD/DY, and the unique DD/E (fragment X = DED and fragment Y = DE). Cross-linkage of DD with fragment E is vulnerable to further cleavage, producing D-dimer fragments. The measurement of D-dimer levels can have clinical utility, in that the absence of circulating D dimer correlates with the absence of massive thrombosis.[40] Ordinarily, in the setting of focal cerebral ischemia, the thrombus load is small and the meaning of any D-dimer elevation is uncertain. The generation of the degradation products has two consequences: (i) incorporation of some of these products into the forming thrombus destabilizes the fibrin network of the thrombus and (ii) reduced circulating fibrinogen and the generation of breakdown products of fibrin(ogen) limits the protection from hemorrhage by hemostatic thrombi.

PLASMINOGEN ACTIVATORS

All fibrinolytic agents are obligate PAs (see Table 2.1). Tissue PA, scu-PA, and u-PA are *endogenous* PAs involved in

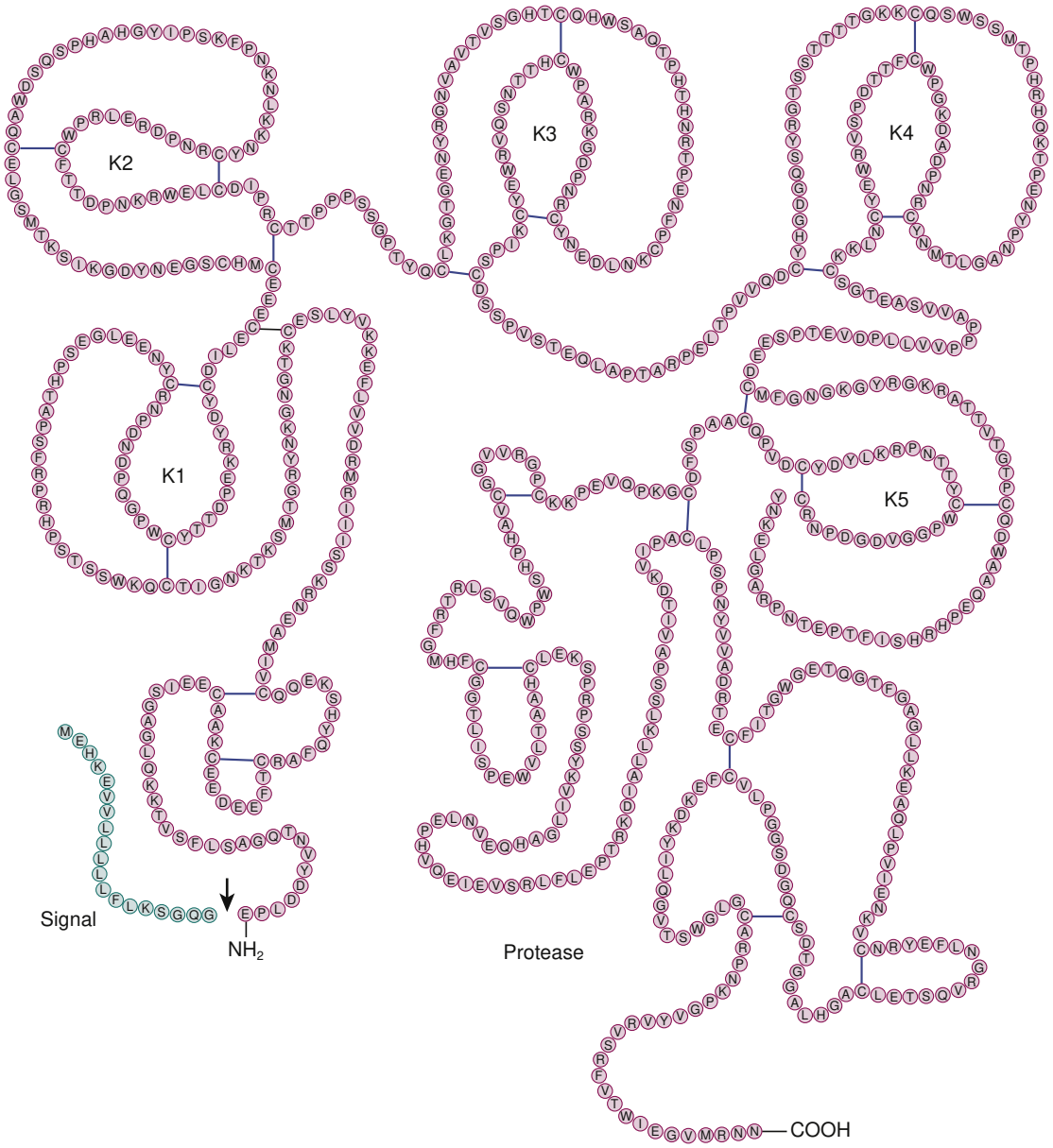

Fig. 2.4. The secondary structure of plasminogen.

physiologic fibrinolysis. Recombinant t-PA, scu-PA, and u-PA, as well as SK, acylated plasminogen streptokinase activator complex (APSAC), staphylokinase (STK), PAs from *Desmodus* species, and other newer novel agents in clinical use (e.g. reteplase [r-PA], and tenecteplase [TNK]), are termed *exogenous* PAs.[38,39] t-PA, scu-PA, and a number of novel agents have relative fibrin and thrombus specificity.[40]

Endogenous Plasminogen Activators

Tissue Plasminogen Activator

Tissue PA is a 70-kDa, single-chain glycosylated serine protease that has four distinct domains—a finger (F-) domain, an epidermal growth factor (EGF) domain (residues 50–87), two kringle regions (K1 and K2), and a serine protease domain (Fig. 2.5).[41] The COOH-terminal serine protease domain contains the active site for plasminogen cleavage, and the finger and K2 domains are responsible for fibrin affinity.[41,42] The two kringle domains are homologous to the kringle regions of plasminogen.

