Published in final edited form as:

J Clin Neurophysiol. 2016 June ; 33(3): 196–202. doi:10.1097/WNP.0000000000000275.

Spreading depolarizations: A therapeutic target against delayed cerebral ischemia after subarachnoid hemorrhage

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Abstract

Delayed cerebral ischemia (DCI) is the most feared mechanism of secondary injury progression after subarachnoid hemorrhage. Initially thought to be a direct consequence of large artery spasm and territorial ischemia, recent data suggest a more complex picture with multiple concurrent and synergistic mechanisms underlying DCI, including microcirculatory dysfunction, inflammation, and microthrombosis. Among these mechanisms, spreading depolarizations (SD) are arguably the most elusive and underappreciated in the clinical setting. Although SDs have been experimentally detected and examined since the late 1970's, their widespread occurrence in human brain was not unequivocally demonstrated until relatively recently. Today we know that SDs occur with very high incidence in human brain after ischemic or hemorrhagic stroke and trauma, and worsen outcomes by increasing metabolic demand, decreasing blood supply, predisposing to seizure activity, and possibly worsening brain edema. Here, we review the causes and consequences of SDs in injured brain. Although much of our mechanistic knowledge comes from experimental models of focal cerebral ischemia, clinical data suggest that the same principles apply regardless of the mode of injury (i.e., ischemia, hemorrhage or trauma). The hope is that a better fundamental understanding of SDs will lead to novel therapeutic interventions to prevent SD occurrence and its adverse consequences contributing to injury progression.

Introduction

Delayed cerebral ischemia (DCI) and infarction after aneurysmal subarachnoid hemorrhage (SAH) are the most dreaded complications facing patients after securing their aneurysm. It is associated with up to 50% of deaths in patients who survive to hospital admission, and leads to significant in-hospital and long-term morbidity (Bederson, Connolly et al. 2009). Given the importance of early detection and treatment, even asymptomatic patients require prolonged ICU observation, which leads to prolonged hospital stays and increased healthcare costs (Roos, Dijkgraaf et al. 2002, Rivero-Arias, Gray et al. 2010). Nimodipine,

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the only FDA-approved drug for the prevention of DCI, is routinely used but with limited efficacy (Allen, Ahn et al. 1983, Pickard, Murray et al. 1989). Therefore, better therapies to prevent and treat DCI are still greatly needed. Elucidation of the underlying cause of DCI would be helpful to that end, but mechanisms remain poorly understood. Large artery vasospasm does not appear sufficient for the development of DCI, as was most recently demonstrated by the failure of the endothelin receptor antagonist clazosentan to improve patient outcomes despite significantly ameliorating the large vessel vasospasm in the CONSCIOUS-2 trial (Macdonald, Higashida et al. 2011). Conversely, nimodipine can improve outcomes in SAH despite its equivocal effect on vasospasm (Macdonald 2016). These data underscore the disconnect between large artery vasospasm and DCI, and point to alternative mechanisms such as inflammation, microcirculatory dysfunction, microthrombosis, and in particular spreading depolarizations.

Spreading depolarizations

Spreading depolarizations (SD) are recurrent waves of intense neuronal and glial mass-depolarization that occur in an apparently spontaneous fashion during and for many days after an ischemic or hemorrhagic stroke, and traumatic brain injury (Bosche, Graf et al. 2010, Lauritzen, Dreier et al. 2011, Ayata and Lauritzen 2015). During an SD, neuronal membrane potentials approach zero due to opening of yet unidentified large conductance, non-selective cation channels, leading to massive transmembrane ion fluxes, dramatic elevations of extracellular K^+ and intracellular Na^+ and Ca^{2+} concentrations, and cell swelling. The extracellular concentrations of virtually all neurotransmitters, metabolites and small signaling molecules show significant changes, including the excitatory amino acid glutamate. Elevated extracellular K^+ is believed to diffuse into the adjacent brain tissue, and, with the help of glutamate, trigger the same depolarization cycle, allowing SD to propagate at a slow pace of ~3 mm/min by way of chemical contiguity. Because high extracellular K^+ and glutamate are critical for wave spread, SDs are limited to gray matter with high neuronal and synaptic density, and do not propagate into white matter where the extracellular space fraction is larger, synapses are sparse, and myelin acts as a barrier (Merkler, Klinker et al. 2009). As near complete depolarization precludes action or postsynaptic potentials, SDs are also associated with depression of all spontaneous or evoked electrophysiological activity (Figure 1). This is what prompted Leão to coin the term ‘spreading depression’ when he serendipitously discovered SD while studying rabbit cortex (Leao 1944). Today these terms are often used interchangeably as the mass depolarization underlying spreading depression and spreading depolarization are one and the same, with the latter term being phenomenologically more apt.

