Mechanisms of Spreading Depression and Hypoxic Spreading Depression-Like Depolarization

GEORGE G. SOMJEN

Department of Cell Biology, Duke University Medical Center, Durham, North Carolina

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current could generate SD-like depolarization, but ordinarily, it is brought about by the cooperative action of the persistent Na\(^{+}\) current \(I_{\text{Na,p}}\) plus NMDA receptor-controlled current. SD is ignited when the sum of persistent inward currents exceeds persistent outward currents so that total membrane current turns inward. The degree of depolarization is not determined by the number of channels available, but by the feedback that governs the SD process. Short bouts of SD and HSD are well tolerated, but prolonged depolarization results in lasting loss of neuron function. Irreversible damage can, however, be avoided if Ca\(^{2+}\) influx into neurons is prevented.

I. INTRODUCTION

A. Scope and Purpose of This Review

Spreading depression, SD for short, is a striking and highly reproducible response of the gray matter of the central nervous system. Its place in and significance for the functioning of the brain and its biophysical mechanism have long intrigued yet eluded researchers. Recent developments have moved us closer to solve the puzzle, and this review attempts to put the pieces in their place. SD is important for at least two reasons. First, it may underlie certain clinical neurological conditions, a matter that is addressed in section IV of this review. But, apart from practical considerations, understanding its mechanism is essential for a complete picture of general neurophysiology.

SD is hardly a new phenomenon; in fact, it has first been described 56 years ago (213). An extensive literature describes its properties, yet attempts to explain its mechanism remained unsatisfactory until recently, in part because the biophysical properties of central neurons were incompletely known and also because of the lack of computational power to test hypothetical proposals. Both these handicaps have gradually been overcome, and believable theoretical treatments, based on reliable laboratory data, have recently emerged. In this review I outline the history and the general features of SD. The emphasis is on data published during the last decade or two. Additional details of the earlier studies may be found in earlier reviews (41, 42, 179, 237, 240, 281, 294, 373, 375).

B. A Note on Terminology

At the core of SD is a rapid and nearly complete depolarization of a sizable population of brain cells with massive redistribution of ions between intracellular and extracellular compartments, which evolves as a regenerative, “all-or-none” type process and propagates in the manner of a wave through gray matter. A similar response occurs in cerebral gray matter a few minutes after interruption of the blood flow or of the supply of oxygen. The pioneer investigators suspected that the same cellular process underlies the potential shifts and ion fluxes induced by hypoxia/ischemia and by SD (128, 214, 237, 427), but others have disputed this identity (399). Other names used to describe the hypoxic event include terminal depolarization (39, 384), anoxic depolarization (AD) (41), and rapid depolarization (397). A semantic objection against applying the term SD to hypoxia-induced depolarization stems from the assumption that the hypoxic process starts at once in a wide area, for if it does not propagate, it should not be called spreading depression. In arguing against this notion, Marshall (237) emphasized that propagation is not the essential feature of the process. Besides, recently, we have found that hypoxic SD-like depolarization actually does start in small foci, and it spreads at about the same velocity as does normoxic SD (6).

We prefer the somewhat cumbersome expression, SD-like hypoxic depolarization (377), abbreviated to hypoxic SD or HSD (6), for the following reasons. Although the sequence of events that leads to the depolarization does differ between SD and HSD, no difference has been detected in the biophysics of the depolarization itself. “Terminal depolarization” is misleading because the hypoxic/ischemic SD-like event is initially quite reversible, and it becomes “terminal” only if it persists beyond a critical period of time. The terms anoxic depolarization and rapid depolarization are not specific. All cells of mammals depolarize eventually in the absence of oxygen, but not all hypoxia-induced depolarizations are SD like. The diagnostic criterion is the accelerating, regenerative, all-or-none type depolarization typical of SD. Even in the neocortex, mild hypoxia causes only a slow, gradual depolarization that is not SD like (54), and this is typical of the spinal cord and of white matter even in severe hypoxia (390, 416). The distinction between SD-like and non-SD depolarization was appreciated already by van Harreveld and collaborators (416, 427).

The similarities and the differences between SD and HSD are discussed in some detail in section vB.