The single-chain form of t-PA is converted to the two-chain form by plasmin cleavage of the arg^{275}-isoleu^{276} bond. Both single-chain and two-chain species are enzymatically active and have relatively fibrin-selective properties. Infusion studies in humans indicate that both single-chain and two-chain t-PA have circulating plasma $t_{1/2}$ values of 3–8 minutes, although the biologic $t_{1/2}$s are longer. Tissue PA is considered to be fibrin-selective because of its favorable binding constant for fibrin-bound plasminogen and its activation of plasminogen in association with fibrin. Significant inactivation of circulating factors V and VIII does not occur with infused rt-PA, and an anticoagulant state is generally not produced. However, if sufficiently high rt-PA dose-rates are employed, clinically measurable fibrinogenolysis and plasminogen consumption can be produced.

Physiologically, secretion of t-PA from cultured endothelial cells is stimulated by thrombin,[37,43] APC,[44] histamine,[37] phorbol myristate esterase, and other mediators.[45] Physical

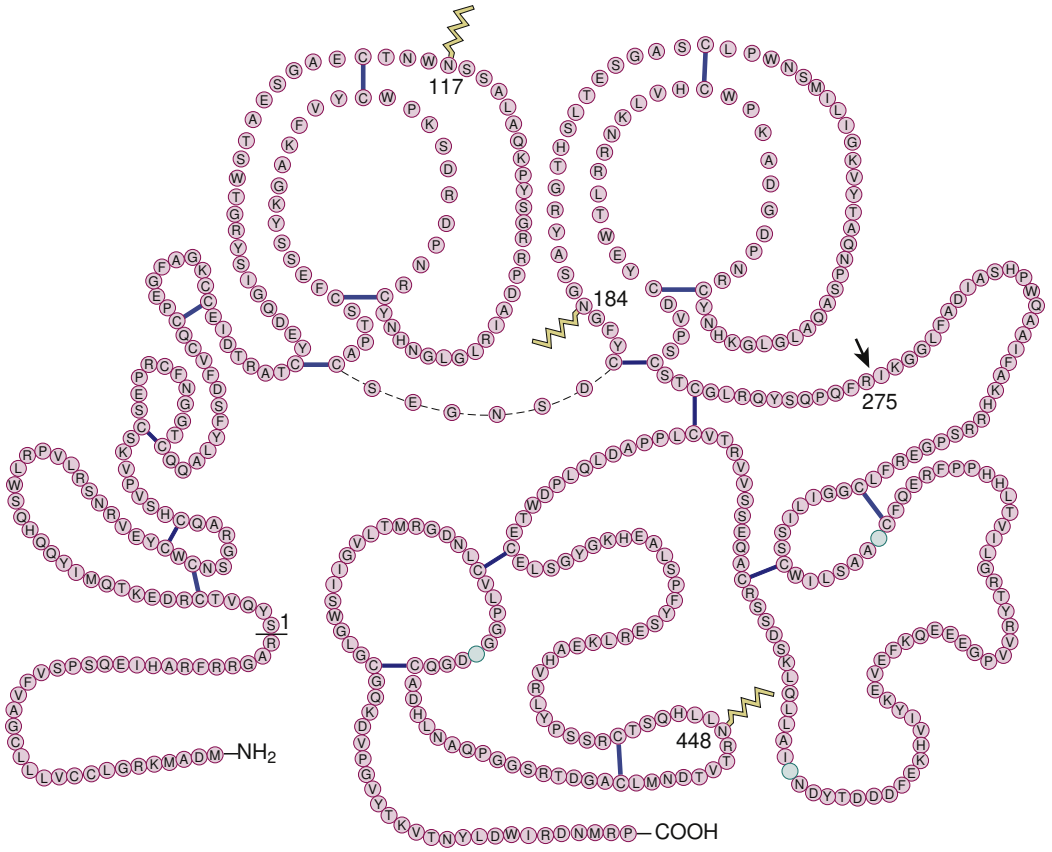

Fig. 2.5. The secondary structure of tissue plasminogen activator (t-PA). Conversion of single-chain t-PA to two-chain t-PA by plasmin occurs at the arg^{275}-isoleu^{276} bond *(arrow)*.

exercise and certain vasoactive substances produce measurable increases in circulating t-PA levels, and 1-deamino(8-D-arginine) vasopressin (DDAVP) may produce a 3-4-fold increase in t-PA antigen levels within 60 minutes of parenteral infusion in some patients. Both t-PA and u-PA have been reported to be secreted by endothelial cells, neurons, astrocytes, and microglia *in vivo* or *in vitro*.[22,46-51] The reasons for this broad cell expression are not known, however.

Urokinase-Type Plasminogen Activator

Single-chain u-PA is a 54-kDa glycoprotein synthesized by endothelial and renal cells as well as by certain malignant cells (Fig. 2.6).[23] This single-chain proenzyme of u-PA is unusual in that it has fibrin-selective plasmin-generating activity[52] and also has been synthesized by recombinant techniques.[53]

The relationship of scu-PA to u-PA is complex: cleavage or removal of lys^{158} from scu-PA by plasmin produces 54-kDa, two-chain u-PA. This PA consists of an A-chain (157 residues) and a glycosylated B-chain (253 residues), which are linked by the disulfide bridge between cys^{148} and cys^{279}. Further cleavages at lys^{135} and arg^{156} produce low-molecular-weight (31-kDa) u-PA.[41] Both high- and low-molecular-weight species are enzymatically active.

The 54-kDa urokinase (u-PA) activates plasminogen by first-order kinetics.[38,53] The two forms of u-PA exhibit measurable fibrinolytic and fibrinogenolytic activities *in vitro* and *in vivo*, and have plasma $t_{1/2}$ values of 9–12 minutes.[54,55] When infused as a therapeutic agent, pharmacologic doses of u-PA lead to plasminogen consumption and inactivation of factors II (prothrombin), V, and VIII. The latter changes constitute the systemic lytic state.

It has been postulated that t-PA is primarily involved in the maintenance of hemostasis through the dissolution of fibrin, whereas u-PA is involved in generating pericellular proteolytic activity by cells expressing the u-PA receptor, which is needed for degradation of the ECM for migration. The roles of these two PAs in central nervous system cell function are not fully understood. However, recent work has provided further insight between the interactions of t-PA and the u-PA precursor.

