SPREADING DEPRESSION, SPREADING DEPOLARIZATIONS, AND THE CEREBRAL VASCULATURE

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Ayata C, Lauritzen M. Spreading Depression, Spreading Depolarizations, and the Cerebral Vasculature. Physiol Rev 95: 953–993, 2015. Published July 1, 2015; doi:10.1152/physrev.00027.2014.—Spreading depression (SD) is a transient wave of near-complete neuronal and glial depolarization associated with massive transmembrane ionic and water shifts. It is evolutionarily conserved in the central nervous systems of a wide variety of species from locust to human. The depolarization spreads slowly at a rate of only millimeters per minute by way of grey matter contiguity, irrespective of functional or vascular divisions, and lasts up to a minute in otherwise normal tissue. As such, SD is a radically different breed of electrophysiological activity compared with everyday neural activity, such as action potentials and synaptic transmission. Seventy years after its discovery by Leão, the mechanisms of SD and its profound metabolic and hemodynamic effects are still debated. What we did learn of consequence, however, is that SD plays a central role in the pathophysiology of a number of diseases including migraine, ischemic stroke, intracranial hemorrhage, and traumatic brain injury. An intriguing overlap among them is that they are all neurovascular disorders. Therefore, the interplay between neurons and vascular elements is critical for our understanding of the impact of this homeostatic breakdown in patients. The challenges of translating experimental data into human pathophysiology notwithstanding, this review provides a detailed account of bidirectional interactions between brain parenchyma and the cerebral vasculature during SD and puts this in the context of neurovascular diseases.

I. INTRODUCTION

In 1944, Aristides A. P. Leão reported a peculiar electrophysiological observation that he serendipitously discovered while studying seizure activity in rabbit cortex (FIGURE 1A). He observed that a sufficiently intense but not necessarily injurious focal electrical or mechanical stimulus trig-
brain in ischemic or hemorrhagic stroke, and traumatic injury (FIGURE 1C), and perhaps in other less common conditions such as transient global amnesia (79, 93, 115, 183, 451). There is strong albeit indirect evidence suggesting that SD is the electrophysiological substrate of migraine aura as well (13). Moreover, data also indicate that SD is not an innocent bystander or an epiphenomenon, but does indeed worsen injury outcomes by vascular and metabolic mecha-
nisms. As such, SD is now a diagnostic and therapeutic target in neurovascular diseases. Numerous studies have explored the bidirectional interactions between parenchymal depolarization during SD and the cerebral vasculature. However, to put the metabolic and vascular effects of SD in proper context, we will start with a brief overview of its electrophysiological properties and the underlying transmembrane ionic and water fluxes.

II. NEUROPHYSIOLOGICAL FEATURES OF SPREADING DEPRESSION

SD is a slowly propagating wave of intense but transient regional depolarization of most neurons and glia (indeed perhaps all cells), lasting up to a minute or more in otherwise normal tissue (40, 63, 455). Because complete membrane depolarization precludes action potentials or synaptic transmission, SD is accompanied by suppression of all spontaneous or evoked electrical activity in that region (Figure 1A), thus prompting Leão to coin the term depression (268-271). In terms of membrane potential, however, the term depression is somewhat of a misnomer as the underlying electrophysiological process is a near-complete and prolonged depolarization (i.e., strong excitation), a fact that should be kept in mind when interpreting its metabolic and vascular concomitants.

As the name implies, the depolarization spreads at a rate of only a few millimeters in a minute, often centrifugally from the point of origin by way of grey matter contiguity, regardless of functional divisions or vascular territories (Figure 1D), although large cerebral arteries can sometimes act as barriers (137). Interruption of grey matter by preexisting lesions blocks the spread, and high astrocyte density slows the propagation (137). By the same token, SD cannot penetrate white matter, and therefore, intervening white matter bundles form barriers to SD propagation. Contiguous spread in grey matter is independent of action potentials or physiological synaptic transmission and, therefore, of long-range axonal connections. Instead, it is dependent on depolarization-induced massive transmembrane ionic and water shifts that locally flood neighboring tissues with depolarizing ions and neurotransmitters that trigger the same depolarization cycle in adjacent cells. This slow, regenerative propagation of the depolarization is primarily driven by neurons and passively followed by astrocytes, since astroglycine [Ca^{2+}], waves temporally lag behind neuronal changes (59, 367).

SD is triggered when a sufficiently strong stimulus simultaneously depolarizes a minimum critical volume of brain tissue estimated to be \(\sim 1\) mm\(^3\) in rodent cortex, in vivo (295), although the critical volume is smaller in cortical slices in vitro (471). The depolarizing stimulus overloads the extracellular K\(^+\) ([K\(^+\)]\textsubscript{o}) clearance mechanisms causing [K\(^+\)]\textsubscript{o} to exceed a critical threshold concentration of \(\sim 12\) mM (162, 180, 190, 280, 295). These thresholds can vary in different species and brain regions depending on neuronal and excitatory synaptic density, and with age, among other factors (43, 294). The inciting event causes a sudden drop in membrane resistance via opening of nonselective large-conductance cation channels, the presence and identity of which are yet incompletely understood. As a result, both intracellular and extracellular ions move along their transmembrane concentration gradients (Figure 1E). Massive K\(^+\) efflux raises [K\(^+\)]\textsubscript{i} from \(\sim 3\) mM at resting state (219, 378, 507) to over \(\sim 30-50\) mM, and sometimes as high as 80 mM, in an all-or-none fashion, in most species, tissues, and model systems (39, 179, 180, 232, 280, 342, 507, 518). This large K\(^+\) efflux is reciprocated by Na\(^+\) and Cl\(^-\) influx that pulls water, causing cell swelling (134, 180, 341, 495, 497-499, 518). Production of intracellular osmotolites presumably contributes to intracellular water accumulation and cell swelling. Extracellular space shrinks by more than 50\%, which accentuates extracellular ion concentrations (178, 308, 370, 518). Depolarization also triggers Ca\(^{2+}\) influx and a more than 10-fold drop in [Ca\(^{2+}\)]\textsubscript{i}, (146, 180, 340), which, along with Na\(^+\) and water influx, leads to release of many if not all neurotransmitters and neuromodulators within the depolarized tissue. Extracellular glutamate, aspartate, glycine, GABA, and taurine concentrations increase during SD (117, 232, 326), and similar increases have been shown for adenosine, catecholamine, and ascorbate levels (277, 278, 325, 391, 465). The massive rise in [K\(^+\)]\textsubscript{i} to levels sufficient to depolarize neighboring cells is the critical factor mediating the contiguous spread of the wave (i.e., reaction-diffusion model of SD propagation; Figure 2) (161, 162, 180). Elevated extracellular levels of the strongly depolarizing excitatory amino acid glutamate...
further fuels SD and facilitates its propagation by activating NMDA receptors (289, 324).

The massive redistribution of ions, water, and neurotransmitters is self-limited. A number of mechanisms, including the Na\(^{+}\)-K\(^{+}\)-ATPase, intracellular buffering of [Ca\(^{2+}\)], reuptake and metabolism or spatial buffering by the astrocytic network, and quite likely vascular clearance, all help restore the homeostasis usually within a minute. The process is in part energy-dependent and strongly stimulates \(O_2\) and glucose consumption. Therefore, in severely energy-compromised tissue, restoration of homeostasis is understandably delayed, with deleterious consequences on tissue viability.

Neurons are like batteries. They store a substantial charge across their polarized cell membranes. In contrast to the highly regulated and brief currents comprising action potentials and synaptic transmission, SD is analogous to shorting out the battery, discharging the entire membrane potential of all cells in a narrow (2–3 mm) strip of brain tissue simultaneously. SD may indeed generate heat in the tissue (250, 251, 473, 489), although changes in blood flow complicate the measurements and interpretation (1, 188). When recorded extracellularly, this massive discharge during SD creates a signature potential shift that can reach up to \(-30\) mV or more in amplitude. Owing to its slow onset and resolution (many seconds), this potential shift is often not detected using traditional high-pass AC amplifiers, and requires unfiltered direct-coupled (DC) amplification; because of this, it has classically been referred to as the DC shift.

These properties distinguish SD from normal electrophysiological activity in the brain. For example, an action potential lasts milliseconds, generates an extracellular potential shift that is microvolts in amplitude, and can propagate meters in 1 s. In contrast, SD lasts up to a minute, generates an extracellular potential shift on the order of millivolts, and propagates only a few millimeters in a minute. It is easily evoked in vertebrates (e.g., mammals, birds, reptiles, amphibians, fish) as well as invertebrates (e.g., locust, cockroach, cephalopods) (43, 266, 290, 302, 341, 388, 391, 393, 399, 530, 531). In human brain, SD was first detected electrophysiologically upon stereotactic injection of concentrated KCl in caudate and hippocampus in neurosurgical patients (448), and then spontaneously in cortex in a brain-injured patient (302). In vertebrates, cortex, striatum, thalamus, cerebellum, brain stem, spinal cord, and retina (Figure 1F) can all sustain SD, albeit with varying degrees of susceptibility. Because in vitro preparations (e.g., retinal cup, brain slices) can readily undergo SD, perfused vasculature is not required for SD generation, propagation, or recovery.

Experimentally, SD can be evoked when a sufficiently strong stimulus raises \([K^+]_o\), above a threshold of \(-12\) mM in a minimum critical volume of grey matter, as noted earlier. This can be achieved chemically by direct application of depolarizing substances, such as concentrated KCl, glutamate receptor agonists particularly of NMDA subtype (496), and Ca\(^{2+}\) or Na\(^{+}\) channel openers (e.g., BAY K 8644, aconitine); electrically by direct cathodal stimulation of tissue; and by mechanical stimulation (e.g., pinprick, focused ultrasound or laser) (230, 381, 407). Other chemical stimuli can also precipitate SD, such as Na\(^{+}\)-K\(^{+}\)-ATPase inhibitors (e.g., ouabain) (21, 76, 292, 449), membrane-impermeable Cl\(^-\) substitutes (293), and metabolic toxins (e.g., cyanide, azide, 2,4-dinitrophenol, fluoride, fluoroacetate, iodoacetate) (41, 49, 145). Indeed, hypoxia and ischemia are potent inducers of an SD-like event termed anoxic depolarization (AD) (441). Moreover, spontaneous and repetitive peri-infarct or peri-injury depolarizations (PIDs) that are often indistinguishable from SD occur in focal ischemic brain. Such injury-induced spreading depolarization waves have been detected in human ischemic or hemorrhagic stroke and trauma (79, 93, 115, 183). It should be noted that in the context of stroke and brain injury, usage of the term SD has become problematic, as it requires depression of spontaneous electrophysiological (i.e., electrocortico-graphic) activity. Because the latter is often already absent in peri-infarct or injured brain, its suppression cannot define or be relied upon to detect injury depolarizations (IDs). The arguably more accurate and definitely more useful term spreading depolarization has aptly been coined as an alternative to describe both (83). For historical reasons, and because the distinction between spreading depressions and spreading depolarizations is less clear in normal brain, we refer to both as SD in this article.

A large number of physiological (e.g., pH, temperature, fasting, hormones), pharmacological (e.g., Ca\(^{2+}\) channel blockers, K\(^{+}\) channel openers, \(\alpha\)-receptor inhibitors, nitric oxide synthase inhibitors) and genetic (e.g., mutations in Cav2.1 P/Q type Ca\(^{2+}\) channel, Notch3 receptor) modulators of susceptibility to and electrophysiological properties of SD have been described (37, 103, 105, 369, 482), while other modulators of neuronal activity and synaptic transmission have been ineffective (e.g., \(\text{GABA}_{\alpha}\) receptor agonists, voltage-gated Na\(^{+}\) channel blockers, AMPA/KA subtype of glutamate receptor antagonists), as reviewed in detail elsewhere (15).

Despite the relatively constant electrophysiological features of SD across a wide range of tissues and species, its vascular effects appear to be highly variable, depending on the species (e.g., rats versus mice), brain region, vascular bed (e.g., large arteries versus precapillary arterioles and capillaries), and experimental conditions (e.g., anesthesia, systemic physiology). However, to put the hemodynamic response in
proper context, we shall first review the metabolic consequences of SD in normal brain.

III. METABOLIC IMPACT OF SPREADING DEPRESSION

As early as 1950s, it was recognized that SD precipitates marked metabolic changes in brain tissue (FIGURE 3). First and foremost, ATP consumption is stimulated, presumably as a result of activation of Na⁺-K⁺-ATPase and other ATP-dependent pumps to restore ionic gradients across neuronal and glial membranes, and to recycle and replenish the neurotransmitters after their release during the depolarization. Although some studies have failed to detect a significant change in ATP levels during SD (42, 43, 226, 237, 238, 262), others have convincingly shown a decrease by as much as 50% (143, 321, 416), along with increased ADP and AMP (262). Adenosine levels are also elevated and correlate with the metabolic burden and mismatch imposed on the tissue by SD (278). The onset of these changes approximately coincides with the DC shift onset (143, 321). Recovery starts at peak DC negativity and prior to hyperemia and is complete within 1–2 min in otherwise normal tissue (321). The ATP/ADP systems are linked through a rapid equilibration in the creatine phosphokinase reaction (110). As a result, creatine phosphate decreases by up to 80% during the peak DC negativity and recovers within 1–2 min (143, 238, 262, 412, 416). Despite these changes in high-energy phosphates, the total energy charge does not appear to be diminished during and after SD (262). These results suggest that while a great turnover takes place, otherwise healthy brain tissue can cope with the challenge.

The restoration of ionic gradients after SD is a challenge for the local energy homeostasis, and the rise in metabolism is among the largest observed in the living brain. Changes in high-energy phosphates are accompanied by a rapid stimulation of glucose consumption. Increased cerebral metabolic rate of glucose (CMRGlu) is detectable as early as the DC shift onset, reaches a peak at repolarization, and returns to normal levels after about 15 min (150, 258). Multiple consecutive SDs can indeed double or triple CMRGlu in broad areas of cortex (163, 226, 320, 332, 433). As a result, tissue glucose decreases by 30–60% and lactate increases by more than twofold at or within a minute of peak depolarization (68, 69, 143, 150, 226, 237, 238, 262, 321, 329, 394, 411, 416). Cumulative changes were detected upon repeated SDs using microdialysis (186). Tissue pH decreases by up to 0.5 units depending on the recording technique and the tissue and species under study (69, 143, 231, 329, 380, 411, 461, 487). In fact, tissue acidification during SD (pH ~6.80–6.90) approaches that observed during ischemia (pH ~6.75). Studies with sufficient temporal resolution also show a preceding alkaline shift of 0.05–0.16 pH units lasting a few seconds at the onset of depolarization (231, 329, 411). It is worth noting that intracellular pH also drops in neurons, but not in astrocytes where it increases (57, 536), and may transiently interfere with neuronal glycolytic phosphorylation due to the pH sensitivity of enzymes such as phosphofructokinase (337, 485).