Although spreading depression has been known since the 1940’s, it wasn’t until the late 1970’s that the occurrence of spreading depression-like waves was discovered during experimental focal cerebral ischemia (Branston, Strong et al. 1977). Four decades later, we now know that SDs occur in most forms of human brain injury (Dreier 2011). Despite our accumulated knowledge base, however, SD is still among the most underappreciated pathophysiological process in the evolution of brain injury. The most important reason for this has been the difficulty in detecting SDs in human brain. Although the amplitude of the electrophysiological signature of SD, the extracellular slow potential shift, is orders of

magnitude larger (up to 30 mV) and longer in duration (up to a minute; Figure 1) than most other electrophysiological events in the brain (e.g., action or postsynaptic potentials), it is almost invisible to conventional transcranial high-pass EEG because SD is slow to develop and resolve (seconds) and therefore filtered out of the EEG, and it is highly focal, occupying ~3 mm strip of brain tissue at any given time and therefore spatially averaged by large leads (Drenckhahn, Winkler et al. 2012). Moreover, injured brain often already has depressed spontaneous electrical activity; therefore, EEG depression cannot be relied upon to identify SD occurrence in injured tissue either. Subdural electrocorticographic recordings in patients with large ischemic or hemorrhagic strokes or severe traumatic brain injury requiring a craniotomy have overcome these obstacles and unequivocally demonstrated the occurrence of numerous SDs in injured human brain. This tremendous contribution by the COSBID investigators (Dreier, Woitzik et al. 2006) has sparked new research to improve the clinical detection of SDs, as well as to target them therapeutically.

Spatiotemporal characteristics of spreading depolarizations

As noted above, SDs occur with high incidence in virtually all forms of acute human brain injury, starting immediately after injury onset in ischemic stroke, to many days and even a couple of weeks after SAH (Lauritzen, Dreier et al. 2011). Full field optical imaging in the experimental setting has shown that recurrent SDs usually, if not always, originate from the boundary zone between the injured and presumably already depolarized tissue and the tissue that is relatively healthy (Shin, Dunn et al. 2006, von Bornstadt, Houben et al. 2015). In focal ischemic stroke, this zone is the peri-infarct tissue surrounding the core, including the ischemic penumbra (i.e., tissue with synaptic silence but preserved neuronal resting membrane potential), and adjacent moderately ischemic (i.e., metastable) brain (von Bornstadt, Houben et al. 2015). In any injured brain, SDs tend to originate repeatedly from the same one or just a few foci within a time frame of hours. However, new foci do appear and previous ones stop generating SDs during acute to subacute stages over days, suggesting that local tissue factors and changing conditions are critical for the timing and origin of SD occurrence.

For decades, the trigger factors that determine when and where an SD originates in injured brain have remained a mystery. However, recent data using multimodal optical imaging indicate that SDs are triggered when the resting state O₂ supply-demand mismatch in peri-infarct tissue is transiently worsened by either reduced supply or increased demand (Figure 2) (von Bornstadt, Houben et al. 2015). For example, systemic hypoxic or hypotensive transients that are highly common in the clinical setting can reduce O₂ and blood supply to already critically hypoperfused and hypoxic peri-infarct tissue, and trigger anoxic depolarization that becomes the origin of an SD. Conversely, functional activation of peri-infarct tissue (e.g., somatosensory stimuli) can increase O₂ demand and extraction, which once again worsens supply-demand mismatch and triggers focal anoxic depolarization at the site of activation, and a propagating SD originating from that site. Although the precise triggers after SAH or traumatic brain injury are not known, the same principle of supply-demand mismatch transients likely apply, regardless of what triggers the transient. Accordingly, the larger the volume of metastable tissue as described above, the higher the chance of SD occurrence.