Recent Considerations of Endogenous Thrombolysis That Suggest Approaches to Thrombotic Stroke

The antithrombotic milieu of the endothelium is maintained in part by secretion of t-PA and the single-chain urokinase PA (scu-PA, pro-UK) and two-chain u-PA (urokinase). As pointed out above, t-PA binds to fibrin and fibrin-bound plasminogen within the thrombus in a ternary complex that efficiently initiates fibrin degradation.[56,57] Plasmin thus generated exposes two new plasminogen binding sites,[58,59] the first of which causes a conformational change in the plasminogen that scu-PA recognizes, which is then activated to plasmin.[60] Plasmin further activates scu-PA to two-chain u-PA, which in turn activates fibrin-bound plasminogen on the second binding site.[61] Hence, t-PA activates one fibrin-bound plasminogen initially and u-PA activates plasminogen on newly exposed binding sites on degraded fibrin in the thrombus.[62,63] This provides a further efficiency to endogenous thrombus lysis and is the basis for potential further refinement of pharmacologic vascular thrombolysis.[64]

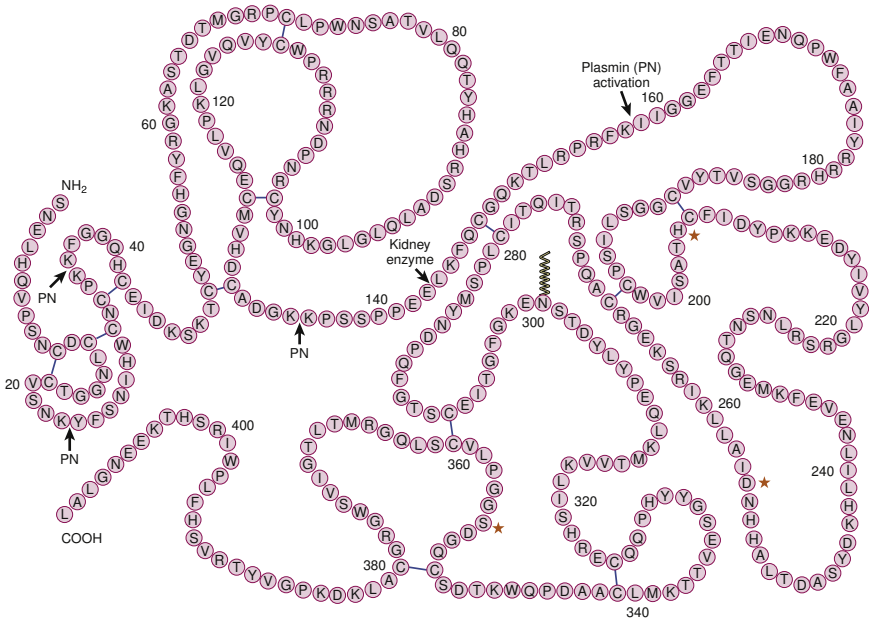

Fig. 2.6. The secondary structure of single-chain urokinase plasminogen activator (scu-PA; 54 kDa). Activation by plasmin takes place at the 158–159 bond *(arrow)*. The *zigzag line* represents the glycosylation site.

Exogenous Plasminogen Activators

Streptokinase

Streptokinase (SK) is a 47-kDa, single-chain polypeptide derived from group C β-hemolytic streptococci. The active [SK-plasminogen] complex converts circulating plasminogen directly to plasmin and undergoes further activation to form the [SK-plasmin] complex. The [SK-plasminogen], [SK-plasmin], and plasmin species circulate together.[65] The [SK-plasmin] complex (not bound by the inhibitor α_2-antiplasmin) and free circulating plasmin degrade both fibrinogen and fibrin and inactivate prothrombin, factor V, and factor VIII.

The kinetics of SK elimination are complex. Antistreptococcal antibodies formed from antecedent infections neutralize infused SK and arise maximally by 4–7 days after initiation of an SK infusion. Therefore, the doses of SK required to achieve steady-state plasminogen activation must be individualized. Plasminogen depletion through conversion to plasmin and by, as yet, poorly understood clearance mechanisms for the [SK-plasminogen] complex can lead to hypoplasminogenemia. Generation of plasmin is limited at both low and high SK infusion dose-rates because of inadequate plasminogen conversion and depletion of plasminogen, respectively.

APSAC (e.g., Anistreplase) was an artificial activator construct consisting of plasminogen and SK bound non-covalently. Fibrin selectivity relies on the fibrin-attachment properties of the plasminogen kringles. The activity of APSAC depends on the deacylation rate of the acyl-plasminogen component. Hydrolytic activation of the acyl-protected active site of plasminogen allows plasmin formation by SK within the complex in the presence of fibrin. From those observations and on the basis of the terminal $t_{1/2}$ of SK and the $t_{1/2}$ for APSAC deacylation, APSAC has a longer circulation time than SK.[66,67] However, despite these clinically favorable characteristics APSAC has not found a place in the treatment of vascular thrombosis.

Staphylokinase

Staphylokinase (STK) is a 16.5-kDa polypeptide derived from certain strains of *Staphylococcus aureus*.[67-69] STK combines stoichiometrically (1:1) with plasminogen to form an irreversible complex that activates free plasminogen. The binding of STK to plasmin has been worked out in detail.[67,69,70] Recombinant STK has been prepared from the known gene nucleotide sequence and has been tested in the setting of acute myocardial infarction (MI), and has been tested preliminarily in focal cerebral ischemia model studies.[71,72]

Plasminogen Activators Derived From *Desmodus rotundus*

Recombinant PAs identical to those derived from the saliva of *Desmodus* species are fibrin-dependent. The α form of *Desmodus* salivary PA (DSPA-α; desmoteplase) and vampire bat salivary plasminogen activator (bat-PA) are more fibrin-dependent than t-PA and may be superior to t-PA in terms of sustained recanalization without fibrinogenolysis.[73,74] The plasma $t_{1/2}$ of DSPA-α is significantly longer than that of rt-PA.[73] A program of studies of desmoteplase as acute treatment for ischemic stroke by several sponsors has so far failed to demonstrate improved outcomes in patients.[75] Recently, treatment of ischemic stroke patients, appearing within 3–9 hours after symptom onset, with desmoteplase was not found to have different outcome (mRS = 0–2) compared to those treated with placebo, and no difference in the hemorrhagic risk or mortality was observed.[76] No additional studies of this compound have been reported.