As with high-energy phosphates, intracellular pH recovers within a minute or two (143). Tissue glucose concentration recovers after ~5 min, followed by a modest overshoot, while extracellular lactate and pH recover in ~10 min (237, 262, 329, 411), although recovery may be incomplete for more than 30 min as shown by microdialysis in one study (186). Glucose recovery is in part accomplished by a rapid increase in blood-brain glucose transfer, which starts during the depolarization prior to the onset of hyperemia without an apparent change in permeability for glucose (150). Indeed, a single SD decreases tissue glycogen by up to 50%,

FIGURE 3. Metabolic impact of SD. A summary diagram showing changes in metabolic indexes as a function of time after SD onset. Blue scale indicates the magnitude of change (darker is bigger change). Some degree of uncertainty exists in the literature in terms of the precise timing and magnitude of metabolic changes. Therefore, we included in the diagram only those indices with reproducible changes reported in the literature. Notably, temporal changes in Po2, CMR02, and NADH have been complex and highly variable among studies, as described in detail in the text, possibly related to differences such as measurement technique, species, experimental conditions, and the hemodynamic response. The latter is an important determinant of supply-demand mismatch and supply limitation.
which is detectable within 2 min, and lasts 10–90 min \( (236, 237, 262, 416) \). To put in context, this degree of glycogen drop is larger than that seen after seizures, moderate hypoxia, or days of fasting and is exceeded only by severe anoxia with depolarization \( (5, 167) \). Glycogen breakdown is a result of the twofold increase in glycogen phosphorylase activity \( (235) \), stimulated by the two- to threefold increase in cAMP levels \( (234) \), both of which appear to peak shortly after DC shift recovery and last more than 10 min \( (235) \). The activation of glycogenolysis is consistent with intracellular alkalinization of astrocytes after SD \( (57) \), which stimulates the glycogen phosphorylase kinase via a rise in cAMP \( (196) \). The sensor may be the soluble adenylyl cyclase, which is highly expressed in astrocytes and becomes activated in response to \( \text{HCO}_3^- \) entry via the electronegic \( \text{Na}^+/\text{HCO}_3^- \) cotransporter. Activated soluble adenylyl cyclase increases intracellular cAMP levels, causing glycogen breakdown, enhanced glycolysis, and the release of lactate into the extracellular space. This process may be recruited over a broad physiological range of extracellular \( \text{K}^+ \) and also during aglycemic episodes, helping to maintain synaptic function \( (58) \). Although neurons may also contain glycogen \( (403) \), astrocytic glycogenolysis is likely to be the predominant source. Recovery of glycogen lags behind all other metabolites including creatine phosphate, glucose, and lactate. Unlike other metabolites, multiple repetitive SDs do have an additive effect on the magnitude of glycogen reduction \( (236, 237) \).

It is well established that SD stimulates oxidative phosphorylation and \( \text{O}_2 \) demand \( (43, 98, 131, 138, 248, 273, 285, 290, 305, 325, 371, 396, 406, 469, 500, 516) \), with the exception of isolated retina preparations \( (501) \). Stimulation-induced increases in cerebral metabolic rate of \( \text{O}_2 (\text{CMRO}_2) \) are controlled by the ATP turnover, which depends on the energy used to fuel the \( \text{Na}^+/\text{K}^-\)-ATPase to reestablish ionic gradients after SD \( (110, 111) \). Therefore, SD-induced rises in CMRO\(_2\) support the increased \( \text{Na}^+/\text{K}^-\)-ATPase activity \( (42, 68, 69, 75, 143, 146, 258, 262, 298, 320, 364, 433, 440) \). The huge rise in cytosolic \( \text{Ca}^{2+} \) after SD depolarizes neuronal mitochondria and may trigger a rise in CMRO\(_2\) via the \( \text{Ca}^{2+} \) uniporter in the inner mitochondrial membrane \( (536) \). In most studies, CMRO\(_2\) increases by up to twofold coincident with the hyperemia \( (131, 371, 406) \). However, there are other data suggesting that during the depolarization (i.e., preceding the hyperemia) there may be a transient impediment to mitochondrial \( \text{O}_2 \) utilization \( (285, 371, 532) \). Spectroscopy has detected a reduced rather than oxidized state of cytochromes \( c \) and \( \text{aa}3 \) (cytochrome \( c \) oxidase, complex IV) during the depolarization, despite apparently sufficient \( \text{O}_2 \) availability \( (464, 520, 521, 523, 528) \). This could be explained by a number of mechanisms including diffusional barrier for \( \text{O}_2 \), compartmentalization of energy metabolism that relies on glycolytic rather than oxidative phosphorylation, or disruption of mitochondrial respiration during SD because of mitochondrial depolarization and swelling which may alter the proton-motive potential \( (10, 133, 138, 203, 233, 285) \). As an exception, the profoundly vasoconstrictive response in the mouse brain severely limits the \( \text{O}_2 \) supply, and by this way CMRO\(_2\) is paradoxically reduced, as discussed in detail later \( (17, 38, 54, 432, 532) \).

In rats, CMRO\(_2\) remains elevated in the post-SD period \( (10–20\% \text{ above resting state}) \) and gradually recovers over a period of \( 2 \) h \( (131, 371) \). This prolonged increase in CMRO\(_2\) may be explained by oxidation of lactate converted to pyruvate and resynthesis of glycogen from glucose for the first \( 10–15 \) min. But this mechanism cannot account for the rise in CMRO\(_2\) from \( 15–60 \) min after the SD, when brain glucose and glucose usage, lactate, and glycogen have returned to normal \( (258, 262) \). This is reminiscent of the small rise in the cerebral oxygen/glucose uptake and consumption ratio during recovery from sensory stimulation that was not explained by delayed consumption of lactate or glucose \( (286) \). Glucose must traverse a series of intermediate metabolic pools before it is used directly for energy metabolism, and the size of the intermediate pools may enlarge during SD. The subsequent increase in CMRO\(_2\) may serve to decrease the size of these pools to resting levels \( (286) \) or to increase anaplerotic formation of glutamate \( (417) \) during SD and subsequent glutamate oxidation \( (192) \). In addition, the prolonged CMRO\(_2\) rise may relate to “oxygen debt,” known from studies of muscle metabolism, which is a persistent rise in oxygen use after exercise that relates to both oxidation of lactate and to replenishment of intermediate metabolic pools \( (288) \). Regardless of its mechanisms, protracted elevation in CMRO\(_2\) is clinically relevant because of its potential contribution to secondary brain damage in acutely injured human brain \( (176) \), where SD occurs in a large proportion of patients \( (79, 93, 115, 116) \).

IV. CEREBRAL HEMODYNAMIC EFFECTS OF SPREADING DEPRESSION

A. Historical Perspective

Leão \( (269) \) was struck by a conspicuous hyperemic response accompanying the SD wave that nearly doubled the pial arterial diameters, occasionally followed by a longer lasting constriction in rabbits. The dilation peaked within a couple of minutes and lasted a few minutes. In a subsequent study, Leão \( (271) \) spectroscopically showed increased oxyhemoglobin concentrations in pial veins during SD, supporting a hyperemic response. Several years later, Van Harreveld and Stamm \( (500) \) used tissue volume change as a surrogate marker for the vascular response in rabbits and reached a different conclusion; they detected cortical volume decrease during the DC shift, interpreted as vasoconstriction, sometimes followed by a slower but longer lasting volume increase suggesting dilation. Using a similar approach, Marshall et al. detected only vasodilation in cats \( (290) \), yet the same group had earlier reported no change in vessel diameters during SD in rabbits and monkeys \( (291) \).
Both studies may have been confounded by tissue volume changes during SD that are independent of perfusion as has been shown in isolated retina (51).

Subsequent studies using the thermocouple approach, where heat transfer by blood flow is monitored, also yielded mixed results. One study showed a hyperemic response in most cats and rabbits, but an initial reduction in blood flow was observed during the DC shift in a few rabbits (498). Another study in cats and rabbits found a highly variable response among animals and brain regions where “blood flow might increase, decrease, or show a biphasic change,” although the response remained relatively uniform within a given region upon repeated SDs (487). The same technique in rats mainly showed hyperemia that started more than a minute after the DC shift onset, without any evidence of hypoperfusion during or after the DC shift (44). It was also associated with increased cortical hemoglobin content as a surrogate measure for cerebral blood volume (CBV) in the same study, detected 3 min after the DC shift and resolved after 5 min. Hyperemia was later confirmed in cats using a thermocouple (290).

Arguably the most telling data among early studies were obtained by flash cortical freezing approach, which minimized trauma by monitoring devices and enabled visualization of both pial and penetrating vessels in histological sections cut parallel to the propagation path of SD in transit across the cortex. All rabbits studied in this manner showed a narrow band of vasoconstriction that spatiotemporally coincided with the DC shift, followed by a much broader area of vasodilation (498). Interestingly, none of the cats studied the same way displayed vasoconstriction even when their gyrencephalic cortex was appropriately sectioned to allow assessment of a sufficiently long path of SD propagation, hinting for species differences (498).

In the next two decades, cortical surface spectroscopy of changes in tissue hemoglobin content as a surrogate for CBV once again showed marked heterogeneity during SD: monophasic CBV reductions or increases during the DC shift, biphasic or triphasic responses, and even no change, have all been reported (280, 297, 300, 301, 307, 398). Therefore, heterogeneity in the observed hemodynamic responses persisted despite methodological advances over time, suggesting a complex response pattern composed of multiple components with independent mediators and modulators. First, we review these components in order of their dominance in the hemodynamic response.

B. Prototypical Cerebral Hemodynamic Response to SD

In most species (e.g., rats, rabbits, cats) and under normal physiological conditions, SD triggers a massive peak hyperemia that dominates the vasomotor response (FIGURE 4; component II) (2, 17, 20, 22, 66, 86, 90, 94, 95, 97, 98, 113, 114, 118, 119, 124, 130, 131, 150, 152–155, 163, 179, 186, 200, 207, 220, 221, 226, 242, 248, 257, 260, 263, 305, 320, 321, 343, 352, 354, 371, 373, 376, 382, 384–386, 389, 392, 394, 406, 410, 422, 424–426, 443, 444, 456, 457, 460, 461, 469, 489–491, 520, 521, 523, 530, 534, 535). Electrophysiological recordings and voltage-sensitive dye imaging coupled to blood flow measurements show that the hyperemia starts 15–20 s after the depolarization onset, and peaks only after full repolarization, usually 1–2 min after DC shift onset (17, 352). The magnitude of hyperemia has been highly variable, between 30–250% cerebral blood flow (CBF) increase, although exceptions certainly exist ranging between no detectable hyperemia (257) particularly in awake animals (95, 96), and up to fivefold CBF increase (327, 426, 427). No doubt such variability is multifactorial, including the species, experimental conditions, and measurement techniques (see below for a detailed discussion). Direct pial artery diameter measurements showed a corresponding dilation (25–120%) in most studies, which appeared to be more prominent in smaller caliber arterioles than larger ones (16, 38, 46, 59, 64, 65, 314, 315, 407, 419–422, 439, 460, 461, 512, 513). Multispectral reflectance, near-infrared spectroscopy, light transmission, and intravascular fluorescence imaging studies also showed hyperemia during SD in rats and cats (FIGURE 5A; component II) (18, 81, 163, 195, 227, 351, 407, 474, 528). Hyperemia was further confirmed by magnetic resonance imaging (MRI), where CBV increased by ~30% for 1–2 min with a latency of 15–20 s after the apparent depolarization onset, and peaks only after full repolarization (71). There is good evidence that peak hyperemia associated with SD leads to functional recruitment of red blood cells into the microcirculation (i.e., reduction in the percentage of capillaries free of erythrocytes; FIGURE 5B; component II) (287, 343). In contrast to arteries and arterioles, venous caliber changes are often inconspicuous, delayed, or absent (38, 59, 419, 460, 461).

The hyperemia usually lasts up to 3 min, and often gives way to a prolonged post-SD oligemia; CBF remains 10–40% below baseline for an hour or longer (FIGURE 4; component IV) (17, 90, 94–97, 114, 119, 124, 130, 131, 152–154, 171, 226, 242, 248, 255, 263, 343, 352, 371, 373, 376, 394, 410, 424, 425, 491, 502, 534, 535). Once again, corresponding constrictions have been observed in pial arteries (407, 421, 512), and reduced CBV by MRI, near-infrared spectroscopy, multispectral reflectance, and intravascular dye imaging (18, 71, 195, 227, 351, 407).

Many studies with sufficient spatiotemporal resolution have also observed an initial hypoperfusion coincident with the DC shift (FIGURE 4; component I) (17, 86, 90, 114, 119, 124, 128, 131, 150, 152, 154, 163, 179, 186, 257, 260, 343, 352, 376, 385, 389, 394, 406, 424, 443, 456, 460, 474, 490, 491). When present, this transient vasoconstrictive tone usually decreases CBF by 5–30% for 5–30 s, and...
can either precede the hyperemia leading to an early dip in CBF, or be superimposed on the rising slope giving the response a notched or jittery upstroke. Corresponding changes in vessel diameter have been detected by intravital microscopy of pial arteries (16, 46, 54, 59, 359, 419, 420). Moreover, several surface reflectance and light transmission studies have detected reduced CBV preceding the hyperemia (FIGURE 5A; component I), or notched or jittery upstroke, in multiple species including rats and cats (98, 163, 227, 299, 303, 305, 306, 359, 407, 442, 444, 474, 479, 481). Indeed, dramatic capillary red cell slowing, flow arrest, and even transient disappearance of red blood cells from capillary segments have all been observed (FIGURE 5B; component I), lasting as long as a minute during SD, without any evidence for focal or diffuse capillary narrowing or constriction, compression or collapse, or occlusion (59, 479, 480, 490, 491, 532). The flow reduction in parenchymal arterioles and capillaries may precede the vasoconstriction in larger pial arteries (359). Altogether, these data indicate that variable degree of vasoconstriction and hypoperfusion occur during the DC shift (I), and long-lasting and severe post-SD oligemia (IV). [Hbtot], PAD, and OIS are normalized to pre-SD baseline. [Modified from Chang et al. (54).]