II. PHENOMENOLOGY OF SPREADING DEPRESSION

A. Early Reports: Electroencephalography and Surface Direct-Current Potential

The first, seminal paper on SD, titled “Spreading depression of activity in the cerebral cortex” (213) appeared in 1944, written by a young and unknown Brazilian inves-
tigator, Aristides Leão, working at the Harvard laboratory of R. S. Morison. Leão wanted to study the cortical electrogram (ECoG) of experimental epilepsy in anesthetized rabbits, but he was distracted from his original goal by an unexpected silencing of the ongoing normal electrical activity that took the place of the anticipated seizure (Fig. 1). The flattening of the ECoG trace crept slowly over the cortex, from one recording electrode pair resting on the cortical surface to the one beside it. According to Leão, SD and propagating focal seizures were related phenomena, generated by the same cellular elements (213), an inference later supported by others (e.g., Ref. 428).

After returning to Rio de Janeiro, using string galvanometer and vacuum tube amplifier, Leão recorded from the cortical surface the “negative slow voltage variation” (here denoted $\Delta V_o$) associated with SD, and the similar voltage shift that occurs after a few minutes delay when the cortex is deprived of blood flow (214). Reaching maximal amplitude of $\sim 15$ mV, this surface potential shift was astonishingly large compared with other brain waves. In a delighted footnote, Leão (214) acknowledged a personal communication by B. Libet and R. W. Gerard, who apparently made the same observation.

### B. Occurrence

Normoxic SD can be triggered by high-frequency electrical pulses (“tetanic” stimulation) or direct current (DC) (“galvanic”), mechanical stimuli such as pressure on or puncture of the cortex, alkaline pH, low osmolarity, and a variety of chemicals (20, 26, 41, 79, 117, 212, 237, 294, 319, 327). Among the chemical agents noteworthy are potassium ions, glutamate, and, in some areas, acetylcholine, because these are normally present in the brain, and ouabain because it raises extracellular $K^+$ concentration.
In general, similar insults can induce SD or provoke seizure discharge, and there are no simple rules by which to predict which of the two will prevail. Severe hypoxia or, more generally, sudden energy failure induces an SD-like response, and “spontaneous” waves of SD emanate from the border of ischemic foci and propagate into the surrounding brain region (43, 125, 149, 256, 336, 388, 437).

Some have contended at first that SD can occur only in cortex that is either diseased or ill treated (237). Although it is true that drying, hypoperfusion, and trauma facilitate SD, it can be provoked in perfectly healthy, well-nourished, oxygenated brain even when it is protected by its normal coverings, and also in the brains of unanesthetized, freely moving animals (44, 45, 176, 178, 247, 248, 294, 425, 430). The same is, of course, true for epileptiform seizures. Moreover, SD has been demonstrated in almost all the gray matter regions of the central nervous system, but it is more readily provoked in some areas than in others. The CA1 sector of the hippocampal formation is perhaps the most prone, closely followed by the neocortex. In the cerebellar cortex and olfactory bulb it is difficult to produce, unless the tissue is pretreated (“primed” or “preconditioned”) by raising $[K^+]_o$, substituting $Cl^-$ by acetate or propionate in the extracellular milieu, or hypotonicity (8, 87, 216, 281, 454). The spinal cord seemed quite “immune” for a long time but, under special conditions, its gray matter can also produce SD-like events (68, 387). In between these extremes are the subcortical gray matter of the basal ganglia and the thalamus, and also the retina, all of which can support SD, if suitably provoked (7, 40, 73, 86, 178, 239, 432). What it is that makes tissue more or less susceptible to SD has not been determined. Various possible reasons are discussed in section III M.

In newborn animals, SD cannot be induced. In rabbit and rat cerebral cortex the capacity to generate SD appears between the 10th and 25th postnatal day in different areas (41, 278, 446). Thereafter the threshold decreases and the amplitude of the associated extracellular voltage shift ($\Delta V_o$) increases until it reaches adult proportions. Hypoxic SD-like depolarization is evident already in 4-day-old rat pups, but the latent period from oxygen withdrawal to the appearance of the SD-like event is extremely long, and the apparent threshold level of $[K^+]_o$ from which the steep, SD-like increase takes off is very high. Then, as the rats mature, the latency shortens and the $[K^+]_o$ threshold is lowered (121, 157, 232). The final level to which $[K^+]_o$ rises is, however, similar in all age groups. The decreasing threshold of SD ignition may have to do with the shrinkage of interstitial space with age (222) or the maturation of transmitter systems (229, 253, 334, 392) (see section III, J, L, and M). In senescent rats, latency becomes even shorter than in young adults (322).