Novel Plasminogen Activators

Efforts to alter the stability and thrombus selectivity of endogenous PAs have led to a growing list of possible pharmacologic agents. Point and deletion mutations in t-PA and u-PA have provided molecules with unique specificities.[77] For instance, t-PA sequences lacking the K1 and K2 domains possess fibrin specificity, normal specific activity, but reduced inhibition by PA inhibitor-1 (PAI-1).[42] In theory, the increased fibrin selectivity might provide greater thrombolytic effect; however, in studies of the use of this agent in coronary artery thromboses, significant advantages did not arise.

For the clinical target of myocardial ischemia, several t-PA mutants with prolonged $t_{1/2}$ and delayed clearance have been devised that may have benefit when infused as a single bolus[78,79]:

- **Reteplase**, a non-glycosylated PA consisting of the K2 and protease domains of t-PA, has a 4.5- to 12.3-fold longer $t_{1/2}$ owing in part to lower affinity for the hepatic cell t-PA receptor.[79,80] It also possesses lower fibrin selectivity.
- **Tenecteplase (TNK-t-PA or TNK)** differs from t-PA at three mutation sites (T103N, N117Q, and KHRR[296–299]AAAA), which alter two glycosylation sites and increase fibrin selectivity. The changes also result in decreased clearance and prolonged $t_{1/2}$.[81] Application of TNK to clinical ischemic stroke has been formally tested in a small trial,[82] based upon limited experimental studies. Recent report of a non-randomized pilot study indicates the feasibility of intravenous TNK treatment within 3–6 hours of symptom onset.[83] In addition to enhanced fibrin selectivity, TNK has relative resistance to inhibition by PAI-1. A recent report has suggested that TNK may be relatively useful as a preparation for endovascular removal of thrombi in a symptomatic artery;[84] however, further controlled examination of this analysis is required.
- **Lanoteplase (n-PA)**, another t-PA mutant with greater $t_{1/2}$, derives from deletion of the fibronectin finger and EGF domains and mutation of asn^{117} to gln^{117}.[78]
- **Monteplase (E6010)** is a t-PA–like construct with moderate fibrin selectivity. This molecule differs from t-PA in the location and organization of disulfide bridges and the complexity of glycosylation.
- **Pamiteplase (YM866)** has fibrin selectivity and specific activity that are nearly identical to those of t-PA, but pamiteplase has a longer $t_{1/2}$.[85,86]

These mutants have been developed for bolus infusion application in the setting of MI.

What advantage delayed clearance or prolonged $t_{1/2}$ of a t-PA mutant may have in acute application in ischemic stroke is yet to be demonstrated.[87] Dose-adjustment studies in patients with stroke have not been reported. One unproven concern with long $t_{1/2}$ molecules is that they may increase the intracerebral hemorrhage risk in the setting of ischemic stroke.

A similar situation exists for other novel PA constructs. These have included single-site mutants and variants of rt-PA and recombinant scu-PA, t-PA/scu-PA and t-PA/u-PA chimerae, u-PA/antifibrin monoclonal antibodies, u-PA/antiplatelet monoclonal antibodies, bifunctional antibody conjugates, and scu-PA deletion mutants.[88-90]

Recently, interest in recombinant pro-UK (scu-PA) has reappeared, based upon a report of its potential utility in acute MI.[91] M5, a single site mutation (K300H) of pro-UK, is more stable in plasma than pro-UK and can remain in its pro-enzymatic form at therapeutic doses.[92,93] The mutation reduces the intrinsic activity of pro-UK five-fold and increases its reactivity to plasma C1 inhibitor, which forms a complex with the enzymatic form. Complex formation potentially reduces the risk of hemorrhage without interfering with the thrombolytic effect.

Sequential Combinations of Plasminogen Activators in Exogenous Thrombolysis

When given following a low dose rt-PA bolus infusion, pro-UK produced acceptable arterial recanalization acutely in coronary artery thrombosis patients compared historically to rt-PA alone.[91] This has been the basis for the development of a more stable pro-UK analogue with a longer circulation time than the wild-type molecule that will be tested acutely in ischemic stroke patients.

REGULATION OF ENDOGENOUS FIBRINOLYSIS

Endogenous fibrinolysis is modulated by several families of inhibitors of plasmin and of the PAs.

In the circulation, α_2-antiplasmin is the primary inhibitor of fibrinolysis, inhibiting plasmin directly. Excess plasmin is inactivated by α_2-macroglobulin. The potential risk of vascular thrombosis then depends on the balance between plasminogen activation and plasmin activity and their respective inhibitors in the circulation.

Thrombospondin interferes with fibrin-associated plasminogen activation by t-PA. Inhibitors of the contact activation system and complement (C1 inhibitor) have an indirect effect on fibrinolysis. HRG is a competitive inhibitor of plasminogen. Generally, though, these physiologic modulators of plasmin activity are overwhelmed by pharmacologic concentrations of PAs.

For SK, APSAC, and STK, circulating neutralizing antibodies appear, which directly inhibit their activation of plasminogen.

α_2-Antiplasmin and α_2-Macroglobulin

Circulating plasmin generated during fibrinolysis is bound by α_2-antiplasmin in the plasma. The two forms of α_2-antiplasmin are (i) the native form, which binds plasminogen, and (ii) a second form that cannot bind plasminogen.[94] Ordinarily, α_2-antiplasmin is found in either plasminogen-bound or free circulating forms. Fibrin-bound plasmin is protected because of its interaction with fibrin and because α_2-antiplasmin is already occupied. Excess free plasmin is bound by α_2-macroglobulin. α_2-Macroglobulin is a relatively nonspecific inhibitor of fibrinolysis that inactivates plasmin, kallikrein, t-PA, and u-PA.

INHIBITORS OF PLASMINOGEN ACTIVATORS AND FIBRINOLYSIS

PAIs also reduce the activity of t-PA, scu-PA, and u-PA by direct binding (see Table 2.2).

PAI-1 specifically inhibits both plasma t-PA and u-PA. PAI-1 is derived from both endothelial cell and platelet sources.[95] Several lines of evidence indicate that the K2 domain of t-PA is responsible for the interaction between t-PA and PAI-1 and that this interaction is altered by the presence of fibrin.[96] PAI-1 is also an acute-phase reactant,[97] and deep venous thrombosis, septicemia, and type II diabetes mellitus, for instance, are associated with elevated plasma PAI-1 levels.