**FIGURE 5.** CBV, capillary flow, and pial artery diameter responses to SD. A: time-lapse subtraction images of transilluminated cortical light transmission show a spreading concentric wave of an initial increase in light transmittance (i.e., CBV reduction, I) followed by a decrease in light transmittance (i.e., CBV increase, II) in a rat (left panel) and a cat (right panel) under urethane/α-chloralose anesthesia. Time after SD induction is indicated on the top left of each image. Arrow: the site of KCl injection. [Modified from Tomita et al. (481).] B: capillary blood flow dynamics during SD studied by line scans using in vivo multi-photon microscopy (top panel) through a closed cranial window in a representative isoflurane-anesthetized rat (postnatal day 15) show blood cell flow arrest (middle panel) during the DC shift (bottom panel) that corresponds to component I. Following repolarization, there was a rebound increase in blood flow (component II). [Modified from Chuquet et al. (59).] C: combined electrophysiological recording and multimodal optical imaging in a representative isoflurane-anesthetized mouse show changes in total hemoglobin ([Hbtot]), pial artery diameter (PAD), hemoglobin O2 saturation (SatO2, %), and optical intrinsic signal (OIS) in relation to the DC shift (field potential; mV). Different vasomotor components (I–IV) are labeled as described in the text as well as in FIGURE 3. Note the severe vasoconstriction and hemoglobin desaturation during the DC shift (I), and long-lasting and severe post-SD oligemia (IV). [Hbtot], PAD, and OIS are normalized to pre-SD baseline. [Modified from Chang et al. (54).]

**FIGURE 4.** CBF response to SD. Sample tracings show a range of CBF responses to SD in the rat (A–C), cat (D), and mouse (E) cortex obtained by laser Doppler (A–H) or speckle flowmetry (I) from different laboratories, selected to demonstrate the variable presence, timing, and magnitude of the four vasomotor components (I–IV) shaping the CBF response as described in text. A–C: CBF responses to single or consecutive cortical SDs and their temporal relationship to the DC shift recorded using intracortical microelectrodes under isoflurane anesthesia. [Modified from Ayata et al. (17) and Sukhotinsky et al. (456).] D: CBF responses to two consecutive cortical SDs 15–20 min apart, and their temporal relationship to the depolarization as imaged using a voltage-sensitive dye under halothane anesthesia. [Modified from Farkas et al. (124).] E and F: CBF responses to cortical SD under halothane anesthesia. [Modified from Fabricius and Lauritzen (119) and Farkas et al. (123).] G: evolution of the CBF response to cortical SDs triggered consecutively every 30 min under halothane anesthesia. Note diminished initial hypoperfusion (component I), augmented hyperemia (component II), decreased latency to component III merging into the hyperemia, and absence of a change in post-SD oligemia (component IV). [Modified from Fabricius et al. (114).] H: CBF response to cortical SD in a cat under urethane/α-chloralose anesthesia. Note the prominent vasmotion at resting state and its disappearance after the SD. [Modified from Piper et al. (376).] I: CBF responses to two consecutive cortical SDs triggered 15 min apart in a mouse under isoflurane anesthesia. Although the pronounced hypoperfusion (component I) dominates the response, all other components are also readily identifiable. The second SD is superimposed on severe post-SD oligemia (component IV) after the first SD, and does not show component I, closely resembling the response to SD in other species. [Modified from Yuzawa et al. (532).]
creases during tissue depolarization is unclear, the phenomenon has critical relevance in the pathophysiology of stroke and other brain injury states (discussed in detail later).

Another inconsistently observed component of the vasomotor response is a second smaller (10–50%) but longer lasting (4–8 min) late hyperemia (or dilation) peaking 3–5 min after the peak hyperemia, forming a characteristic hump (FIGURE 4; component III) (114, 119, 124, 152, 154, 200, 352, 376, 385, 394, 406, 419, 422, 520, 521). In addition, a mild hyperemia (~10%) (120) and a much delayed oligemia (~30%) (360) have been shown to follow at 1–2 h and 3 days later, respectively, although the latter was in response to numerous SDs triggered consecutively over a couple of hours. Lastly, an early rise or fall in CBF preceded the DC shift sometimes by up to a minute in some studies (17, 456), possibly vascular conduction of the vasomotor signal ahead of the approaching wave front (38).

Despite the comparable hemodynamic responses in rats, rabbits, and cats, SD may evoke a different response in mice, with a CBF decrease of 60–70% may accompany the SD bits, and cats, SD may evoke a different response in mice, despite the comparable hemodynamic responses in rats, rabbits (e.g., 100–200% CBF increase). Nevertheless, hyperemic responses have been detected in human brain during migraine aura and after brain injury, as summarized below in detail (169, 194, 519).

In summary, data so far suggest that the hemodynamic response to SD is composed of multiple opposing vasomotor influences at different stages of the SD wave (FIGURE 6), variations in the presence, amplitude, and timing of which account for much of the heterogeneity of the hemodynamic response. The prototypical CBF response has at least four distinct vasomotor components: I) an initial hypoperfusion or constriction coincident with the DC shift; II) a peak hyperemia or dilation that emerges during repolarization and reaches a maximum after complete DC shift recovery; III) a smaller late hyperemia or dilation that develops 3–5 min after SD; and IV) a post-SD oligemia or constriction lasting an hour or more. Each component is an aggregate response originating from one or more vascular compartments. Throughout this review, we will use these four terms uniformly when referring to hemodynamic components I–IV to minimize confusion. Undoubtedly oversimplified, the framework nevertheless serves as a starting point to better understand the sources of heterogeneity of the hemodynamic responses in the literature, and to dissect the dilator and constrictor mechanisms, mediators and modulators (197).

C. Potential Sources of Heterogeneity in the CBF Response to SD

A number of factors may account for the heterogeneity in the hemodynamic response among studies. For example,

![FIGURE 6](http://physrev.physiology.org/)
Resting CBF on which SD is superimposed is another important source of heterogeneity. SD-induced peak hyperemia (component II) is often smaller, and the initial hypoperfusion (component I) is more prominent, when resting CBF and vessel calibers are elevated (e.g., acetazolamide, inhalational anesthesia, dimethyl sulfoxide) than when they are reduced (e.g., barbiturate or propofol anesthesia, indomethacin, hypocapnia, or post-SD oligemia after a preceding SD) (71, 95, 257, 394, 460). Although anesthetics can alter SD susceptibility (74, 191, 200, 240, 241, 375, 404, 443, 466), they do not appear to have a consistent direct effect on the hemodynamic response (443); both absent and potent peak hyperemic responses have been reported in unanesthetized animals in different studies (95, 424, 443). Nevertheless, anesthesia can indirectly influence the hemodynamic response by changing resting CBF (128) or systemic blood pressure (see below).

Arguably one of the overlooked confounders of the hemodynamic response to SD is inadvertent triggering of one or more SDs during surgical preparation (54, 478). Although repeated SDs are highly reproducible electrophysiologically, the hemodynamic response to the first SD occurring in naïve tissue is decidedly different than the response to subsequent SDs occurring less than 30–45 min apart, superimposed on post-SD oligemia caused by the preceding SD. During subsequent SDs, initial hypoperfusion (component I) is diminished, peak hyperemia (component II) tends to get larger, late hyperemia (component III) gradually increases in magnitude and merges with the peak hyperemia that precedes it, and post-SD oligemia (component IV) is only minimally augmented (FIGURE 4, C, D, AND G) (22, 71, 114, 124, 152, 163, 227, 352, 406, 427, 443, 474). In the mouse, subsequent SDs triggered within 30 min are superimposed on a severe post-SD oligemia (40% of baseline CBF) (17, 532). Therefore, the initial hypoperfusion coupled to the DC shift is minimal or absent, and instead of a transient normalization of CBF, there is an ostensibly monophasic peak hyperemia that represents 100% increase relative to the post-SD oligemic baseline (FIGURE 4F). After this apparent hyperemic phase, CBF returns to approximately the same post-SD oligemia level (17, 532). Similarly, pial arterial diameter measurements have shown diminished vasoconstrictive responses with each SD triggered in rapid succession (54). In all species, if SDs are repeated more than 1 h apart, the response resembles that in the naive cortex. Therefore, failure to detect and recognize the occurrence of SD during experimental preparation may be a confounder. For example, CBF measurements using methods involving electrode insertion into the tissue (e.g., hydrogen clearance, microdialysis) have yielded abnormally low CBF values. A careful analysis of possible explanations has eliminated tissue injury as a cause, and instead identified SD triggered during electrode insertion as the culprit (24, 478, 502). Indeed, craniotomy is a frequent cause of SD, especially in susceptible species and under favorable experimental con-
ditions (e.g., barbiturate anesthesia), and can profoundly alter the hemodynamic responses to SD (54). Because the initial hypoperfusion virtually disappears after the first SD, it is the most frequently missed vasomotor component of the CBF response (469). The absence of post-SD oligemia and presence of abnormal vascular reactivity (see below) in some studies may also be due to a preceding undetected SD (248).

Systemic physiological parameters (e.g., arterial blood pressure, \(P_O_2\)) can dramatically change the shape and the magnitude of the vasomotor response to SD. Systemic hypotension in rats, even when it is relatively mild within the autoregulatory range, brings out and augments the initial hypoperfusion (component I) during the DC shift, and diminishes the peak hyperemia (component II), while prolonging both components; systemic hypoxia has a similar effect, albeit milder (FIGURE 7A) (123, 198, 445, 456, 457). Systemic hypercapnia has also been reported to diminish the peak hyperemia, possibly because SD was superimposed on an elevated resting CBF (523). Systemic hyperglycemia appeared to augment both the peak hyperemia and the post-SD oligemia (515, 523). Therefore, it is critical to monitor all systemic physiological parameters during SD experiments, in vivo. Lastly, aging has been reported to diminish the magnitude of all vasomotor components in rats (2 versus 8 mo old) (123), and to a lesser extent the initial hypoperfusion in mice (19 mo old) (432).

V. MECHANISMS, MEDIATORS, AND MODULATORS OF THE CBF RESPONSE TO SPREADING DEPRESSION

The mediators and permissive modulators of the hemodynamic response to SD have been subject to much investigation. Because SD markedly stimulates ATP, glucose, and \(O_2\) consumption, the hemodynamic response, particularly the peak hyperemia, was thought to reflect metabolic coupling. The fact that hyperemia starts during the DC shift when tissue glucose reaches a nadir (321), and that its magnitude is inversely related to resting CBF (257), further supported the notion that it is a reactive hyperperfusion state such as after a period of ischemia. However, providing a surplus of oxygen and glucose during SD did not diminish the CBF response (276, 523), nor did oxygen or glucose shortage augment it (457), casting doubt over this simplistic view.

Indeed, there are ample data suggesting that, to the contrary, SD causes neurovascular uncoupling (54, 371), and that the hemodynamic response is not titrated to match the increased metabolic demand. The principle (i.e., lack of substrate sensing) also applies to neurovascular coupling during more physiological levels of activation (54, 132, 133). The CBF response to SD is likely a result of direct cell-cell contact or via diffusible mediators within the neurovascular unit. Neurovascular unit is a conceptual ensemble consisting of many cell types, including astrocyte end feet, parenchymal neuronal projections, perivascular nerves, pericytes, smooth muscle cells, and the endothelium. Hence, a multitude of potential vasoactive substances are released during SD (160, 441), many of which have been implicated in shaping the hemodynamic response (FIGURE 8; TABLE 1). The mechanisms likely differ among vascular compartments (e.g., pial versus parenchymal arterioles and capillaries), further adding to the complexity.

A. Cellular Constituents of the Neurovascular Unit and the Vasomotor Response to SD

1. Astrocytes

Although all cell types within the neurovascular unit are likely to contribute in a temporally coordinated manner, astrocytes are critically positioned to regulate the cerebrovascular tone in response to events taking place in brain parenchyma. Astrocytes can trigger vasodilation or constriction in a context-dependent manner (159, 275, 318,
During normal (i.e., functional) neuronal activation, metabotropic glutamate receptor activation induces Ca\(^{2+}\)/H\(_{11001}\) elevations in astrocytic end feet. Although these inositol 1,4,5-trisphosphate receptor (IP\(_3\)R)-generated Ca\(^{2+}\)/H\(_{11001}\) rises are too slow to explain alone the relatively rapid increase in CBF that accompanies normal neuronal activation (328, 537), prolonged neuronal and glial depolarization during SD can certainly recruit astrocytic mechanisms of vascular regulation. Astrocytic mediation of neurovascular coupling is generally slower than direct neuronal mechanisms, but its relevance is likely to be amplified during the sustained depolarization of SD (52).

Astrocytic Ca\(^{2+}\) elevations stimulate arachidonic acid production by phospholipase A\(_2\), which can then be converted to dilator epoxyeicosatrienoic acids (EETs) by the epoxygenase CYP42C11 (6), constrictor 20-HETE by CYP4A in vascular smooth muscle (328), or dilator prostaglandin E\(_2\) by cyclooxygenase-1, to relay the vasomotor signal to the vessel wall. The magnitude of end feet Ca\(^{2+}\) transients, background nitric oxide synthase (NOS) activity, tissue O\(_2\) availability, and extracellular lactate and adenosine levels may all play a role in determining the polarity of the vasomotor effect (159, 275, 328, 468, 537). In immature rats, fast astrocytic Ca\(^{2+}\) elevations during SD trigger vasoconstriction of intracortical arterioles, in vivo (59), instead of the anticipated dilation (159, 537). The data suggest that the astrocytic control of vasomotor response is context dependent and that there may be subcellular compartmentalization of astrocytic Ca\(^{2+}\) elevations with specific downstream targets and mediators.

Astrocytes are also critical for perivascular K\(^+\) signaling as a complementary mechanism of neurovascular coupling. Astrocytic Ca\(^{2+}\) waves triggered by neuronal activation open Ca\(^{2+}\)-sensitive large conductance K\(^+\) (BK) channels clustered on the end feet, which elevates perivascular [K\(^+\)], to hyperpolarize vascular smooth muscle via inward rectifying K\(^+\) (K\(_{IR}\)) channels (129, 377). Although the contribution of end feet BK channels to SD-induced vasomotor re-

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**FIGURE 8.** Propagation of SD across the dense cerebral vascular network and the neurovascular unit. SD invading the tissue profoundly impacts all cells and constituents of the cerebrovascular unit. The intense pandepolarization causes massive extracellular ionic, neurotransmitter, and metabolic changes. The cerebrovascular response is the result of numerous complex processes and interactions within the unit, mediated by key cell types and molecular signals, some of which are listed. The large number of mediators and modulators simultaneously released from multiple different cell types has made it extremely difficult to dissect the role of individual factors in the hemodynamic response, further complicated by the heterogeneity of the normal response among studies. Therefore, we have refrained from attempting a contrived representation of the vasomotor actions of each potential mediator or modulator, and instead summarized the available data in TABLE 1. EDHF, endothelium-derived hyperpolarizing factor; PACAP, pituitary adenylate cyclase activating peptide; NE, norepinephrine; NPY, neuropeptide Y; Ach, acetylcholine; SP, substance P; NK-A, neurokinin A.
sponse is not known, the mechanism can depolarize vascular smooth muscle if \([K^+]_o\) rises to sufficiently high levels (>20 mM), providing bipolar modulation (149).