For a while it was debated whether SD can occur in the highly convoluted cortex of primates, especially in humans. Indeed, the smooth cortex of rats and rabbits produces SD more readily than that of cats, whereas the monkey brain is relatively resistant though by no means immune (41, 430). Šramka et al. (386) recorded SD-like potential shifts in the hippocampal formation of human patients during stereotactic surgery. Against this contention McLachlan and Girvin (252) failed to evoke SD in the exposed cortex of patients, using electrode configurations and current intensities similar to those that consistently provoked SD in rat cortex. This failure may have to do with the anesthesia of the patients (307). Mayevsky et al. (249) saw the unmistakable signs of recurrent SD in at least one patient suffering of severe head injury whose cortex was monitored with an implanted multiple probe. There is no doubt that hippocampal and cortical tissue slices prepared from human brain fragments removed during neurosurgery do generate both SD and HSD (Fig. 2 and Refs. 4, 17, 175, 376). Nor is SD limited to mammals.

FIG. 2. Spreading depression (SD) and hypoxic SD-like depolarization (HSD) in slices of human hippocampus. A: extracellular potential during SD provoked by a small drop of solution containing high $K^+$ concentration on the slice at some distance from the recording. B: extracellular potential ($V_{ec}$) and potassium concentration during hypoxia. [Recordings by P. G. Aitken, J. Jing, and J. Young; surgically removed human tissue supplied by A. Friedman. See also Aitken et al. (4).]
It has been recorded in bird brain (238) and in the cerebellum and retina of a variety of vertebrates (108, 139, 187, 212, 239, 454).

C. Focally Recorded Sustained Potential Shifts and Extracellular Current Flow

The potential shift recorded through DC-coupled amplification from the exposed cerebral cortex of cats, rats, or rabbits during SD has a maximal amplitude of $-5$ to $-15$ mV (214, 215). The initial surface-negative wave is followed by a smaller but more prolonged positive phase. When recorded by extracellular microelectrodes inserted into the gray matter of the neocortex or hippocampus, the $\Delta V_o$ can be biphasic or triphasic, with the main component again negative relative to a distant ground, and reaching $-15$ to $-30$ mV. The white matter beneath the cortical gray becomes positive, while the cortex itself undergoes the negative wave (215). Ochs (294) inferred that apical dendrites were preferentially involved in the generation of the voltage shift. Current source density analysis in hippocampal formation of anesthetized rats and in organ cultures confirmed that during SD the main current flows inward in layers containing the dendritic trees of pyramidal neurons during the negative phase of the $\Delta V_o$ (Fig. 3) (185, 435). The direction of the current related to SD flows in the opposite direction compared with the current underlying tonic-clonic seizure discharges, which is inward in the neuron soma layers (104, 378, 435).

The onset of the $\Delta V_o$ is usually preceded by increased neuronal excitability (110) or “fast activity” in the ECoG trace (330). In the hippocampus, the prodromal excitation is manifested in a shower of “population spikes,” representing synchronized firing of neurons (136). Rosenblueth and García Ramos (330) emphasized that the $\Delta V_o$ itself has all-or-none character: once it is started, its ultimate magnitude is independent of the triggering stimulus.

Several investigators concluded that SD is a complex phenomenon resulting from the interaction of various processes (42, 78, 187, 199, 298, 330). As seen in Figures 2A, 3, 5A, and 6, the negative $\Delta V_o$ rapidly attains an early peak followed either by a less negative plateau or, after a brief decline or “notch,” a slow, second negative maximum. We (134) have called this upside-down peak-and-hump waveform the “inverted saddle.” It is frequently evident in recordings of both SD and HSD, in retina (78), neocortex (124, 238), cerebellar cortex (281) and most prominently in stratum (st.) radiatum of CA1 region of the hippocampus (134, 136), in brain in situ as well as tissue slices in vitro, and it is accentuated by current source density analysis (Fig. 3) (435). When recordings were made simultaneously from st. pyramidale and st. radiatum of hippocampal CA1 sector, the $\Delta V_o$ invariably started earlier and ended later in the layer of the dendritic trees than among the cell somata. It was the later, slower “hump” at the rear of the “saddle” that was much more pronounced in st. radiatum. During microdialysis of the N-methyl-D-aspartate (NMDA) antagonist drug (±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), the late phase was suppressed near the dialysis source, but the early, sharp peak was unaffected and continued to propagate. As the early peak moved away from the source of CPP, the late, slower component reemerged (Fig. 6) (134). It seemed that the first peak and the following hump or plateau of the $\Delta V_o$ were expressions of two distinct ion currents, and NMDA-controlled channels were responsible only for the second of the two. As we shall see...
in section III, this is not exactly correct; rather, the first peak is probably generated by a brief, intense surge of both NMDA current ($I_{\text{NMDA}}$) and persistent sodium current ($I_{\text{Na}}$) while the later phase is indeed due mainly if not exclusively to the more sustained flow of $I_{\text{NMDA}}$.