Once an SD originates, it propagates throughout the tissue surrounding the injury and often some distance into surrounding healthy brain tissue. In fact, in the architecturally more simple lissencephalic rodent brain, SD often propagates throughout the ipsilesional cortex and may even penetrate subcortical gray matter structures (Eikermann-Haerter, Yuzawa et al. 2011). In gyrencephalic brains, however, propagation patterns can be extremely complex (Santos, Scholl et al. 2014). Modulated by the gyri and sulci, as well as pial vessels that can act as barriers to propagation, radial or spiral spread patterns and reverberating (i.e., circling) waves commonly occur in gyrencephalic brains (Figure 3). Such complex spatiotemporal propagation patterns add to the difficulties of interpreting surface recordings obtained by subdural electrodes in human brain.

Making matters worse, the pattern of propagation evolves in an unpredictable fashion depending on changes in the intrinsic tissue susceptibility to SD over time. For example, tissue exposed to an SD becomes relatively resistant and less conducive to another SD, thereby channeling subsequent waves on to a different path of propagation. In both gyrencephalic and lissencephalic brains, such spatiotemporal evolution of SD propagation can predispose to reentrant SD clusters (akin to ventricular tachycardia) where an SD wave reenters and propagates through a given brain region several times in a short time span, imposing tremendous metabolic burden and paradoxical vasoconstriction (Eikermann-Haerter, Lee et al. 2012). In this way, the adverse impact of a single SD wave can be effectively multiplied if it reverberates and propagates through the same tissue, exposing that tissue to an unusually high number of depolarizations.

Importantly, genetic factors modulate susceptibility to SD occurrence and propagation in ischemic brain. For example, transgenic knock-in mice expressing human familial hemiplegic migraine type I mutations show markedly higher incidence of SDs and more frequent reentrant and circling SDs during focal cerebral ischemia, and cortical SDs more frequently propagating into subcortical grey matter structures (striatum, thalamus, hippocampus) (Eikermann-Haerter, Yuzawa et al. 2011, Eikermann-Haerter, Lee et al. 2012, Eikermann-Haerter, Lee et al. 2015).

Impact of spreading depolarizations on injury outcome

Spreading depolarizations exacerbate tissue injury in several ways. First, SDs are energetically highly costly, even more so than epileptic activity. Restoration of normal transmembrane ion gradients during repolarization requires ATP, and after an SD all metabolic indices show intense activation. ATP and phosphocreatine levels drop, O₂ and glucose consumption is increased, tissue glucose availability and glycogen are reduced (Sarrafzadeh, Santos et al. 2013) and may take up to an hour to restore (Ayata and Lauritzen 2015). Unsurprisingly, such heavy metabolic demand is often not met sufficiently in injured brain, and repolarization is often prolonged beyond the usual rate of under 1 minute in healthy brain. Indeed, parts of critically ischemic tissue may fail to ever recover from the depolarization and get incorporated into the infarcted core.

Exacerbating matters further, SDs have a strong vasoconstrictive effect on ischemic brain tissue (Shin, Dunn et al. 2006, Dreier, Major et al. 2009, Hinzman, Andaluz et al. 2014),

which, when combined with the tremendous metabolic burden, worsens the supply-demand mismatch and is highly detrimental to the survival of already metabolically compromised brain tissue (Bosche, Graf et al. 2010, von Bornstadt, Houben et al. 2015). In this way, SDs often expand irreversibly injured tissue in a stepwise manner (Shin, Dunn et al. 2006, Nakamura, Strong et al. 2010, Ayata and Lauritzen 2015).

In addition to worsening supply-demand mismatch, SDs activate inflammatory cascades (Jander, Schroeter et al. 2001, Urbach, Bruehl et al. 2006, Grinberg, Milton et al. 2011, Grinberg, Dibbern et al. 2013), disrupt the blood-brain barrier (Gursoy-Ozdemir, Qiu et al. 2004), and predispose to epileptic discharges (Fabricius, Fuhr et al. 2008, Dreier, Major et al. 2012, Lapilover, Lippman et al. 2012, Lapilover, Lippmann et al. 2012), all of which may contribute to worsening clinical outcomes (Lauritzen, Dreier et al. 2011).