Lastly, swelling of astrocytic end feet during SD has been hypothesized as a potential mechanism to compress the microvasculature and restrict perfusion, which may explain the capillary flow arrest during SD (8, 479, 480). Capillary narrowing can have a dramatic effect on cerebrovascular resistance. However, the phenomenon has not been observed in studies with sufficient spatiotemporal resolution (469, 532, 536), and further studies are needed to address this issue.

2. Parenchymal neurons

Neurons have direct vasomotor effects. Both pyramidal and interneurons regulate vasomotor tone and contribute to neurovascular coupling upon synaptic glutamate release and raised neuronal \([Ca^{2+}]_i\). For example, somatosensory stimulation induces hyperemia in part via activation of cortical pyramidal cell cyclooxygenase-2 (272, 345). In addition, cortical GABAergic interneurons receive input from subcortical cholinergic, catecholaminergic, and serotoninergic efferents and make direct contacts with local microvasculature to induce dilation by vasoactive intestinal peptide and nitric oxide, and constriction by somatostatin and neuropeptide Y (53, 108, 225). Activation of noradrenergic input from locus ceruleus on to the astrocytic end feet around the blood vessels reduces resting CBF (62, 72, 365, 379). However, data on direct neuronal control of vasomotor responses during SD are sparse. For example, inhibition of controlled neuronal vesicular exocytotic neurotransmitter release by tetrodotoxin or tetanus toxin did not alter the vasomotor response to SD (59). In contrast, topical application of tetrodotoxin at concentrations that abolished the electrocorticogram reduced resting CBF and augmented both the initial hypoperfusion and the post-SD oligemia in another study (114).

3. Perivascular nerves

Pial (i.e., extraparenchymal) arteries are richly innervated by perivascular nerves originating from superior cervical ganglion (sympathetics containing norepinephrine and neuropeptide Y), sphenopalatine, otic and internal carotid ganglia (parasympathetics containing acetylcholine, vasoactive intestinal polypeptide, and NOS), and trigeminal ganglion (sensory nerves containing calcitonin gene-related peptide, substance P, neurokinin A, and pituitary adenylate-cyclase activating polypeptide) (172). As with parenchymal neurons discussed above, perivascular nerves are in contact with the arteries and capable of directly regulating the vasomotor tone. Sympathetic nerve activation constricts the

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<th>Hemodynamic Component</th>
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<tr>
<td>Initial hypoperfusion/constriction (component I)</td>
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<td>Preceding SD</td>
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<td>Voltage-gated (Na^+) channel inhibition</td>
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<td>Peak hyperemia/dilation (component II)</td>
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<td>Voltage-gated (Na^+) channel inhibition</td>
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<td>Adenosine A1 receptor inhibition</td>
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<td>Cyclooxygenase inhibition</td>
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<td>Post-SD oligemia/constriction (component IV)</td>
<td>Voltage-gated (Na^+) channel inhibition</td>
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<td>Hyperglycemia</td>
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SD, spreading depression; \([K^+]_o\), extracellular \(K^+\) concentration; CBF, cerebral blood flow; NO, nitric oxide; CGRP, calcitonin gene-related peptide; \(mPTP\), mitochondrial permeability transition pore; 5-HT, serotonin.
spatial arteries, particularly large caliber vessels, while parasympathetic nerves are dilators (29, 102, 462). Sympathectomy reportedly has no effect on the peak hyperemic response to SD in the rat either acutely or chronically, although the spatiotemporal resolution of the methods may not have been sufficient to resolve early or late constrictive components in this study (44). In contrast, both sensory nerve transection and parasympathectomy diminished the peak hyperemic response to SD by 20–30% without altering the hypercapnic hyperemia or the DC shift (389). Importantly, a chronic lesion was required, suggesting that distal degeneration of perivascular nerve fibers is essential, and that local depolarization of perivascular nerve endings triggers the release of vasoactive substances, rather than a nerve conduction-dependent effect via efferent activity from the ganglia. Indeed, both elevated \([K^+]_o\) and glutamate can potently trigger the release of vasoactive substances from perivascular nerve endings (312). Consistent with these data, acute systemic administration of capsaicin or direct trigeminal ganglion stimulation to transiently deplete vasoactive peptides in sensory nerve endings also transiently diminished the peak hyperemia by 20–30% (22).

4. Endothelium

Endothelium does not appear to directly mediate or modulate the vasomotor response to SD. For example, inhibition of endothelium-dependent hyperpolarizing factor production by cytochrome P-450 epoxygenase inhibitor miconazole, direct endothelial disruption by intravenous phorbol 12,13-dibutyrate, or endothelial dysfunction induced by insulin resistance did not alter the hyperemia or the DC shift during SD (427). Of note, endothelial cells do change their morphology (e.g., swelling, contraction) acutely in response to local oxygenation changes (212, 213); however, such changes have not been observed or reported during SD, in part due to insufficient spatial resolution of the imaging techniques.

5. Pericytes

Pericytes are critically positioned to regulate capillary blood flow, and pericyte contraction in response to oxidative stress reportedly contributes to the no-reflow phenomenon after focal ischemia (527). One might therefore speculate that the initial hypoperfusion during the depolarization (component I) and the post-SD oligemia (component IV) may be produced by pericyte constriction. However, high spatiotemporal resolution microvascular imaging has thus far failed to detect any focal constriction of capillaries during SD, in vivo (469, 480, 532). Moreover, pial artery constriction observed during and after SD (512) cannot be explained through a pericyte mechanism. Nevertheless, these modified vascular smooth muscle cells may contribute to CBF regulation under physiological conditions. Recent data suggest that neuronal activity and the neurotransmitter glutamate evoke the release of messengers that dilate capillaries by actively relaxing pericytes (170), although not all studies support a role for pericytes in functional hyperemia (127). Future work is expected to shed light on pericyte signaling to regulate capillary diameter during SD and the possible importance of pericytes for local blood flow control.

B. Vasoactive Substances Released During SD

Numerous vasoactive substances are released during SD, largely inferred from studies on the effect of blockers of synthesis, release, and interaction with vascular receptors. In the following section, we review the data that mainly stem from pharmacological interventions, i.e., without directly monitoring the activity of a specific pathway targeted by the drug. This weakness, inherent to in vivo dissection of physiological mechanisms, is difficult to circumvent and the matter is complex because SD evokes a rise in almost any mediator or modulator that has been measured so far. These substances differ in a number of important aspects. Some are released from stores, while others require de novo synthesis. Some are released in the vicinity of the vasculature, while the levels of others rise dramatically in the parenchyma and may diffuse or be siphoned to the vasculature. Some directly mediate a response, while others play a permissive role. And some have effects that last seconds, while others trigger changes that can last hours. The complexity is brought about by the intensity, duration, and multicellular nature of the pandepolarization and ion fluxes during SD. Concentrations of vasoactive substances often are exceedingly small and difficult to directly measure. Parallel slice experiments are most commonly required because the vasoactive compounds, especially the lipophilic ones, disappear within the brain’s largest sink, the blood flow (131). Therefore, our understanding of vascular mediators and modulators is based on indirect evidence, mainly from pharmacological or ablation studies.

1. Ions and channels

During SD \([K^+]_o\) rises ~10-fold from 3–4 mM to ~30–60 mM (FIGURE 1), and all cells within the neurovascular unit are likely exposed, including vascular smooth muscle. \([K^+]_o\) elevations up to 20 mM hyperpolarize smooth muscle membrane via activation of \(K_{\text{aq}}\) channels, in particular \(K_{\text{aq}}2.1\), and reduce \([Ca^{2+}]_o\), and muscle tone (157, 224, 246, 309). The dilation response to low elevations of \([K^+]_o\) appears to be specific for cerebral vessels (157, 402). When \([K^+]_o\) exceeds 20 mM, direct smooth muscle membrane depolarization, \([Ca^{2+}]_o\), rise, and constriction ensue, reaching near maximum above 60 mM (157, 310, 402). Of note, cerebral arteries are more sensitive to the constrictive effects of elevated \([K^+]_o\) compared with peripheral vasculature (e.g., mesenteric artery), and perhaps more importantly, human
pial arterioles are more sensitive to $[K^+]_o$ than either rabbit or rat pial arteries (e.g., EC$_{50}$ and EC$_{max}$ 18 and 45 mM in human, versus 37 and 109 mM in the rat, respectively), suggesting that the constrictive effect of $[K^+]_o$ elevation may be stronger in human brain (402). Similar species differences in the cerebrovascular constrictive response to high $[K^+]_o$ have also been reported between rats and mice, where isolated mouse basilar arteries were more sensitive to $K^+$-induced constriction compared with rats (17).

Taking into account the vasoactive properties of $K^+$, $[K^+]_o$ changes during SD are expected to initially increase CBF, and subsequently to constrict the resistance vessels when $[K^+]_o$ exceeds ~20 mM. Because the $[K^+]_o$ surge during SD is tightly coupled to the extracellular DC potential shift (FIGURE 1E), one can use the DC shift as a surrogate marker to temporally correlate $[K^+]_o$ changes to CBF. As such, the initial hypoperfusion coincident with the DC shift may reflect the constrictive influence of $[K^+]_o$ on the cerebral vasculature. The fact that not all animals and SDs show initial hypoperfusion during the DC shift may be because spatial $K^+$ buffering prevents the perivascular $[K^+]_o$ reaching more than 20 mM, or because concurrent release of dilator substances counteracts the vasoconstrictive influence. There appears to be competition between dilators and constrictors during the DC shift; $K^+$ is the leading constrictor known released during the DC shift, and pharmacological inhibition of opposing dilators such as nitric oxide (NO) can bring out the constrictive effect of $K^+$. Peak hyperemia starts during the recovery of $[K^+]_o$ and peaks after complete restoration of $[K^+]_o$. Vasomotor changes closely resembling the response to SD have indeed been reproduced by focally applying high-$K^+$ pulse with a micropipette in cats (359), although it is not clear whether this is a direct effect of $K^+$ or the result of an SD-like cascade of events triggered by the pulse injection.

An important consideration is how elevated $[K^+]_o$ during SD gets into contact with the vasculature to exert its vasomotor effects. Astrocytic end feet completely encase parenchymal microvasculature. Astrocytes also play a critical role in restoring the $[K^+]_o$ during SD by uptake and spatial buffering, and may release $K^+$ into the perivascular space through large conductance Ca$^{2+}$-activated $K^+$ (BK) channels which are capable of carrying large $K^+$ fluxes. However, inhibition of BK channels by charybdotoxin did not have an effect on the CBF response to SD (426). Microinjections of KCl into the cortex can raise $[K^+]_o$ comparable to levels evoked by stimulation, but do not achieve the same magnitude of CBF increases that develop in response to stimulation (48). The data suggest that mediators other than $[K^+]_o$ are also important for activity-dependent rises in CBF (47). Passive diffusion is a possibility in superficial vasculature within the Virchow-Robin space and the interstitial fluid. However, parenchymal $[K^+]_o$ changes during SD may be unlikely to have significant influence on larger pial vasculature, because pial perivascular $[K^+]_o$, reportedly does not increase significantly during SD, and topical superfusion of up to 12 mM $K^+$ does not reproduce the hemodynamic changes during SD such as the large hyperemia (420). Alternatively, $K^+$-induced local vasomotor changes can be conducted undiminished for more than a millimeter away via endothelial direct coupling, and therefore, it may be hypothesized that parenchymal $[K^+]_o$ changes trigger upstream pial arterial diameter changes during SD (201). In support of this, conducted vasodilation has been observed during SD (38).

Effects of $K^+$ channel modulators on the CBF response to SD have also been studied. Topical application of ATP-dependent $K^+$ channel (K$_{ATP}$) inhibitor glibenclamide augmented the peak hyperemia by ~30% in two studies, without altering the resting CBF or the DC shift in rats (200, 426). K$_{ATP}$ channels are activated and hyperpolarize the cells when ATP/ADP levels drop, such as during hypoxia or hypoglycemia, and during SD, and are expected to increase CBF. Therefore, K$_{ATP}$ inhibition is expected to promote depolarization, and although the target cell type (i.e., neurons versus vasculature) responsible for glibenclamide effect is unclear, it is unlikely to be the smooth muscle. In contrast, inhibition of mitochondrial K$_{ATP}$ by 5-hydroxycanoeato did not alter SD-induced CBF changes in rats (200).

There are much less data on voltage-gated Na$^+$ and Ca$^{2+}$ channels. In one study, voltage-gated Na$^+$ channel inhibitor tetrodotoxin, which does not suppress SD, reduced resting CBF, augmented initial hypoperfusion, increased the peak hyperemia relative to the reduced baseline, and augmented post-SD oligemia (114). And lastly, L-type Ca$^{2+}$ channel inhibitors lomerizine and flunarizine have been reported to dose-dependently attenuate post-SD oligemia when administered systemically (425). The effect, however, may be nonspecific via direct smooth muscle relaxation, as these drugs also elevate resting CBF and inhibit pharmacological vasoconstriction.

Cerebral arteries and arterioles are responsive to perivascular pH (245). Acid pH dilates and alkaline pH constricts pial arterioles (246, 310, 509). During SD there is a brief extracellular alkaline transient (~0.1 pH unit lasting a few seconds) at the onset of depolarization, and this is followed by a larger and longer-lasting extracellular acidification (~0.3–0.4 pH unit lasting a few minutes); the latter is indeed capable of dilating the pial arteries by ~20%, and increase CBF by ~100%. Although these pH shifts could contribute to the initial hypoperfusion and the subsequent hyperemia evoked by SD (114), CBF changes showed no consistent relationship to pH changes in one earlier study (487). Superfusion with artificial CSF, which abolished the hypercapnic vasodilation presumably by buffering the pH changes, did not diminish the pial dilator response to SD (420, 421). Although these data argue against a direct role
of pH in the pial arterial response to SD, a contribution at the parenchymal arteriolar level cannot be excluded. Another plausible mechanism by which high levels of external lactate (68) may contribute to the hyperemia independent of pH is inhibition of transporter-mediated uptake of vaso- dilator prostaglandin E₂ from the extracellular space (159, 371).