PAI-2, which is found in a 70-kDa form and a 47-kDa low-molecular-weight form, has a lower Ki for u-PA and two-chain t-PA. PAI-2 is derived from placental tissue, granulocytes, monocytes/macrophages, and histiocytes.[98] This inhibitor probably plays little role in the physiologic antagonism of t-PA, and is most important in the utero-placental circulation.[99] The kinetics of PA inhibition by PAI-2 differs from that for PAI-1.

PAI-3 is a serine protease inhibitor of u-PA, t-PA, and APC found in plasma and urine.

Thrombin-activable fibrinolysis inhibitor (TAFI) is an endogenous inhibitor of glu-plasminogen and therefore fibrinolysis. TAFI is a precursor of plasma carboxypeptidase B and, when activated by thrombin in the plasma, produces an antifibrinolytic effect.

CLINICAL CONSEQUENCES OF THERAPEUTIC PLASMINOGEN ACTIVATION

PAs given at pharmacologic doses significantly alter hemostasis and have been used as treatments of acute vascular thrombosis. u-PA, SK, and occasionally t-PA produce systemically detectable fibrin(ogen) degradation, measured by a fall in fibrinogen concentration, and a reduction in circulating plasminogen and α_2-antiplasmin (through binding of the plasmin generated). Both u-PA and SK inactivate factors V and VIII, which contribute to the "systemic lytic state" or "anticoagulant

state." Fragments of fibrin(ogen) interfere with fibrin multimerization and contribute to thrombus destabilization, whereas the circulating fragments, hypofibrinogenemia, and factor depletion produce an anticoagulant state that limits thrombus formation and extension. The clinical consequences of u-PA or SK infusion include a progressive decrease or depletion of circulating plasminogen and fibrinogen, prolongation of the aPTT due to significant fibrinogen reduction, and inactivation of factors V and VIII. With repletion of these elements the anticoagulant state may be transient.

Platelet function can also be affected. Clinical studies of rt-PA have demonstrated prolongation of standardized template bleeding times.[100] In experimental systems, infusion of rt-PA produces greater hemorrhage.[101] Furthermore, t-PA is known to cause disaggregation of human platelets through selective proteolysis of interplatelet fibrin, which is inhibitable by α_2-antiplasmin.[102] Lys-plasminogen and glu-plasminogen can potentiate the platelet disaggregatory effect of rt-PA. It is likely that the risk of intracerebral hemorrhage that attends PA infusion involves disruption of sustained platelet aggregation and lysis of fibrin formed at sites of vascular injury.

LIMITATIONS TO THE CLINICAL USE OF FIBRINOLYTIC AGENTS FOR ISCHEMIC STROKE

The clinical setting in which PAs are used is an important and relevant variable for both the efficacy and the reduction of hemorrhagic risk. Intracerebral hemorrhage is a known risk of the clinical use of PAs. The use of rt-PA in pharmacologic doses in the acute setting of ischemic stroke must conform to the original report,[6] as confirmed subsequently,[103] and in the package insert (see https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/103172s5203lbl.pdf).

An abbreviated summary of the strict contraindications to the use of fibrinolytic agents includes (i) a history of previous intracranial hemorrhage, (ii) septic embolism, (iii) malignant hypertension or sustained diastolic or systolic blood pressure in excess of 180/110, (iv) conditions consistent with ongoing parenchymal hemorrhage (e.g., gastrointestinal source), (v) pregnancy or parturition, (vi) a history of recent trauma or surgery, and (vii) known acquired (e.g., from anticoagulant use) or inherited hemorrhagic diatheses. These contraindications currently apply to the use of rt-PA in selected patients with ischemic stroke less than 3 hours after symptom onset as well as other approved clinical indications for the use of rt-PA, u-PA, or SK. Somewhat different selection criteria were used for the 4.5 hour entry window in the subsequent randomized placebo-controlled study ECASS III.[9]

PLASMINOGEN ACTIVATORS IN CEREBRAL TISSUE

Although current clinical focus is on the use of PAs as therapeutic agents for vascular reperfusion, cerebral tissue also generates and uses PAs. PA activity has been associated with brain tissue development, vascular remodeling, cell migration, neuron viability, tumor development, and vascular invasion in the central nervous system. However, the pathways involved are still under study.

In normal cerebral tissue, t-PA antigen is expressed by microvessels similar in size to those of the vasa vasorum of the aorta.[21] Expression of PA activity has been reported in non-ischemic tissues of mice, spontaneously hypertensive and Wistar-Kyoto rats, and primates.[104] Sappino et al. described the localization of t-PA and protease nexin (PN)-1 in the adult mouse brain,[105] while u-PA mRNA has been shown to be expressed in the adult brain.[50] Tissue-type PA and u-PA are secreted by endothelial cells, neurons, astrocytes, and microglia *in vivo* or *in vitro*.[46-51] u-PA mRNA is expressed in neurons and oligodendrocytes during process outgrowth in the rodent brain.[105] Although t-PA is expressed by neurons in many brain regions, extracellular proteolysis seems confined to specific, discrete brain regions. Studies suggesting that t-PA can mediate neurodegeneration during excitotoxicity or following focal cerebral ischemia in the hippocampus have opened a discussion about whether PAs play roles in cellular viability outside the fibrinolytic system in the circulation.[106] Strickland and colleagues have summarized studies indicating the involvement of t-PA on CNS cellular function and experimental focal ischemia outcomes.[107] Other more recent summaries have highlighted specific aspects of this data.[108,109]

Plasminogen generation is confined to discrete regions of the CNS.[105] Early during focal ischemia, activators of plasminogen are expressed by microvessels and adjacent neurons (e.g., u-PA);[25] however, there is little evidence yet that plasmin activity *per se* is generated in the ischemic territory. Although the loss of basal lamina components are compatible with its action,[28] other proteases are generated that can account for this. In addition, evidence of local plasminogen activation has been shown by in situ zymography.[110] Proteolytic fragments of matrix constituents (e.g., laminin) have been associated with enhanced excitotoxicity in the CNS in experimental settings.[111] The roles for t-PA, while not overtly upregulated in non-human primate ischemia,[25] have been implicated in neuron survival and injury.[112]