Not surprisingly, there is ample experimental evidence suggesting that SDs worsen tissue and functional neurological outcomes, but the clinical evidence in acute brain injury is even more telling (Dreier, Woitzik et al. 2006, Hartings, Strong et al. 2009, Sakowitz, Kiening et al. 2009, Bosche, Graf et al. 2010, Hartings, Bullock et al. 2011). Specifically in SAH, human studies show a strong association between frequency of spontaneous SDs and delayed infarcts (Dreier, Woitzik et al. 2006). Furthermore, in patients with SAH SDs that transiently decrease pO_2 (i.e., increased consumption and reduced delivery) are associated with delayed neurological deficits, whereas SDs that transiently increase pO_2 (due to the hyperemic effect) are not (Bosche, Graf et al. 2010), suggesting that not only are SDs important in the pathogenesis of DCI, but that the nature of the hemodynamic response to SD is a critical determinant of the final impact.

Spreading depolarizations as a therapeutic target

All accumulated evidence to date strongly suggests that prevention of SDs and mitigating their adverse consequences would be beneficial in brain injury, at least in the acute setting. Because SDs occur for many days after injury, especially after SAH, there is a wide therapeutic window for intervention to prevent secondary injury progression and lesion growth. Although there is ample data to support this notion, there has never been any clinical study to test it. A major challenge that has prevented translational efforts is the need for invasive monitoring (i.e., subdural or intracortical recordings) to detect SD occurrence as done by the COSBID collaboration over the past decade (Strong, Hartings et al. 2007, Dohmen, Sakowitz et al. 2008, Bosche, Graf et al. 2010, Hinzman, Andaluz et al. 2014).

Although an argument can be made that SDs occur close to 100% of all patients with ischemic stroke, and up to 80% of patients after SAH at some point after the injury, most acute inhibitors of SDs have neurological side effects including sedation that can obscure the clinical exam, obligate endotracheal intubation, and require longer intensive care unit (ICU) stays. This necessitates, as a first step, a smaller, focused cohort, and targeted therapy with proximate readouts (e.g., SD occurrence rate) to determine efficacy in a proof-of-concept phase I trial. Although advanced ICU monitoring and management may somehow allay concerns about depression of mental status, the development of non-invasive tools to detect SD, such as high density surface EEG with advanced data processing, or optical tools like near-infrared or diffuse correlation spectroscopy, would be a big translational step forward.

The development of new therapeutic modalities that can inhibit SDs without neurological side effects will be equally important to advance clinical translation. There are numerous potential therapeutic targets that can reduce susceptibility to SD (Ayata 2009, Ayata 2013, Costa, Tozzi et al. 2013, Sanchez-Porras, Zheng et al. 2015). However, many require prolonged or chronic treatment for weeks for their efficacy to emerge (e.g., migraine prophylactic drugs) (Eikermann-Haerter, Lee et al. 2015) and others are not yet in clinical use (e.g., $\text{Ca}_v2.1$ P/Q-type voltage gated Ca^{2+} channel inhibitors) or do not penetrate CNS at high-enough concentrations.

Among clinically feasible pharmacological SD inhibitors, the NMDA receptor antagonist ketamine is most promising. Ketamine has been shown to abruptly suppress SDs in human brain injury and SDs in gyrencephalic brains (Sakowitz, Kiening et al. 2009, Sanchez-Porras, Santos et al. 2014). NMDA antagonists reduce infarct volumes and improve neurological outcomes in experimental models of ischemic stroke (Shin, Dunn et al. 2006).

The anticonvulsant lamotrigine appeared to shown good efficacy in suppressing SDs after a single dose (Eikermann-Haerter, Lee et al. 2015). Unfortunately, however, clinically this drug has to be initiated at low doses and titrated up over weeks to avoid serious potential side effects. Sigma-1 agonists (e.g., dextromethorphan) are another promising class of drugs that can inhibit SDs in the experimental setting (Shin, Dunn et al. 2006) and may be clinically feasible.