2. NO

Owing to its rapid and potent vasodilator effect, NO has arguably been the most intensely investigated molecule as a potential mediator of the CBF response to SD. During physiological neurovascular coupling, neuronal NOS-derived NO acts as a permissive factor in the cortex (i.e., blocking effects of NOS inhibition are reversed by NO donors), and as a mediator in the cerebellum (3, 525). Glutamate release, NMDA receptor activation, and massive Ca²⁺ influx during SD are predicted to stimulate neuronal NOS, which is attached to the NMDA receptor via the scaffolding protein, postsynaptic density protein 95 kDa (408). NMDA receptor activation stimulates NO release (121, 317, 347). Stimulation of NO synthesis and release during SD is indirectly supported by a drop in L-arginine levels and a rise in cGMP, the downstream effector (117, 143). Direct polarographic measurements during SD in rats and cats have indeed been reported to detect a sharp two- to threefold increase in tissue NO levels coincident with the DC shift, followed by a sharp decrease that coincided with the hyperemia; NO levels then decreased to a post-SD plateau lower than baseline for at least 10 min (382, 383, 385, 387). Importantly, peak NO increases were gradually reduced upon repeated SDs, unlike the gradually augmented hyperemic response to recurrent SDs (385). Unfortunately, the results showing NO release have not been reproduced by other groups, and it is still uncertain to what extent tissue NO rises during SD. In addition to cortical interneurons, perivascular nerves, and the endothelium may contribute NO during SD (112, 204, 205, 348).

Inhibitors NOS have been tested to dissect how NO mediates or modulates the CBF response to SD, with highly conflicting results. Given the permissive role of NO for vascular reactions in the cerebral cortex, it is not surprising that the effect of NOS inhibition during SD is ambiguous. In some studies, systemic NOS inhibitor N-nitro-L-arginine methyl ester (L-NAME) completely abolished the CBF response to SD in cats, without altering the single unit burst firing that was used to monitor SD occurrence, but the lack of proper control experiments make it difficult to assess the data (154, 385). As expected, L-NAME increased systemic blood pressure, reduced resting NO levels, and significantly diminished the biphasic NO response to SD (385). Although L-NAME has antimuscarinic effects that may be confounding, N-nitro-L-arginine (L-NA), which is a more specific NOS inhibitor, also reversibly suppressed SD-induced peak hyperemia by up to 40–70% when applied topically or systemically in rabbits (65, 66, 314, 315). Moreover, 7-nitroindazole (7-NI), a relatively specific inhibitor of neuronal NOS in vivo, also reduced the peak hyperemia to SD by 35%, without a significant change in resting CBF or systemic blood pressure, implicating a neuronal origin for NO (66). Lastly, NOS inhibition also attenuated NMDA-induced dilations (121), but the dilations could in part be due to SD in that model (16). Several other studies failed to detect any effect of NOS inhibition on the CBF response to SD. For example, the same group that found potent inhibition of hyperemia by L-NAME in cats failed to show an effect in rats using an otherwise similar experimental model, despite complete inhibition of the increase in NO levels during SD (382). Other rat studies also failed to detect any inhibition of peak hyperemia after systemic or topical L-NA or L-NAME; peak hyperemia to repeated SDs was in fact even larger (118, 427, 428, 520, 534). NOS inhibition was verified by direct enzyme assays as well as by absent endothelium-dependent vasodilation and attenuation of hypercapnia-induced rises in CBF in a subset of these studies, and others that have used similar doses and administration routes observed similar lack of effect on CSD-induced increases in CBF. Therefore, insufficient enzyme inhibition or systemic versus topical administration do not appear to explain the discrepant results. Besides the obvious possibility of experimental pitfalls and caveats, one potential source of variation is the fact that NOS inhibition can reduce resting CBF by up to 10–50%, and the magnitude of peak hyperemia critically depends on whether preinhibition or pre-SD CBF is taken as the denominator. This, however, does not explain all variability in reported effects. There is more consistent data suggesting that NO counteracts the vasoconstrictive tone that develops during SD. NOS inhibition augmented the initial hypoperfusion and diminished the peak hyperemia without significantly altering the other vasomotor components of CBF response described earlier (FIGURE 7, B AND C) (86, 94, 114, 445). This effect was subsequently confirmed in mice (17). Altogether the data suggest that NOS inhibition augments the initial hypoperfusion, which would be consistent with the sharp increase in tissue NO levels during the DC shift.

Indeed, resting NO levels may modulate the cerebrovascular response by augmenting the effect of other vasodilators, and diminishing the effect of vasoconstrictors released during SD (87, 322). For example, NOS inhibition, or NO scavenging using hemoglobin, severely augmented the vasoconstrictive response to elevated [K⁺]ₒ (35 mM) to the extent that spontaneous and prolonged SDs developed that caused severe vasoconstriction which reduced the perfusion level to <20% of baseline for up to an hour (86). SDs induced during topical L-NA with elevated [K⁺]ₒ (35 mM) evoked a severe and 5- to 10-fold prolonged hypoperfusion coupled to a similarly prolonged DC shift; peak hyperemia was also diminished and prolonged (90). These changes were partially reversed by NO donor S-ni-
trosol-N-acetylselenamine (SNAP), Ca^{2+} channel blocker nimodipine, and to a lesser extent direct vasodilator papaverine (86, 90). Similarly, in the absence of NO, extracellular ionic changes that cerebral blood vessels are exposed to during SD caused vasoconstriction in isolated middle cerebral arteries, in vitro (518). NO may also hinder vasoconstriction by inhibiting cytochrome P (CYP)-450 o-hydroxylase activity, which catalyzes the conversion of arachidonic acid (AA) to 20-hydroxyeicosatetraenoic acid (20-HETE) (328, 395, 459), as described below.

These data clearly suggest that ambient NO modulates vasoconstriction during SD. In support of a permissive role for NO in SD, application of the NO donor SNAP or cell-permeable cGMP analog 8-bromo-cGMP, to mimic steady resting levels of NO availability, restored the dilations in response to elevated [K+]o, after NOS inhibition had transformed the response into constriction in isolated cerebral arteries, in vitro (156). Lastly, topical or systemic pretreatment with L-arginine, the substrate for NO synthesis, ameliorated the post-SD oligemia in rats, and enhanced the late hyperemia (component III) that followed the peak hyperemia (FIGURE 7C) (114). These observations point to a functional downregulation of the NO-cGMP pathway following SD. The central role for NO in the protracted vascular changes following SD is further corroborated by the observation that topical application of NO donors or 8-Br-cGMP restored resting CBF during post-SD oligemia to values measured before SD (410). In contrast, systemic glyceryl trinitrate infusion did not alter the CBF response to SD, despite apparently enhanced NO availability at resting state and increased production during SD (386).

3. Arachidonic acid metabolites

Prostanoids are abundantly produced by astrocytes and possibly other cells types in the neurovascular unit, such as pyramidal neurons that express cyclooxygenase-2 (331). SD is a well-documented trigger for rapid arachidonic acid production, presumably due to activation of phospholipase A2 via elevated [Ca^{2+}]i, through NMDA receptors (262). Perivascular prostaglandin E2, I2, and F2a levels increase after a single SD by 30–90%; the increase is even larger after multiple consecutive SDs (50–300%), which also increases thromboxane B2 (421, 422). Importantly, vasoconstrictor prostaglandin F2a shows the largest increase. Although the time course of prostanoid release is not known, it is likely to be slower than the rapid hyperemic components of the hemodynamic response. Indeed, although systemic indomethacin can completely abolish the prostanoid release during SD, it does not diminish the peak hyperemia at doses that achieve complete inhibition of cerebrovascular cyclooxygenase, making this component unlikely to be mediated by prostanoids (257, 426–428). To the contrary, indomethacin markedly augmented the pial arteriolar dilation and the peak hyperemia to SD in several studies, not fully explained by the reduction in resting diameters and CBF induced by indomethacin (95, 315, 419, 422). In addition to the augmented peak hyperemia, indomethacin abolished post-SD vasoconstriction (421).

More recently, lipoxygenase products have been investigated, because of their vasoactive properties, and because their CSF levels appear to be linked to delayed cerebral ischemia and poor outcomes after aneurysmal SAH (67, 222) where injury depolarizations akin to SD occur spontaneously (93). Within the neurovascular unit, arachidonic acid released from astrocytes by Ca^{2+}-activated phospholipase A2 is converted to its main vasoconstrictor metabolite 20-HETE by a cytochrome P-450 enzyme (CYP4A) in vascular smooth muscle (328). Indeed, 20-HETE levels doubled after SD in brain slices, peaking within 20 min and lasting up to 2 h, congruent with the time course of post-SD oligemia (131). The prolonged 20-HETE elevation is interesting in light of the much shorter [Ca^{2+}]i, and arachidonic acid rise and phospholipase A2 activation during SD, implicating an alternative mechanism such as binding of the phospholipid derivative 20-HETE to an intracellular store (e.g., fatty acid binding protein) during SD-induced synthesis (517), followed by slow but sustained release after SD. When tested in vivo in rats, HET0016, a specific inhibitor of 20-HETE synthesis (222, 323), blocked the increase in 20-HETE levels and significantly ameliorated post-SD oligemia by more than half, without altering the resting CBF, PO2, and CMRO2, or changes in these parameters during the SD (131). These data suggested that an astrocyte-dependent mechanism might cause the post-SD oligemia. Importantly, 20-HETE levels increase by 10-fold in CSF after subarachnoid hemorrhage in rats (222), and by 6-fold in rat cerebral arteries during hypertension (144), underscoring the broad physiological impact of this vasoconstrictor metabolite.

Altogether these data suggest that both cyclooxygenase and lipoxygenase products of the arachidonic acid metabolism derived from astrocytes exert a vasoconstrictive influence during and after SD. In support of this, blocking arachidonic acid production by an inhibitor of phospholipase A2 markedly diminishes astrocyte-mediated constriction of parenchymal arterioles during the depolarization, as shown by in vivo two-photon microscopy (59). Lastly, a direct interaction between NO and arachidonic acid metabolites in cerebrovascular regulation has been proposed (395, 459). In one study where such an interaction was sought during SD, NOS inhibition diminished the peak hyperemic response, and contrary to the expectations, this was prevented by indomethacin (315). The importance of these findings, however, is not clear.

4. Peptide neurotransmitters

Calcitonin gene-related peptide (CGRP) is a potent, endothelium-independent vasodilator that is present in perivascular trigeminal sensory nerve endings in experimental animals, and more sparsely in humans (102, 151, 281). Cor-
tical CGRP release has been detected during exposure to elevated [K\(^+\)]\(_o\) (26–40 mM) both in vivo in anesthetized rats and in vitro in rat brain slices (26 mM) (100, 483). Topical CGRP antagonist (CGRP\(_{8-37}\)) reversibly diminished SD-induced pial artery vasodilation by \(~40\%\), albeit at relatively high concentrations, without altering resting diameter in rats, rabbits, and cats (64, 389, 513). When administered after chronic sensory denervation, CGRP\(_{8-37}\) was ineffective, confirming its specificity (389). Importantly, CGRP antagonists appeared to inhibit SD-induced intrinsic optical signals in one study (483), while others have reported no change in propagation speed, in vivo (64, 513). Therefore, whether CGRP modulates SD remains to be studied using standard electrophysiological methods.

Endothelin is another vasoactive peptide that is primarily released from the endothelium with potent constrictive as well as neuronal effects. Endothelin receptors are amply expressed by vascular smooth muscle, endothelium, neurons, and glia. Although endothelin is capable of triggering SD, in vivo, it fails to do so in brain slices, in vitro (85), suggesting a perfusion-dependent effect likely vasoconstrictive ischemia. Blockade of endothelin receptors did not alter the cerebrovascular response to SD, arguing against a role for this mediator in the initial hypoperfusion and post-SD oligemia components of the CBF response to SD (153).

5. Small molecule neurotransmitters

Acetylcholine dilates cerebral vessels via endothelium-dependent and independent mechanisms largely via muscarinic receptors when applied topically (247, 313, 316, 414). In one study, muscarinic receptor antagonist atropine diminished SD-induced peak hyperemia by \(~40\%\); atropine inhibition was largely prevented after chronic trigeminal and parasympathetic denervation (389). In another study, atropine was reported not to change the CBF response to SD (95). Serotonin (5-HT) is an indoleamine neurotransmitter with complex vasmotor properties, including endothelium-dependent vasodilation via 5-HT\(_2\) receptors (12). Central serotoninergic nerves also project onto pial and parenchymal arteries and may play a role in the cerebrovascular response to SD (101). The 5-HT\(_{2A/2C}\) antagonist ritali- nerin reportedly decreased SD-induced peak hyperemia by \(~40\%\), but also prolonged the hyperemia duration as well as the DC shift, without significantly altering the hypercapnic hyperemia in rats (155). In contrast, 5-HT\(_{1D/1B}\) agonist zolmitriptan augmented the peak hyperemia amplitude albeit at very high doses, and did not affect the DC shift. Adenosine, a purine metabolite of ATP, markedly increases in response to hypoxia and metabolic activation, as well as during SD (217, 277, 400). Although adenosine is a vasodilator when applied topically (510), adenosine A1 receptor inhibitor 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) has been reported to augment rather than inhibit SD-induced peak hyperemia (200). However, changes in baseline flow may in part be responsible for the apparent effect. Much less is known on the involvement of norepinephrine and epinephrine in the cerebrovascular response to SD. Perivascular application of these biogenic amines causes dose-dependent pial constriction via \(\alpha\)-adrenergic receptors (244, 511), and in one report, the \(\beta\)-adrenergic receptor blocker propranolol did not alter the CBF response to SD (95). Because glutamate receptor inhibitors suppress SD, it has been difficult to assess its role in the cerebrovascular response (261). It is well known that glutamate receptor activation leads to vasodilation (126). This could be mediated by parenchymal depolarization and neuronal NOS activation via NMDA receptors (121, 122, 317), rather than being a direct vasomotor effect (181), although activation of non-NMDA (i.e., AMPA) subtype of glutamate receptors may be involved as well. Indeed, AMPA receptor activation is the main mechanism in neurovascular coupling during normal physiological activity, NMDA receptors are only involved during strong stimulation (199). Future studies will need to examine if vascular responses during SD involve glutamate receptors other than NMDA receptors. Lastly, it should be remembered that glutamate agonists can trigger SD (266), which may in part contribute to the CBF responses (16).