Plasminogen Activators and Neuronal Functions

PAs participate in CNS development.[105,113] It is not surprising that as many cells harbor receptors for PAs, the PA system could play distinct roles in CNS development and function. u-PA has been shown to participate in (i) forebrain postnatal development (along with u-PAR), (ii) neuron and axonal growth in the CNS,[113] and (iii) epileptogenesis (along with u-PAR).[114,115] In experimental systems under normoxia t-PA is synthesized by neurons and appears to participate in (i) hippocampal neuron function and responses,[116] (ii) epileptogenesis,[115,117] and (iii) excitotoxic injury of neurons.[49] Microglia appear to require t-PA for proper function in phagocytosis.[118]

Tsirka et al. have demonstrated that deletion of t-PA prevents the excitotoxic generation of neuron injury (in the hippocampus).[49] In contrast, it had been suggested that rt-PA (alteplase) promotes neuron injury during ischemic stroke. Wang et al. reported that injury volumes were significantly smaller in t-PA$^{-/-}$ mice (129/Sv and C57 Bl/6 backgrounds) subject to transient ischemia, compared with wild-type companions.[119] In both strains infusion of human rt-PA at 0.9–1.0 mg/kg increased infarction volumes.[119] High t-PA doses (10 mg/kg) increase MMP-9 levels in brain.[120] The increase in injury volume has been attributed directly to neuron injury by the ability of rt-PA (alteplase) to potentiate *N*-methyl-D-aspartate (NMDA) receptor signaling,[121] evidence of direct proteolytic cleavage of the NR1 subunit of that receptor by rt-PA,[121] or t-PA expression in the hippocampus and amygdala.[117] Concerns have been raised that the proteolytic activity could be associated with the serum in which cells were grown and/or the supraphamacologic concentrations of human rt-PA used in the mouse preparations (e.g., 10 mg/kg). Alternatively, murine cells could be more sensitive to the human rt-PA, as species controls have not been reported. The role(s) and the mechanisms of PA action in individual reports are often difficult to define, in part because the methodologies and the settings of experimental testing have often not been fully described. In another setting, modulation of the NR2B component of the NMDA receptor by rt-PA (alteplase, 100 μg/mL) increased ethanol-withdrawal seizures in mice (C57 Bl/6 background).[117]

Further technical concerns have appeared. Yi et al. have demonstrated that reduction in infarction volume in an middle cerebral artery (MCA) occlusion model in the Sprague-Dawley rat occurred when rt-PA (alteplase), the S478A mutant of t-PA, or denatured rt-PA were given by intracerebroventricular injection compared to control.[122] It has also been noted that low-molecular-weight contaminants (potentially L-arginine) in commercial preparations of human rt-PA (alteplase) could cause cell toxicity, and similarly contaminants in plasmin preparations could stimulate neuron Ca^{+2} flux.[123] Those studies suggest that non-fibrinolytic off-target effects may be responsible for the increased injury observed with high concentrations of human rt-PA in murine model systems. How these observations relate to ischemic stroke is uncertain.

Many studies have not taken into account the importance of species differences with regard to coagulation system activation. Korninger et al. have demonstrated that for thrombus lysis, non-human systems require a 10-fold higher concentration of human rt-PA than human-relevant thrombus lysis systems *ex vivo*.[124] This applies to vascular thrombosis. Often, with non-thromboembolic models of MCA occlusion, the use of rt-PA has been associated with an increase in infarction volume.

In the non-human primate no change in infarction volume was observed at several doses of rt-PA (alteplase or duteplase) infused intravenously.[125] Furthermore, Overgaard et al. had demonstrated significant reduction in infarction volume with rt-PA at 10 mg/kg following ischemia in rat models of MCA occlusion.[126-128] Those observations suggest that in rat strains thrombus lysis is feasible resulting in reduction in infarction volume, while in mouse strains the rt-PA concentrations achieved are toxic.

In culture, injury to cells occurs consistently at suprapharmacologic concentrations of rt-PA (del Zoppo GJ, Gu Y-H, personal observation; and,[129-131]). Furthermore, there is no clear indication that rt-PA results in a worsening of the injury territories in human stroke patients, independent of hemorrhage, who are treated appropriately.

Therefore, further investigation of the interactions of the PA system and its substrates within the neurovascular unit is required to understand better the roles of this system.

Plasminogen Activators and Cerebral Microvessel Integrity

A clinically relevant notion proposed is that rt-PA can increase cerebral vascular permeability and the risk of hemorrhage by increasing the vascular matrix degradation. Work has focused on the matrix metalloproteinases (MMPs) and other proteases with matrix protein degrading activities.

Loss of the basal lamina matrix[28,132-137] and rapid reorganization of microvessel endothelial cell and astrocyte matrix adhesion receptors occurs during focal ischemia.[28,135,138-140] Heo et al. first described the acute appearance of pro-MMP-2 in ischemic tissue, and the association of pro-MMP-9 with hemorrhagic transformation in the primate.[141] Rosenberg et al. explored the role(s) that gelatinases play in permeability barrier loss, neuron injury, and the evolution of infarction.[142-145] Within the ECM, collagen IV, laminin, and fibronectin decrease significantly during focal ischemia.[28,140]

A plausible explanation for the cerebral vascular ECM changes seen following MCA occlusion is the acute appearance of active matrix-cleaving proteases in the ischemic territory. Four families of matrix-altering enzymes acutely increase following MCA occlusion in the non-human primate: (i) (pro-)MMP-2 and (pro-)MMP-9,[141] and the activation system for pro-MMP-2,24 (ii) serine proteases, including u-PA and thrombin,[24,146] (iii) cathepsin-L,[135] and (iv) heparanase.[28,135] Their individual involvement in brain injury is now certain.[135,137,141,143,147-152] However, no study to date has shown a clear causal relationship; their involvement has been mostly circumstantial.