More recently, transcutaneous vagus nerve stimulation has been shown to non-invasively suppress KCl- or electrical stimulation-induced SD susceptibility within 30 minutes of a single treatment in otherwise normal rats (Chen, Ay et al. 2015). Vagus nerve stimulation also improves tissue and neurological outcome in animal models of ischemic stroke (Ay, Lu et al. 2009, Ay, Sorensen et al. 2011), and is FDA approved in refractory epilepsy with excellent clinical feasibility. It remains to be seen whether vagus nerve stimulation can also suppress SD occurrence in injured brain.

Another important consideration to suppress SD occurrence is to minimize the factors that predispose to a higher incidence of SDs in injured brain. In this regard, systemic physiological parameters are critical. For example, higher plasma glucose levels are associated with a lower susceptibility to SD in rats (Hoffmann, Sukhotinsky et al. 2013), and hypoglycemia and hyperglycemia have been associated with higher and lower incidence of SDs, respectively, in ischemic stroke (Nedergaard and Astrup 1986, Strong, Smith et al. 2000). Similarly, hypoxic and hypotensive transients trigger SDs (von Bornstadt, Houben et al. 2015) and normobaric hyperoxia effectively blocks SD occurrence (Shin, Dunn et al. 2007, Shin, Oka et al. 2014) in focal ischemic stroke. Therefore, while maintaining normoxemia and normotension is important, normobaric hyperoxia and induced hypertension may afford additional benefit in further reducing PID incidence, as well as their deleterious effects. It should be noted, however, that normobaric hyperoxia may lose its beneficial effect in the presence of endothelial dysfunction, such as in elderly individuals with multiple vascular risk factors (Shin, Oka et al. 2014). Finally, functional activation of the cortex, for example by somatosensory stimuli, can also trigger SDs in focal ischemia

(von Bornstadt, Houben et al. 2015), and minimizing unnecessary stimulation during the acute stroke stage may be beneficial.

SD occurrence in animal models of brain injury

The occurrence of SDs is not uniform in experimental models. For example, while 100% of all ischemic stroke models show recurrent SDs, spontaneous SDs have never been observed in rodent models of pure SAH (e.g., cisternal blood injection). SDs observed in the endovascular puncture model of SAH are likely to be triggered by an inadvertent ischemic stroke induced by the perforation procedure. Even in a collagenase model of intracerebral hemorrhage in pigs, where recurrent spontaneous SDs have been recorded during the first 6 hours (Mun-Bryce, Wilkerson et al. 2001), focal cerebral ischemia may be the trigger underlying SDs.

Similarly, traumatic injury creates focal ischemic zones by directly disrupting the cerebral vessels or creating a prothrombotic state, to then trigger SDs. Therefore, focal ischemia may be a shared mechanism that triggers SDs after ischemic, hemorrhagic or traumatic injury. The lack of SD occurrence in animal models of SAH contrasts with human SAH where numerous SDs occur. As a potential explanation, severe delayed cerebral ischemia is much less common in rodents than man. Nevertheless, because SDs do develop in human ischemic or hemorrhagic stroke including SAH, and in trauma, lack of SD occurrence in animal models of hemorrhage or trauma poses a difficulty for investigational purposes only.

Concluding remarks

Delayed cerebral ischemia and neurological deficits remain a significant clinical problem with poorly understood pathophysiology. To further improve outcomes, we need a better understanding of SD as an understudied cause of delayed cerebral ischemia and neurological deficits that may integrate many of the proposed mechanisms. Animal models that better recapitulate the natural history of human SAH, as well as more practical and less invasive methods to detect SDs will be critical for our translational efforts.

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Figure 1. Spreading depolarizations in normal and injured brain

(A) Electrophysiological tracings of SD showing ECoG depression spatiotemporally coincident with a negative extracellular direct coupled (DC) potential shift recorded by two serial intracortical microelectrodes (E1, E2) in the rat. The DC shift is typically ~20–30mV in amplitude, lasts up to a minute and propagates at a speed of ~3–4mm/min. SD is triggered by topical KCl application. (B) Electrophysiological tracings showing large and often prolonged DC shifts associated with peri-infarct SDs recorded by two intracortical microelectrodes in the mouse. Note the terminal depolarization at the end of the recording in

both electrodes sequentially (arrowhead). Ischemia was induced by middle cerebral artery occlusion (MCAO). Gray shaded area shows the typical distribution of perfusion defect after MCAO. (C) Sample ECoG and DC potential tracings from human brain showing two propagating SDs after traumatic brain injury recorded by subdural electrode strips. ECoG traces are bipolar between adjacent leads on the strip. DC traces are referential to an extracranial reference. Note the sequential involvement of electrode pairs by SD (courtesy of Dr. Jed Hartings). Modified from (Ayata 2009) and (Ayata and Lauritzen 2015) with permission.