6. Free radicals

Free radical production by glial cells has been demonstrated during the post-SD period (164, 165, 503), although not all studies agree (86). A few studies have tested free radical scavenger antioxidants (e.g., tirilazad, superoxide dismutase, oxyypurinol) on pial artery diameter and CBF responses to SD and did not observe an effect (96, 152, 314), although post-SD oligemia was reportedly prevented by tirilazad in one study (171). It should be noted that under artificial conditions creating a spreading ischemia pattern (i.e., reduced NO availability and elevated [K\(^+\)]\(_o\)), SD was associated with significant free radical production, presumably a result of tissue hypoxia and ischemia (86). However, its significance for the hemodynamic response is unclear.

7. Mitochondrial permeability transition pore

SD has many similarities to cerebral ischemia: during SD, Ca\(^{2+}\) accumulates in neurons and astrocytes (23, 59), free fatty acids are released (262), and neuronal mitochondria depolarize (536). However, as noted above, there are divergent views on oxygen free radical formation during SD, which is a hallmark of brain damage (86), and recurrent waves of SD do not induce irreversible neuronal injury in otherwise normal rat brain (334). Nevertheless, Ca\(^{2+}\) accumulation and free fatty acid release all favor formation of the mitochondrial permeability transition pore (mPTP), an important mechanism of mitochondrial dysfunction and apoptosis (135). Blockade of mPTP prior to, during, or following episodes of brain ischemia can rescue mitochondrial function, O\(_2\) metabolism, and tissue integrity, suggest-
ing that these signaling pathways contribute importantly to impairment of function in models of acute brain diseases (135, 458). Whether mPTP modulates the generic features of SD and its impact on cerebral vasculature has recently been tested (373). The mPTP blockers cyclosporin A, and its more specific derivative NIM811 (514), did not affect the electrophysiological or metabolic features of SD, but augmented the peak hyperemia and ameliorated post-SD oligemia (373). Neither resting CBF nor thromboxane-induced vasoconstriction was altered, ruling out a nonspecific dilator effect. The findings suggest that a transient opening of mPTP may occur in a small population of highly Ca\textsuperscript{2+}-sensitive mitochondria, most likely in interneurons (189) with slow recovery of function. This mechanism may explain part of the vascular impairment following SD (373).

C. Vascular Propagation of Vasomotor Responses

Intercellular gap junctions (e.g., connexin 40) directly couple endothelial and smooth muscle cells to allow electrotonic and chemical spread of vasomotor signals along the vessel length as a mechanism to induce adaptive changes upstream to the activated segment (201, 338, 484, 526). Given the fact that parenchymal substances released during SD are much diluted by the time they reach the pial vasculature (e.g., [K\textsuperscript{+}], ~4–5 mM) (420), and that superfusion with artificial cerebrospinal fluid, which inhibits hypercapnic pial vasodilation, fails to alter the response to SD (420), it is likely that pial vasomotor responses observed during SD are at least in part conducted upstream from the penetrating arterioles, or that the main mechanisms occur at a depth that is not affected by changing the superfusion fluid. Indeed, vascular conduction of vasomotor responses, mostly dilator, has been suggested during SD in both mice and rats (38). The conducted arteriolar response was ~500 \textmu m and 10 s ahead of the depolarization wave front, independent of the baseline vessel diameter or the direction of SD propagation in relation to the blood flow direction, and never skipped across segments or from one artery to another, arguing against endothelial shear stress or conduction via perivascular nerves as propagation mechanism.

VI. IMPAIRED CEREBROVASCULAR REACTIVITY AFTER SPREADING DEPRESSION

SD not only has direct vasomotor effects, but it also influences physiological cerebrovascular responses. In the post-SD period vascular responses to most if not all physiological and pharmacological stimuli are markedly blunted. For example, vasoreactivity to perivascular pipette application of acidic or basic solutions (pH 6.87–7.57), varying concentrations of K\textsuperscript{+} (0–6 mM), adenosine, and bradykinin, was greatly diminished for at least 75 min after an SD when studied by intravital microscopy in rats and cats (512). Because solutions were applied locally onto the pial arterioles, diminished reactivity was likely due to changes within the vessels, rather than the parenchyma. Indeed, isolated vessels harvested after they were exposed to an SD in situ showed much diminished reactivity to pH and [K\textsuperscript{+}], changes (415). Even the response to the direct vasodilator papaverine appeared to be diminished after an SD in one study (130), although responses to acetylcholine, CGRP, NMDA, K\textsubscript{ATP} channel opener aprikalim, and adenylyl cyclase activator forskolin were reportedly preserved after SD in rabbits (46, 80), suggesting that the vasomotor paralysis may not apply to all species or mediators.

A single SD impairs both resting and stimulus-evoked neurovascular coupling. In the wake of a cortical SD in the rat, both CMR\textsubscript{Glu} and CMR\textsubscript{O2} are preserved or increased when CBF is reduced by 30% or more, indicating flow-metabolism uncoupling at resting state (258, 373). Similar findings have been reported after a single SD in the cat using PET imaging (242), and using optical imaging in the mouse (54), where CMR\textsubscript{O2} becomes O\textsubscript{2} supply-limited in part due to the pronounced post-SD oligemia (532). Interestingly, neurovascular uncoupling has also been reported in subcortical structures (e.g., hippocampus) after a cortical SD, although it is not clear whether this was due to subcortical propagation of the cortical SD (327). In addition to the resting state supply-demand mismatch, cortical activation-induced hyperemic responses to transcallosal or somatosenory stimulation are also diminished after an SD, without a corresponding change in the electrophysiological or CMRO\textsubscript{2} response, suggesting impaired neurovascular coupling with intact neurometabolic coupling (166, 371). A persistent reduction in the activity of interneurons containing vasoactive intestinal polypeptide (VIP) and NO may be a potential mechanism for the uncoupling (108, 239, 371). More recently, inhibition of mitochondrial permeability transition pore formation and calcineurin activation by cyclosporin, FK506, and NIM811 restored neurovascular coupling after SD, without altering the cortical electrical activity, resting CMRO\textsubscript{2}, or the CMRO\textsubscript{2} increase during and after SD (373). A possible mechanism of action of cyclosporin and FK506 may be enhanced phosphorylation of neuronal NOS, which is required for normal neurovascular coupling (363), especially since data suggest a functional downregulation of NO/cGMP pathway after an SD (410). In contrast, inhibition of 20-HETE synthesis by HET0016 did not restore neurovascular coupling after an SD despite the fact that post-SD oligemia was ameliorated, suggesting that different mechanisms control resting CBF and activation-induced hyperemia during post-SD oligemia (131).

Hypercapnic hyperemia is also abolished or markedly diminished in the wake of an SD, starting as early as 15 min, and lasting anywhere between 20 min to 12 h in different studies and species (46, 114, 119, 130, 152, 248, 253, 376,
perivascular tissues detected as early as 3 h after SD and the BBB. Indeed, SD caused plasma protein leakage into membrane and tight junction proteins, and disruption of MMP-9 activation was associated with loss of basement as 15–30 min after an SD when examined in tissue sections. Increased by more than 10-fold starting within 6 h, peaking at revealed that cortical SD increased MMP-9 gene expression increased with a similar time course. Importantly, these changes were prevented by an MMP inhibitor and absent in MMP-9 knockout mice (168). In another study, 2 h of repetitive cortical SD induction increased the number of pinocytic vesicles in endothelial cells in the rat, which may be another mechanism contributing to BBB disruption after SD (529). However, not all studies agree with BBB disruption after SD. For example, an earlier study has failed to detect any plasma protein extravasation from pial arteries 4 h after SD (73), and more recently, plasma protein extravasation after 1 h of SD induction by topical KCl appeared to be a direct effect of KCl rather than SD itself as extravasation rapidly diminished as a function of distance to KCl application site, and was absent more than 5 mm away (356).

VIII. METABOLIC SUPPLY-DEMAND MISMATCH DURING SPREADING DEPRESSION

As reviewed in detail above, SD markedly stimulates energy consumption and glycolytic and oxidative metabolic rates. Whether this creates a critical supply-demand mismatch and metabolic stress has long been debated. To that effect, SD induces hypoxia inducible factor 1α expression in rat cortex (504). Although exposure to numerous repetitive SDs does not cause acute injury in otherwise healthy brain with normal perfusion and oxygenation at baseline (334), long-term or cumulative effects are certainly possible. Two surrogates that have been studied as markers of metabolic stress are tissue O₂ availability and mitochondrial redox state. The data altogether suggest that SD indeed causes O₂ supply-demand mismatch in brain tissue, but the magnitude of mismatch may be small in tissue with normal perfusion at resting state and a hyperemic response to SD, and large-enough to create a hypoxic state in tissue with hypoperfusion at resting state and in the absence of a hyperemic response to SD.

A. O₂ Availability

Most but not all studies show a decrease in O₂ availability (i.e., PO₂ or mixed arteriovenous hemoglobin O₂ saturation) during the DC shift, sometimes preceded by a brief increase (20, 54, 131, 185, 248, 251, 285, 290, 304, 371, 406, 469, 486, 490, 491, 500). Reduced supply and/or increased demand may limit tissue O₂ availability during SD (131, 371, 469, 532). While reduced O₂ availability can be explained by vasoconstriction in some species and experimental models (54, 227, 406, 500, 532), similar PO₂ dips were also observed in the absence of vasoconstriction and even in the presence of hyperemia, and were accompanied by increased tissue Pco₂, thus implicating increased O₂ use (131, 248, 371, 469). Upon repolarization, when the hemo-
dynamic response becomes predominantly hyperemic, O₂ availability may increase, which could suggest luxury 
arterialization (18, 20, 89, 98, 248, 271, 406, 500, 520, 521, 
523), even though some studies have reported tissue hypoxia (131, 371, 469). By luxury arterialization or perfusion
we mean an overabundant cerebral perfusion relative to the 
motoric needs of the brain tissue (254).

The observation of “pink veins” (i.e., veins with a high 
hemoglobin O₂ saturation) (269) poses an apparent para-
dox with low tissue Po₂ during SD. One theoretical mecha-
nism by which this could happen is increased capillary 
transit time heterogeneity, where the linear velocity of red 
blood cells in a subset of capillaries is too fast for O₂ to be 
extracted efficiently (i.e., decreased O₂ extraction fraction), 
leading to relative tissue hypoxia (215). The possibility re-
 mains to be tested.

When peak hyperemia is absent, Po₂ increase is also absent 
(304, 532). In the post-SD period, tissue O₂ availability 
remains significantly reduced for up to 2 h as a result of 
the post-SD oligemia that leads to a reduction in tissue Po₂ (18, 
54, 131, 185, 195, 371, 532). Despite this reduction in 
supply, CMRO₂ is increased after an SD for up to 2 h. In 
summary, O₂ delivery and availability can be reduced dur-
ing the DC shift because of reduced delivery (initial hypo-
perfusion) and/or increased capillary transit time heteroge-
eity, and after CSD because of a reduced delivery (post-SD 
oligemia). In the mouse, severe initial hypoperfusion 
during the DC shift and subsequent post-SD oligemia are associ-
ated with marked increases in O₂ extraction fraction, and 
reductions in O₂ saturation as well as the CMRO₂ mea-
sured by minimally invasive optical imaging tools (54, 532).

B. Mitochondrial Redox State

SD generates a rise in both aerobic and anaerobic metabo-
lism, but it is less clear whether this results in a shortage of 
ATP in otherwise normal cortex and a change in the redox 
state (469). One way to address this is by monitoring the 
mitochondrial redox state. Monitoring NADH and FAD 
fluorescence has been commonly used to measure changes 
in mitochondrial redox state. The origin of NADH is largely 
cytosolic, while FAD fluorescence is exclusively mitochon-
drial (36, 249), the vast majority of which resides in neu-
rons (216), and reflects the balance between the “reduced” 
and “oxidized” forms of these cofactors (488). Stimulation 
of oxidative phosphorylation, or elevating ADP levels, in-
duces a shift from NADH (i.e., reduced) to NAD⁺ (i.e., 
oxidized), and from FADH₂ to FAD, whereas stimulation of 
glycolysis and the tricarboxylic acid cycle shifts the bal-
ance in the opposite direction (488). Therefore, NADH and 
FAD fluorescence intensities change, respectively, with gly-
colytic and oxidative metabolic rates.

The reported patterns of NADH response to SD have been 
variable. While many studies detected a monophasic de-
crease in NADH fluorescence (i.e., oxidation) (252, 280, 
296, 297, 299, 303, 305, 307, 397, 398, 444, 464), a mono-
phasic increase, or biphasic responses with an initial de-
crease and a subsequent increase or vice versa, have all been 
reported in various species (50, 81, 185, 187, 304, 390, 
442, 443, 532). A longer lasting NADH increase followed 
in the wake of SD in some studies, coinciding with the 
post-SD oligemia (50, 185, 532). Regardless of the direction 
of change, the NADH redox shifts were fairly large in mag-
nitude, approaching the decrease induced by hyperbaric 
hyperoxia or mitochondrial uncouplers, or the increase 
e.g., 2-fold) induced by anoxia or inhibitors of mitochon-
drial respiratory chain, in vivo (187, 296, 300, 301, 311, 
390). Because ouabain significantly slows down the NADH 
oxidation rate during SD, stimulation of Na⁺-K⁺-ATPase is 
likely to be a significant contributor to the increase in energy 
consumption (252).

Variable NADH response patterns are a result of superim-
posed glycolytic and oxidative responses with variable mag-
nitudes and time course. Upon depolarization, rapid glyco-
lytic surge tends to increase NADH production, but with 
the onset of hyperemia during and after repolarization, ox-
idative phosphorylation increases NADH consumption. If 
the rate of glycolytic NADH production exceeds the rate of 
NADH consumption by the electron transport chain, the net 
result would be NADH accumulation. The direction of 
change during different phases is context sensitive, i.e., de-
termined by the balance between O₂ supply and demand, 
which is a function of the resting blood flow and metabolic 
supply-demand balance, as well as the blood flow response 
and the supply-demand reserve capacity, both of which can 
vary greatly under different experimental conditions (e.g., 
in vivo versus in vitro, species, presence or absence and type 
or depth of anesthesia, arterial Po₂ and blood pressure, 
presence of ischemia). In the absence of O₂ supply limita-
tion, metabolic stimulation during SD decreases NADH 
(i.e., oxidation). If O₂ supply is limited, NADH increase 
(i.e., reduction) becomes prominent (306, 397, 409, 445, 
488, 532). Inhibition of NOS has a similar effect (445). In 
support of this, the vast majority of SDs that spontaneously 
occur in the partially ischemic peri-infarct penumbra tissue 
(i.e., PIDs) are associated with NADH increase and are 
transformed into NADH decrease only when the PID prop-
agates into adjacent nonischemic tissue where O₂ is not 
supply-limited (193, 452, 454).