In the setting of experimental focal ischemia, it is not known whether the proteases are released in active form and degrade microvessel ECM directly or are activated from the inactive precursors released from cellular or matrix sources. The inactive gelatinase pro-MMP-2 is released from vascular endothelium and pro-MMP-9 is released from PMN leukocytes, monocytes, microglia, pericytes, and other cells during inflammation. pro-MMP-2 is activated by membrane bound MT1- and MT3-MMP, plasmin, and other proteases. Considerable experimental work employing focal ischemia models has focused on the active gelatinases.[120,145,153] In the primate MMP-2 antigen is found throughout the ischemic core acutely,[24] but only the inactive pro-MMP-2 form is observed by high-sensitivity zymography.[141] Less than 1% of total MMP-2 in ischemic basal ganglia appears to be active.[141]

It has been suggested that hemorrhage observed with rt-PA use in murine focal cerebral ischemia models is caused by the generation of MMP-9 by rt-PA in the ischemic tissue.[154] Data to support this claim have been developed in murine models;[120,131,155-157] however, recently this notion has been countered in another model system.[157] This question remains unresolved and may depend upon technical issues.[11]

Technical issues confound confirmation of matrix-cleaving activity in tissue derived from ischemia models, including (i) retention of plasma from unperfused brain samples, (ii) the presence of hemorrhage, (iii) activation of samples during protease extraction, (iv) inconsistencies in assigning molecular masses to active forms, and (v) the absence of sufficient details in the preparation methods to be certain. Species differences in protease expression during focal ischemia between primate (pro-MMP-2) and mouse strains (pro-MMP-9) accentuate this problem.[135,149] Gene deletion studies provide only an indirect impression of the possible impact of specific matrix proteases on evolving ischemic injury,[120,149,150,153,158] and are subject to significant limitations. These include compensatory changes during development, several MMP-$9^{-/-}$ constructs with different phenotypes, failure to identify other protease families, unknown cell sources, and the appearance of similar injury phenotypes with different gene constructs (e.g., within the PA family, for instance).[159] These concerns argue strongly for identifying the exact enzyme pathways and their cell sources in the CNS during injury.

Plasminogen Activators in Experimental Cerebral Ischemia

Focal cerebral ischemia rapidly increases the endogenous expression of u-PA and PAI-1 within striatal tissue of the primate.[25,141] Endogenous t-PA decreases transiently as it binds PAI-1, but otherwise does not change. u-PA is an indirect activator of pro-MMP-2, which is also generated early following MCA occlusion.[24] It has been postulated that loss of basal lamina integrity contributes to hemorrhagic transformation of the evolving infarction.[28,140] Whether exogenous PAs contribute to the loss in microvessel integrity in this manner is under study.

A limited number of experimental studies have tested the ability of PAs to increase arterial recanalization. Improved clinical (behavioral and/or neurologic) outcomes have been reported in rodent models of focal cerebral ischemia treated with PAs (mostly rt-PA) very soon after thromboembolism. Early infusion of rt-PA in a rabbit multiple-thromboembolism model demonstrated significant improvement in clinical outcome in comparison with untreated controls.[160] The use

of rt-PA with putative inhibitors of PMN leukocyte adhesion supports this notion, although differences among rt-PA cohorts were observed in various experimental sets. In an rt-PA dose-rate study in a nonembolic non-human primate stroke model, no significant difference in motor-weighted neurologic outcome was observed, compared with controls.[125] However, another study demonstrated a significant reduction in infarction volume after reperfusion of the MCA territory in one model.[161]

PLASMINOGEN ACTIVATORS AND RECANALIZATION IN ISCHEMIC STROKE

Experimental and clinical studies indicate that timely restoration of blood flow to the ischemic cerebral parenchyma is required for improved clinical outcome. The substrate and condition requirements of PAs have supported their potential use in cerebrovascular ischemia. Angiographic studies have provided valuable information about the anatomy of the vasculature, the magnitude of thrombus burden, and the success of recanalization with PAs.[3,162-164] u-PA and rt-PA appear to contribute to arterial reperfusion as anticipated by their known activities (Table 2.3).

Intervention With Plasminogen Activators

The frequency of successful arterial recanalization appears to be greater when the PA is administered by the intra-arterial route within the brain-supplying arteries to the ischemic territory than by intravenous delivery (see Table 2.3). That observation is consistent with the notion that enhanced efficacy may be due to higher local concentrations of the PA at the thrombus surface. However, this has not been shown in the clinical setting.

Only a handful of studies have prospectively compared recanalization rates in PA-treated patients with a matched control group.[4,162,165] In those studies, recanalization was significantly greater in patients receiving the PA for angiographically proven occlusion of the MCA. In a phase II study of recombinant scu-PA (pro-UK), the recanalization frequency was significantly improved by the co-administration of heparin,[162] and was confirmed in a follow-on open phase III study.[165] Many, but not all, subjects in those studies in whom early recanalization was documented experienced clinical improvement. Lack of clinical improvement despite recanalization may be influenced by longer times to reperfusion, poor perfusion, and/or poor collateralization although this issue is unproven.

Mechanical disruption with either catheter-type devices or ultrasonography has been employed to enhance recanalization in limited clinical series. High ultrasound frequencies have been shown to alter the properties of the fibrin network to increase transport of rt-PA into the structure, increase thrombus penetration,[166] increase rt-PA binding to fibrin,[167] and to increase flow through fibrin gel in *in vitro* systems. Fibrin disaggregation can also occur. It has been postulated that such high frequencies will also cause injury to the brain parenchyma and to the vessel wall structure.

Endovascular Interventions

Quite recently, interest has turned to the subpopulations (~25%) of stroke patients with proximal MCA, internal carotid artery (ICA), or carotid "T" occlusions with a low likelihood of acute recanalization by rt-PA.[3] Direct intra-arterial thrombus retrieval has been shown to effect recanalization and in some cases significant clinical improvement.[168-174] Among these studies, intravenous thrombolysis has been employed as the trial comparator in many,[168-175] and/or has been employed as an adjunct to the endovascular procedure.[168-175] While current practice is evolving, the benefit of PAs as adjuncts to endovascular treatment is not proven at this time.

PLASMINOGEN ACTIVATORS AND CEREBRAL HEMORRHAGE IN ISCHEMIC STROKE

Acute rt-PA administration in ischemic stroke can be complicated by the development of symptomatic parenchymal

TABLE 2.3 Plasminogen Activators in Acute Ischemic Stroke: Carotid Territory.