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Figure 2. The concept of peri-infarct hot zones susceptible to SD initiation by supply-demand mismatch transients

(A) Three-dimensional rendering of a hypothetical CBF defect upon focal arterial occlusion, depicting the severely ischemic depolarized core, the synaptically silent penumbra, and the metastable hot zone surrounding it. The narrow critical CBF range defines the hot zone specifically in a mouse distal MCAO model under isoflurane and nitrous oxide anesthesia. Many factors (e.g., genetic, environmental, pharmacological) likely modulate this critical CBF range where the tissue is more susceptible to SD triggering. Therefore, in different species, under different experimental conditions, the critical range may be different. (B)

Two-dimensional (i.e., top-down) view of the same perfusion defect. In the metastable hot zone, increased demand during functional activation of the tissue worsens the supply-demand mismatch to trigger a PID. Because penumbra is electrophysiologically silent, by definition it cannot be activated upon somatosensory stimulation; therefore, it is only susceptible to reduced O₂ supply during hypoxic and hypotensive transients to trigger a PID. Core is already depolarized, and therefore, already in an SD state. Modified from (von Bornstadt, Houben et al. 2015) with permission.

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Wave initiation patterns

Wave evolving patterns

Figure 3. Spatiotemporally complex SD initiation and propagation patterns in gyrencephalic brain

SD is triggered using topical application of concentrated KCl and detected using full-field optical imaging of cortical hemodynamic changes as a surrogate marker. After one stimulation, the most common pattern is radial (a1). It expands and finds limiting sulci and vessels (a2); the wave then evolves into two semi-planar waves that continue their propagation (a3), or decay at some point. Irregular waves with open rings are produced sometimes after stimulation (b1). They commonly appear as a second or third wave after stimulation. An area with a KCl gradient is created. A maximum vascular constriction

occurs at the center of this gradient and a functional blocked area where no SD can diffuse may be seen. On one side of the KCl gradient, an irregular wave grows and moves mainly in one direction far from the stimulation point (b1–2). It expands and creates a solitary broken radial wave (b3). Another pattern seen was an irregular wave cycling the stimulation site (c1–3). On one side of the KCl gradient, an irregular wave grows (c1) and moves following the periphery of the KCl gradient (c2–3). The spiral is able to create more single semi-planar waves that move away from the stimulation site (c3). The evolving patterns are semi-planar waves (d), spiral waves (e–f) and reverberating waves. The complexity of the wave morphology is increased by collisions (h1–2) and anatomical blocks, such as vessels and sulci (i1–2). Adopted from (Santos, Scholl et al. 2014) with permission.

Figure 4. Heterogeneity of CBF response to SDs in focal ischemic brain depending on local steady state perfusion

(A) A hypothetical diagram showing the transformation of the CBF response (upper graphs) to peri-infarct SDs recorded in tissue with increasing severity of ischemia as shown on the CBF profile across focal ischemic tissue (lower graph). In non-ischemic tissue, SD evokes a predominantly hyperemic response (a), whereas in mildly ischemic penumbra (b) a biphasic response is observed. In moderately ischemic penumbra (c), the response is mainly a monophasic hypoperfusion. In the severely ischemic core-penumbra junction (d), both the DC shift and the hypoperfusion may not recover completely after an SD. Horizontal bars

represent the DC shift during SDs (modified from (Ayata and Lauritzen 2015) with permission). (B) Speckle contrast images from a representative experiment showing the abrupt expansion (>100%) of severely hypoperfused cortex (i.e., 20% residual CBF; shown in dark grey) by a single SD event after acute distal middle cerebral artery occlusion in the mouse. The imaging field covered the entire right hemisphere. Modified from (Shin, Dunn et al. 2006) with permission.

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