It should be noted that NADH fluorescence measurements 
can be confounded by changes in tissue hemoglobin concen-
tration (i.e., cerebral blood volume) and light scattering 
properties during the depolarization (i.e., transient cell 
swelling) (472), and both the magnitude and the direction 
of change in above-mentioned studies should be interpreted 
with caution, despite the standard correction by subtracting 
the reflected light intensity from the fluorescent signal. 
Two-photon microscopy, which is less prone to such con-
founders, has revealed a spatially heterogeneous NADH response during SD in mice (469). In the vicinity of capillaries, where O$_2$ is presumably more available, NADH displayed a marked decrease (~20%), whereas farther away from the capillaries it increased (~25%). Following repolarization, all regions showed uniform and lasting NADH increases. In a more recent study using two-photon microscopy, NADH responses were again multiphasic and spatially heterogeneous, but did not show a relationship to individual capillaries (532). Nevertheless, the NADH response differed between the capillary bed and the tissue in the vicinity of a penetrating arteriole. Within the capillary bed NADH mainly increased during the depolarization and remained elevated throughout the imaging, whereas in the vicinity of an arteriole NADH was multiphasic but remained close to baseline, suggesting higher O$_2$ availability from the nearby vessel. Altogether, the data from these two studies suggest that NADH changes during SD are spatially related to the local O$_2$ availability, which appears to be dependent on the distance to arterioles in normal cortex, and distance to capillaries in hypoperfused cortex (405).

### IX. ROLE OF VASCULATURE IN SPREADING DEPRESSION RECOVERY

Although it is believed that the mechanism of SD recovery is an energy-dependent cellular restoration of transmembrane ionic gradients by uptake and possibly spatial buffering, and that severe energy compromise delays and even prevents SD recovery and repolarization (147), there are a number of observations that are incongruent with this mechanism as well. First, reduced cerebral perfusion pressure by systemic hypotension or arterial stenosis markedly prolongs the DC shift (e.g., more than doubled at a blood pressure of ~40 mmHg) even when resting perfusion is maintained near normal levels (i.e., >90% of baseline) by autoregulation (105, 456, 457). In contrast, mild systemic hypoxia (P$_O_2$ ~45 mmHg) did not significantly prolong the DC shift as long as the arterial pressure and cerebral perfusion were maintained within a normal range (456, 457). Indeed, there was no correlation between tissue P$_O_2$ and the duration of the DC shift, and excess tissue O$_2$ or glucose availability by systemic hypoxia (arterial P$_O_2$ ~400 mmHg) or hyperglycemia (blood glucose ~400 mg/dl) did not restore prolonged DC shift durations to normal during hypotension (198, 457). Similarly, SD recovery in isolated retinal preparation is independent of O$_2$ availability, albeit with substantially elevated glucose levels (501). Moreover, pharmacological interventions that diminish the hyperemic response to SD also prolong the DC shift (153, 353), and the durations of peri-infarct SDs are prolonged in hypoperfused penumbra despite relatively mild ischemia (202, 229). These data suggest that tissue perfusion per se may be critical for the restoration of ionic homeostasis. Because the recovery of SD is critically dependent on restoration of [K$^+$]$_o$, there may be mechanisms of K$^+$ clearance that are not energy dependent (140–142, 174). Astrocytic spatial buffering through intercellular junctions can be an efficient mechanism for clearance of small rises in [K$^+$]$_o$ (i.e., <10 mM) during normal physiological activity that occur within small volumes of tissue (e.g., less than a few hundred microns) at any given point in time. However, the ability of this mechanism to spatially redistribute the [K$^+$]$_o$ among astrocytic network is likely overwhelmed when a large strip of brain tissue (i.e., ~3 mm wide) is simultaneously exposed to over 40 mM of [K$^+$]$_o$ during an SD. An alternative route of K$^+$ dissipation may be the capillary network, which densely penetrates the brain tissue with an average intercapillary distance of ~50 μm. Therefore, the capillary network is the largest sink within the brain tissue that can contribute to the vascular clearance of [K$^+$]$_o$. Indeed, astrocyte endfeet tightly ensheath the capillaries and are ideally positioned to clear parenchymal [K$^+$]$_o$ to the vasculature (129, 336, 366). Although the cerebrovascular endothelium is highly impermeable to K$^+$ on the luminal side (70, 177), active or facilitated transport systems for K$^+$ do exist on the abluminal endothelial membranes and get recruited to pump K$^+$ into the capillaries upon elevated [K$^+$]$_o$ (27, 28, 32, 107, 158). The mechanism becomes physiologically relevant at [K$^+$]$_o$ that is reached during SD based on capillary K$^+$ permeability of 3 × 10$^{-7}$ cm/s and capillary area of 200 cm$^2$/g (175, 177). In this way, a drop in capillary perfusion or an increase in capillary transit time heterogeneity, such as during hypotension, may impact cerebrovascular [K$^+$]$_o$ clearance even if it does not create energy shortage. This may explain the transformation of CBF response from hyperemia to a biphasic response during hypotension (as discussed above), as well as in ischemic penumbra (see below). Reduced vascular [K$^+$]$_o$ clearance together with an augmented vasoconstrictive tone and reduced intraluminal pressure to keep the vessels open may also explain the prolonged DC shift durations during hypotension in the absence of energy deficit (456, 457). In summary, direct vascular regulation of extracellularionic milieu (i.e., vascular clearance of [K$^+$]$_o$) has implications for not only SD recovery and brain injury states, but also for migraine triggers (182). Lastly, it is worth noting that the perivascular space, suggested as a potential route of cerebral waste clearance through bulk CSF flow (210, 211), may also contribute to [K$^+$]$_o$ clearance during SD. However, bulk CSF flow is limited to the perivascular rather than interstitial space (463), and passive diffusion is too slow to explain the dissipation of [K$^+$]$_o$ during SD by this route (140, 142). Although SD-induced vasomotor and cell volume changes undoubtedly impact the perivascular space, the relevance of this phenomenon for SD recovery is unclear.

### X. SPREADING DEPRESSION UNDER PATHOLOGICAL CIRCUMSTANCES

The complex cerebrovascular response to SD is further influenced by the physiological state of the tissue. Pathologi-
cal states, such as reduced perfusion pressure, ischemia, and subarachnoid hemorrhage, all transform the hemodynamic response to SD from hyperemia into hypoperfusion (i.e., inversion) by augmenting the constrictive component I.

A. Focal Cerebral Ischemia

PIDs akin to SD occur with high frequency in focal cerebral ischemia in all species studied to date (34, 104, 332, 333). They arise in an ostensibly spontaneous fashion in the periphery of the infarct (333), in tissue that is functionally and metabolically compromised but not yet irreversibly damaged, the so-called ischemic “penumbra” (11). Recent data show that PIDs are indeed triggered by episodic drops in metabolic supply (e.g., hypoxic or hypotensive transients) or increases in metabolic demand (e.g., functional activation) that cause supply-demand mismatch transients in susceptible peri-infarct hot zones (506). Consequently, both hyperglycemia and hyperoxia can suppress PIDs (430, 454). Reduced tissue perfusion delays the recovery of DC shifts and [K⁺], elevations during PIDs (35). Therefore, PIDs are prolonged in ischemic penumbra. However, when they propagate into normally perfused tissue, PIDs become indistinguishable from SDs in amplitude, duration, and propagation speed (115, 202, 319). Infarct expansion correlates with the number of PIDs (55, 282, 319, 467) or with their cumulative duration (77). SDs artificially evoked in the ischemic hemisphere accelerate infarct growth proportional to their number (19, 45), and suppression of PIDs can ameliorate injury (148, 206, 332, 431). The basis for this relationship may lie in the imbalance between metabolic work load and delivery of O₂ and glucose, i.e., the CBF.

In focal ischemic penumbra, PIDs predominantly cause hypoperfusion secondary to vasoconstriction in all species studied to date, including mice, rats, and cats, as well as humans. In contrast, when PIDs propagate into the nonischemic tissue, they are associated with a peak hyperemia indistinguishable from SD. The deeper into the ischemic territory, the stronger the hypoperfusion response, and the more severe the loss of perfusion (26, 61, 218, 243, 284, 431, 450, 470). In mildly ischemic regions, the response is often biphasic with a conspicuous initial hypoperfusion (component I) followed by peak hyperemia (component II) (FIGURE 9). In more severely ischemic regions, the initial hypoperfusion becomes more severe, and peak hyperemia disappears. Therefore, the response becomes a monophasic hypoperfusion. Indeed, the loss of ability to mount a hyperemic response during the early stages of ischemia predicted tissue deterioration (128). The onset of hypoperfusion follows the onset of depolarization rather than precedes it, confirming that it is the effect of depolarization but not the cause (450). For the same reason, the steal phenomenon that has been proposed as one mechanism by which PIDs can worsen ischemic tissue perfusion (374) is unlikely to be a direct contributor (431). Adjacent to the ischemic core the hypoperfusion may become permanent if the depolarization fails to recover (197, 330, 431, 450), marking the stepwise expansion of ischemic core into the penumbra. Thus the vascular signature depends on the properties of the underlying tissue microenvironment. As such, PIDs are associated with more severe O₂ supply-demand mismatch in ischemic penumbra compared with SD in normal tissue (20, 452, 522), and the oligemic response contributes to ischemic injury rather than being an epiphenomenon (330, 450). Pharmacological (e.g., NMDA receptor antagonist MK-801) or physiological (normobaric hyperoxia) suppression of PIDs improves tissue perfusion and affords neuroprotection in ischemic brain in mice (429, 431). Interestingly, the same has not been observed with MK-801 in rats possibly due to a lower PID frequency in rats than in mice, and systemic hypotension with MK-801 as a potential confounder (206, 362).

The duration of hypoperfusion correlates directly with the duration of PID. Because more severe perfusion deficits in penumbra are associated with longer lasting depolarizations, their vasoconstrictive effect is also prolonged, which slows down and often prevents repolarization, creating a vicious cycle in penumbra. Indeed, when a PID fails to recover in critically hypoperfused tissue (i.e., when a portion of penumbra is incorporated into the core), its vasoconstric-
tive effect also becomes permanent. Since anoxic depolarization (AD) is a permanent (or at least a very long-lasting) SD-like state, it is associated with permanent loss of perfusion in focal ischemic core (431). This is also observed in global ischemia (175, 434). The mechanism may be relevant for the no-reflow phenomenon (7), possibly linked to the vasoconstrictive effect of elevated \( [K^+]_o \), that invariably accompanies SD, PID, and AD (7, 508). Pericytes may contribute (170). The permanent vasoconstrictive effect of AD may also explain the close correlation between MRI indexes of reduced cerebral blood volume and final tissue infarction in acute stroke in humans (446).

The exact mechanisms that transform the predominantly hyperemic CBF response into hypoperfusion in ischemic brain are unknown. By definition, focal arterial occlusion decreases the perfusion pressure in the territory supplied by the occluded artery. Intravascular perfusion pressure is a major determinant of the hemodynamic response to SD. As noted earlier, reduced perfusion pressure such as in systemic hypotension or proximal arterial stenosis or occlusion augments or unmasks the initial hypoperfusion (i.e., component I of the hemodynamic response) and diminishes the magnitude and prolongs the duration of subsequent dilator component (component II). Accordingly, hypotension transforms the CBF response in rats from monophasic hyperemia into a biphasic response (456, 457). Carotid artery occlusion also transforms the CBF response in the same manner (25) even when the resting CBF is maintained within normal range by autoregulatory mechanisms (207, 444, 445).

It is unclear, however, whether reduced luminal perfusion pressure in ischemic penumbra can alone explain the hypoperfusion response observed during PIDs, or whether tissue hypoxia or ischemia also independently transforms the hemodynamic response. In support of the latter, the response to SD also becomes vasoconstrictive after pial vein occlusion in rats, where perfusion pressure is not expected to decrease (361). In fact, CBF response to SD remains transformed and diminished at the infarct edge even 1 day after transient focal cerebral ischemia followed by successful reperfusion in rats, once again suggesting that perfusion pressure is not the only determinant of the transformation of response (428). Therefore, mechanisms other than simple reduction in intraluminal perfusion pressure appear to play a role as well. One can speculate that NOS activity may be diminished in ischemic tissue in part due to reduced \( O_2 \) availability as a substrate for \( NO \) synthesis. This is supported by data showing that pharmacological NOS inhibition transforms the CBF response similar to that in ischemic tissue (17, 114). Alternatively, maximum autoregulatory vasodilation may be approached in ischemic penumbra, limiting any further dilation during SD and unmasking the vasoconstrictive component. Lastly, \( [K^+]_o \) plays a significant role in determining the morphology of the vascular response during injury depolarizations (246, 310). Resting \( [K^+]_o \) is often elevated in ischemic and otherwise injured brain tissue, which augments vasoconstriction during PIDs, as we have learned in the setting of subarachnoid hemorrhage.