Study	Year	Agent	Patients (*n*)	Δ (T-0)[a] (hours)	Recanalization (%)	Total Hemorrhage (%)	Symptomatic Hemorrhage (%)
INTRA-ARTERIAL DELIVERY							
del Zoppo et a.l[1]	1988	SK/u-PA	20	<24	90.0	20.0	0.0
Mori et al.[2]	1988	u-PA	22	<7	45.5	18.2	9.1
Matsumoto et al.[181]	1991	u-PA	39	<24	59.0	33.3	—
PROACT[162]	1997	scu-PA/h	26	<6	57.7	42.3	15.4
		C/h	14	<6	14.3	7.1	7.1
Gönner et al.[182]	1998	u-PA	33	<6	58.0	21.2	6.1
PROACT II[165]	1999	scu-PA/h	121	<6	65.7	35.2	10.2
		-/h (IV)	59	<6	18.0	13.0	1.8
INTRAVENOUS DELIVERY							
Yamaguchi[183]	1991	rt-PA	58	<6	43.1	20.7	—
del Zoppo et al.[3]	1992	rt-PA	93 (104)[b]	<8	34.4	30.8	9.6
Mori et al.[4]	1992	rt-PA	19	<6	47.4	52.6	—
		C	12		16.7	41.7	—
von Kummer and Hacke[184]	1992	rt-PA	32	<6	53.1	37.5	9.4
Yamaguchi et al.[5]	1993	rt-PA	47 (51)	<6	21.3	47.1	7.8
		C	46 (47)		4.4	46.8	10.6

[a]Time from symptom onset to treatment.
[b]Intention to treat.
C, Control or placebo; *h*, heparin; *IV*, intravenous; *rt-PA*, recombinant tissue plasminogen activator; *scu-PA*, single-chain urokinase plasminogen activator; *SK*, streptokinase; *u-PA*, urokinase-type plasminogen activator.

hemorrhage. A number of randomized studies have documented the increased risk of symptomatic hemorrhagic transformation associated with intravenous infusion of PAs.[6-8] Rates of symptomatic hemorrhage for hemispheric stroke in the cerebral artery territory range from 3.3% to 9.6% in this setting.[3,7-9,162] In addition, the development of symptomatic hemorrhage in rt-PA-treated patients contributed to mortality in properly controlled trials, including the National Institute of Neurological Disorders and Stroke (NINDS) study,[6-8] and ECASS-3.[9] Overall, however, those well-designed trials have shown significant neurologic benefit from the use of systemic rt-PA.

Clinical features that have been associated with higher intracerebral hemorrhage risk in the setting of PA use include advancing age and signs of early infarction on initial cranial computed tomography. Early signs of infarction may reflect otherwise undetectable injury to the matrix of the microvascular bed.[6-8] Increased time to treatment, low body mass (higher relative rt-PA dose), diastolic hypertension, older age, early signs of ischemia, and the use of rt-PA are associated with the risk of intracerebral hemorrhage.[3,176,177] From recent perfusion-weighted imaging (PWI) and diffusion-weighted imaging (DWI) studies subgroups of patients receiving rt-PA have been identified for whom the risk of hemorrhage is increased.[178,179] This accords with evidence that the depth and duration of focal ischemia is a contributor to the ultimate cerebral hemorrhage risk during exposure to PAs.[180]

These latter features of focal ischemia also accord with the observation of microvessel matrix degradation in the ischemic territories observed in experimental systems.[27,135,141] The possibility that these processes are augmented by PA exposure (e.g., rt-PA) and interactions with the baseline metabolic environment of the tissue (e.g., hyperglycemia) have not been sufficiently explored.

Both tissue injury and pharmacologic interventions can augment the risk of hemorrhage. Despite the higher risk of hemorrhage associated with rt-PA, a robust clinical benefit results with proper use of this agent.[6] The results of two randomized trials of intra-arterial recombinant scu-PA are consistent with the effect of anticoagulation (heparin) to increase the risk of symptomatic cerebral hemorrhage with scu-PA.[162,165] A significant excess of symptomatic hemorrhages and a significant increase in recanalization rate occurred in patients receiving the higher heparin dose. Nonetheless, there is no firm evidence so far that the increase in hemorrhage associated with the use of PAs is related to greater recanalization. Early infusion of a PA in selected patients is associated, however, with a decrease in the enhanced hemorrhage risk.[3]

CONCLUSION

Thrombus development and the processes of endogenous thrombus-remodeling and dissolution involve discrete well-understood biochemical pathways. They require the interaction of the vasculature and its lining, platelet activation, and the activation of coagulation. These processes are responsible for thrombotic occlusion of brain-supplying arteries that is a cause of ischemic stroke.

Antithrombotic agents are derived from naturally occurring endogenous factors or agents that interfere with individual steps in the pathways of thrombosis.

As an example, the pharmacologic use of PAs is based upon the known activities and properties of *endogenous* PAs, and the purification of proteins with PA activity from natural sources. The acute use of PAs for dissolution of cerebral arterial thrombi and recanalization of the occluded artery during ischemic stroke has devolved from an understanding of PAs and their actions.

The interactions between PAs and the evolving ischemic cerebral tissue are still incompletely understood. However, it is clear that (i) dissolution of vascular thrombi in the CNS can be achieved acutely with PAs, (ii) rt-PA delivered acutely can cause significant clinical improvement, and (iii) increased intracerebral hemorrhagic risk accompanies PA use in this setting. Vascular injury is a necessary component of hemorrhage both with ischemic stroke and the use of antithrombotic agents, including PAs. Current unknowns regarding the generation of intracerebral hemorrhage include (i) whether the PA (e.g., rt-PA) can cause vascular matrix dissolution, (ii) whether, where, and how rt-PA can stimulate matrix protease generation, (iii) the timing of these events in the clinical setting, and (iv) non-vascular contributors. A growing understanding of non-vascular PA effects covers brain development, individual cerebral cell activities, and neuron injury, specifically. Also, work proceeds to understand non-vascular roles of coagulation factors in the CNS.

The outcomes of these studies require the high-quality application of the scientific method, as in the study of the thrombus pathways.

The complete reference list is available at www.expertconsult.inkling.com.

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