### B. Subarachnoid Hemorrhage

Although spontaneous SDs reminiscent of PIDs have not been reported in animal models of subarachnoid hemorrhage, conditions mimicking subarachnoid hemorrhage do transform the peak hyperemic response to induced SDs (86). For example, topical application of hemoglobin, presumed to act as an NO scavenger, transforms the response from a monophasic peak hyperemia into a biphasic response with an initial hypoperfusion followed by diminished hyperemia. When topical hemoglobin is combined with elevated resting \( [K^+]_o \) (20 mM), the hemodynamic response to SD transforms into a severe monophasic hypoperfusion (80% CBF decrease). The combination of topical hemoglobin and 35 mM resting \( [K^+]_o \), indeed triggers spontaneous SDs that are accompanied by severe reduction of CBF to ~15~20% of baseline, the so-called spreading ischemia that lasts anywhere from 10 min to an hour. The condition resembles the vicious cycle during PIDs in ischemic core and penumbra, i.e., initial hypoperfusion (component I) severe enough to interfere with repolarization (90). Interestingly, combination of topical hemoglobin or NOS inhibition with either low glucose solution (0.8 mM) or endothelin-1 (ET-1, 100 nM) also triggered spreading ischemia (84, 368); however, ETA or ETB receptor inhibitors did not prevent spreading ischemia triggered by NOS inhibition combined with 35 mM resting \( [K^+]_o \) (368). The ionic milieu during spreading ischemia, when reproduced in vitro, also constricts isolated MCA when NOS is inhibited (518). Spreading ischemia was proposed as a model for migrainous infarction, or in the pathophysiology of mitochondrial encephalopathy, lactic acidosis, and stroke (MELAS). Despite the severe transformation, the hemodynamic response to SD can be normalized by restoring \( [K^+]_o \) and washout of hemoglobin, by nimodipine coapplication (86), and partially by adenosine coapplication (92). Indeed, normalization of the microvascular hemodynamic response to SD by nimodipine may be one mechanism to explain its clinical benefit in preventing the delayed ischemic neurological deficits after subarachnoid hemorrhage, since large artery vasospasm was not reversed (475).

Subarachnoid blood has also been shown to directly transform functional neurovascular coupling in brain slices via increased amplitude of spontaneous astrocytic \( Ca^{2+} \) oscillations, augmenting the \( K^+ \) efflux through BK channels both at rest and during neural activation (228). As a result, \( [K^+]_o \) approaches and exceeds the threshold at which its normal dilatory effect via \( Kir \) channels is transformed into a direct constrictive effect on vascular smooth muscle, effec-
tively inverting the response. Whether the same mechanism occurs in vivo or transforms the hemodynamic response to SD after subarachnoid hemorrhage has not been tested.

C. Traumatic Brain Injury

Investigation of SD in the setting of trauma has been limited. Spontaneous SDs occurring after traumatic cortical injury were uncommon in one study and occurred mostly during the first hour of injury (505). The CBF response to such spontaneous SDs showed a prominent initial hypoperfusion during the depolarization and a diminished peak hyperemia. Interestingly, artificially (KCl) induced SDs after injury did not worsen the outcome in this model, setting a contrast with focal cerebral ischemia and suggesting compromised perfusion as a critical factor in SD-induced damage in injured brain.

XI. SPREADING DEPRESSION AND CEREBRAL VASCULATURE IN HUMAN BRAIN

Although there is ample evidence indicating the occurrence of SD in human brain, data on the hemodynamic response to SD are limited to SD occurring under pathological conditions such as migraine and brain injury, which may not fully represent normal brain. Moreover, it is highly probable that recordings in subjects with brain injury have missed the first SD occurring in naive tissue, the hemodynamic response to which may significantly differ from the response to subsequent SDs. These potential caveats notwithstanding, a number of studies have attempted to characterize the CBF response to SD in human.

A. Migraine

SD is the pathophysiological substrate of migraine aura and a trigger for headache (13). Aura may be any neurological disturbance that appears shortly before or during the development of a migraine headache, and may have different features depending of the brain regions involved in SD (253, 256). Hemodynamic studies during migraine attacks have revealed a reduction in CBF, termed spreading oligemia, that spatiotemporally closely followed the development of neurological symptoms (263, 265, 267, 346, 350, 357, 436–438). Spreading oligemia most commonly propagates from the occipital lobe into the parietal and temporal lobes at a rate of 2–3 mm/min, the maximal CBF decrease being above the ischemic level (i.e., 20–40% reduction). The original findings of a spreading oligemia have been confirmed with SPECT, PET, and functional MRI techniques (169, 264, 524). A delayed hyperemia (~20%) has also been reported following oligemia during headache resolution (~5 h after attack onset) in a small cohort of migraineurs (9), reminiscent of the delayed hyperemia following the post-SD oligemia reported in experimental animals (120). However, a preceding hyperemia during the aura stage, corresponding to the peak hyperemia (component II) in experimental animals, has been detected in only a small subset of migraineurs (169, 357). This may be because the hyperemia is more prominent in relative hypoperfusion states usually not present in humans (257), or because it is very brief and occupies a narrow strip of tissue (Figure 10) and may have been missed by the poor spatiotemporal resolution of clinically available noninvasive techniques, or partial volume averaging. It is of course possible that hyperemia may not be a prominent feature of the CBF response to SD in human brain, although studies from injured human brain suggest otherwise (see below). In regions showing oligemia during an attack, CO2 reactivity and functional hyperemia are attenuated (169), and autoregulation during blood pressure changes is preserved (4, 265, 267, 435), as the vascular signature of SD in humans, as it is in animals (255, 376).

Oligemia occurs during familial hemiplegic migraine attacks as well (214), often associated with more severe hypoperfusion approaching ischemic levels (136). However, the spatiotemporal evolution of oligemia did not show the typical spread pattern observed in classical migraine with aura. It was rather chaotic, appeared and disappeared faster and unpredictably, and was associated with superimposed large amplitude fluctuations in CBF sometimes showing hyperemia (136, 208). Recently, longitudinal imaging revealed a biphasic pattern with multifocal oligemia within hours of attack onset, and hyperemia after 18 h (209). Although an intrinsic vasomotor instability was suggested to explain these observations, enhanced SD susceptibility and propagation speed and the propensity for reentrant SD...
waves in this disease may also be responsible (103, 104, 106, 493, 494).

The obvious difficulty of capturing patients during an attack notwithstanding, angiographic vasospasm has not yet been demonstrated during oligaemia. Its absence may indicate increased resistance at the pial or parenchymal arteriolar level (437, 512). Because pial vasculature represents an anastomosing network, it is difficult, based on local changes in pial arteriolar diameters, to produce persistent changes in CBF downstream from the brain surface by local pial vasoconstriction (423). In contrast, a slight constriction of penetrating arterioles embedded in cortical tissue through which the SD propagates can explain the reduction in CBF (344). Interestingly, elevated plasma endothelin-1 levels have been reported during the first few hours of migraine attacks with or without aura that may provide a mechanism for increased parenchymal cerebrovascular resistance (125, 139).

B. Brain Injury

For several decades, it was speculated whether PIDs occur in human ischemic stroke (176), and anecdotal evidence has appeared (302). The hypothesis was systematically tested using subdural electrodes in patients with malignant MCA infarcts, a condition that occurs in ~10% of patients with supratentorial infarcts (33) that necessitates hemicraniectomy because of risk for herniation (358). Numerous PIDs were detected over the course of 7 days after stroke in most if not all stroke patients, and more than 90% of the total of depolarization events occurred in temporal clusters (78). As in experimental animals, PIDs increase the infarct size in human brain, demonstrated in a patient with malignant stroke who showed lesion progression by sequential MRI exactly in the zone experiencing around a hundred PIDs over a period of 5 days (330). Intraoperative visualization of the human cerebral cortex in the peri-infarct region of malignant hemispheric stroke showed multiple PIDs with a mixed pattern of CBF responses. More than 70% of events were coupled to hyperemia, and a smaller subset showed biphasic CBF responses or a monophasic hypoperfusion (519). Altogether the types of CBF responses coupled to PIDs in this cohort of malignant hemispheric infarct were consistent with experimental data (283, 330, 431, 450), suggesting that PIDs can be associated with both protective and unfavorable hemodynamic responses.

A similar pattern of CBF changes ranging from monophasic transient peak hyperemia to monophasic permanent hypoperfusion were observed coupled to SDs after subarachnoid hemorrhage as well (88). Although arterial vasospasm is a much-feared complication of aneurysmal subarachnoid hemorrhage, whether arterial vasospasm alone can explain the delayed ischemic neurological deficits days after the initial bleeding has been unclear. Patients with aneurysmal subarachnoid hemorrhage have a high incidence of SD events, and there is a clear correlation between SD occurrence and development of delayed ischemic neurological deficits (93). Indeed, small cortical infarcts are more common than large territorial infarcts corresponding to major cerebral arteries after aneurysmal subarachnoid hemorrhage, suggesting that SD events might contribute to delayed ischemia (91). In one study of 13 patients with aneurysmal subarachnoid hemorrhage, SDs were observed in 12 patients, associated with an increase, no change, or a decrease in CBF, with corresponding changes in tissue Po2 (88). Consistently, progressively hypoxic responses developed during clusters of SDs in patients with delayed ischemic neurological deficits, which were explained both by changes in local CBF due to microcirculatory dysfunction and to augmented metabolism (31). SDs also occur with high frequency in patients with traumatic brain injury and intracerebral hemorrhage (115, 184, 451). Recent systematic investigation of human traumatic brain injury revealed heterogeneous hemodynamic responses to SD ranging from monophasic hyperemia to monophasic hypoperfusion, mimicking the response patterns in peri-infarct tissue as well as after subarachnoid hemorrhage (194), as has been noted in earlier small studies (302).

In summary, SDs have been shown to worsen tissue perfusion in injured human brain, much like in experimental animals, as a potential mechanism leading to detrimental outcomes in stroke, intracranial hemorrhage, and traumatic brain injury (355). This is not surprising because human cerebral arteries are more sensitive to the vasocostrictive effects of elevated $[K^+]o$ compared with rat and rabbit arteries (402). Altogether the data suggest a bidirectional relationship leading to a vicious cycle in injured brain: injury radically transforms the neurovascular response to SD, and in return the transformed neurovascular response exacerbates the tissue injury by both decreasing CBF and metabolic supply and by increasing the energy demand (364). Therefore, SD should be considered a therapeutic target in injured brain (453).

XII. PRIMARY VASCULAR EVENTS TRIGGERING SPREADING DEPRESSION

Data over the past few years have strongly suggested a reverse direction of causality as well, where primary vascular events can lead to SD occurrence. For example, transient vascular microembolic events caused by air microbubbles, microspheres, or cholesterol microcrystals can all trigger SD without causing permanent brain injury (349). The mechanism involves brief ischemia severe enough to induce anoxic depolarization and has been implicated as a trigger for attacks of migraine with aura in patients with patent foramen ovale, particularly in the setting of agitated saline test during diagnostic echocardiography (418, 447). The
theory has been challenged by the failure of patent foramen ovale closure in improving migraine in a pilot randomized trial (82), and lack of an association between patent foramen ovale and migraine in an older population (401). Another interesting possibility is the release of one or more factors from the vasculature to trigger or predispose to SD either by directly acting on neurons and glia, or by causing severe constriction and transient ischemia as above. Indeed, endothelin is a potent vasoconstrictor peptide released primarily by the endothelial cells, and when administered exogenously, it is capable of triggering SD, in vivo but not in vitro, likely via a constrictive or ischemic mechanism through ETA receptors (85, 223). It remains to be determined whether endothelial irritation can induce endothelin release as a potential vascular trigger of SD. Lastly, the clinical association between several genetic vasculopathies and migraine with aura (e.g., CADASIL, RVCL, or mutations in COL4A1) has raised the possibility of a primary vascular determinant of SD susceptibility or induction. However, at least in CADASIL, the phenotype appears to involve parenchymal hyperexcitability (105).

XIII. ON THE EVOLUTIONARY ORIGINS OF VASOCONSTRICTIVE RESPONSE TO SPREADING DEPRESSION

Teleologically, vasoconstriction to PIDs is a highly paradoxical response in ischemic brain as it leads to further loss of perfusion. One has to remember, however, that ischemic stroke is a disease of modern age, an exceedingly short time span in evolutionary history. This is because ischemic stroke is largely a disease of elderly and is relatively rare in young adults; although it is among the major causes of death and disability today, this was not the case a mere millennium ago. Back then and until the beginning of 20th century, human life was much shorter (average global life expectancy <30 yr), and the major form of intracranial pathology that humans were exposed to was presumably traumatic brain injury and hemorrhage. In fact, pediatric strokes are more often hemorrhagic (279). Even today, spontaneous strokes are quite rare in the majority of mammals and phylogenetically lower animals that have been studied in sufficient detail, suggesting that our brains have never been evolutionarily challenged by ischemic strokes to force the natural selection of resistant genotypes. As indicated above, PIDs are in many ways analogous to SDs observed after intracerebral or subarachnoid hemorrhage and traumatic brain injury, and in case of the latter it makes a lot more sense for cerebral vasculature to constrict and minimize hemorrhage, rather than dilate to improve brain perfusion which would be more appropriate in ischemic stroke. Our brain simply does not know the difference between a PID in ischemic brain and an SD in traumatized brain. Because traumatic injury had more evolutionary influence, brain tissue assumes the latter and reacts as such when the tissue homeostasis is disturbed in some way (e.g., ischemia).

Even in the normal brain (i.e., migraine), the invariable component of the cerebrovascular response to SD is post-SD oligemia that lasts up to an hour and is observed across species; the peak hyperemia during SD, which has traditionally attracted more attention, are highly variable in direction as well as magnitude, and last only a couple of minutes. It appears from these observations that vasoconstriction is the more biologically and clinically relevant and deleterious response to intense brain depolarizations.

XIV. SUMMARY AND CONCLUSIONS

SD represents a massive pandepolarization of brain tissue with profound vascular and metabolic consequences. It is an evolutionarily conserved intrinsic ability of neuronal membranes to undergo such depolarizations upon intense excitation or metabolic failure. We here provide a detailed overview of the complexity of cerebrovascular regulation during and after SD. The multitude of vasoactive substances simultaneously released from neurons and astrocytes makes it one of the most difficult vasoactive responses to dissect physiologically and pharmacologically. We have, therefore, refrained from contrived and reductionist simplifications of the vasomotor actions of each potential mediator or modulator, and instead provided a detailed summary of our knowledge base for the reader to draw upon. The image we would like to impress upon the reader is one of chaos, where countless cells and signaling pathways are simultaneously recruited and perturbed. Our job is then to seek targets that can be utilized to better understand the neurovascular processes and alter the course of events during SD. The topic is not only of theoretical but also clinical relevance since SD occurs in patients with migraine and acutely injured cortex, with severe outcome implications in the latter. The goals of ongoing neurovascular research in SD are to suppress the depolarization events, to facilitate substrate supply at a time when the tissue depolarizes, and to prevent the post-SD oligemia that may relate to vasospasm after SAH. The search for vasoconstrictor mechanisms is ongoing and of great importance for our understanding of the neurovascular function and the approach to treat neurovascular disorders. Our knowledge of the loss of vascular reactivity after SD and the persistent impairment in neurovascular coupling is also incomplete and of similar importance. New research tools, such as optical imaging, will be essential and are likely to yield critical data on the contribution of SD to neurovascular disorders, and facilitate new concepts and drugs to mitigate the vascular consequences of SD.

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