D. Ion Fluxes During SD

Independently from one another and at about the same time, Brinley and Kandel (37) and Krivánek and Bures (196) demonstrated the overflow of potassium from the cortical surface during SD. After the invention of ion-selective microelectrodes it became possible to measure ion concentrations in live tissue. Vyskočil et al. (434) reported for the first time the very large increase in $[K^+]_o$ during both SD and HSD.

The unparalleled increase in $[K^+]_o$ (35, 221, 232, 434) is accompanied by a precipitous drop in $[\text{Cl}^-]_o$, $[\text{Na}^+]_o$, and $[\text{Ca}^{2+}]_o$ (78, 124, 128, 187, 281, 283, 373, 448), suggesting that $K^+$ leaving cells is exchanged against $\text{Na}^+$ and $\text{Ca}^{2+}$ that are entering (281, 360). $[\text{Ca}^{2+}]_o$ decreases from its normal level of 1.2–1.5 mM to <0.3 mM. Cations are not exchanged one for one between intra- and extracellular solutions, for the reduction in $[\text{Na}^+]_o$ is greater than the increase in $[K^+]_o$ (267). The concomitant drop in $[\text{Cl}^-]_o$ indicates that some of the $\text{Na}^+$ entering the cells is accompanied by $\text{Cl}^-$. Nicholson (281) suggested that the deficit in extracellular anions is made up by anions leaving the cytosol. Indeed, organic anions, including glutamate, have been shown to be released during SD (71, 81, 395, 397, 420), although some of the glutamate comes from glial cells (170, 171, 394). An exact and complete balance sheet of all ingredients displaced during SD is yet to be completed, however.

The unusual magnitude of the changes in extracellular ion concentrations created the impression that intra- and extracellular ion concentrations equilibrate during SD, and this idea was bolstered by the nearly complete depolarization of neurons during SD (57, 137, 391; see sect. III H). The volume of the cytosol is, however, so much larger than that of the interstitial space that cells need to give up but a fraction of the $K^+$ they contain to achieve a manyfold rise in $[K^+]_o$. Calculations based on the simultaneously recorded levels of $[\text{Na}^+]_o$ and $[K^+]_o$ and the known fractional volume of the interstitial space in hippocampus indicate that a much reduced but still substantial transmembrane $K^+$ concentration gradient remains standing during HSD (267) (Figs. 4A and 7D).

**FIG. 4.** Membrane potential with the simultaneously recorded extracellular ion concentration changes and calculated intracellular ion concentrations of CA1 pyramidal neurons in hippocampal slices. $A$ and $B$ are from two different neurons. $V_i - V_o$, the extracellular voltage shift was subtracted from the intracellular potential change to obtain the true transmembrane potential change; $[K^+]_o$, and $[\text{Na}^+]_o$, extracellular ion concentrations measured with ion-selective microelectrodes; $[\text{Na}^+]_i$, and $[K^+]_i$, intracellular ion concentration changes calculated twice, assuming either that glial cells participate equally with neurons in the ion exchange, or that the ion content of glial cells remains constant. Truth very probably lies between these limits. [From Müller and Somjen (267).]
E. Tissue and Cell pH

During SD, extracellular pH (pHo) first becomes alkaline and then acid (183, 184, 219, 223, 281, 369, 404, 449). During hypoxia or ischemia, strong tissue acidosis begins well before HSD, but the onset of HSD is marked by a brief alkaline transient that interrupts the acid shift (124, 184, 401).

The local acidosis outlasts the ΔV, and is related to the production of excess CO2 and acid metabolites, especially lactic acid (64, 195, 341), by-products of increased metabolic activity required for the restoration of the ion distributions (42). The origins of the alkaline shift are less clear, and several factors may contribute to it. The production of ammonium ions appears to be one factor (183). The more moderate increase of pH induced by electrical stimulation (without SD) has been attributed to the extrusion of HCO3- at GABA receptors-controlled channels (163), plus an uptake of H+ in response to glutamate-induced depolarization. The latter mechanism is calcium dependent, and it is believed to be caused by countertransport exchanging Ca2+ for protons, which compensates depolarization-induced calcium influx (114, 362, 363). This, however, does not completely account for the alkaline shift associated with SD, which does occur even in Ca2+-free medium (255). The extracellular alkaline transient is accompanied by alkalization of glial cytoplasm, while neurons become acid (55, 56). Glial alkalization is in part a function of membrane potential and may be caused by transmembrane proton flux (55, 113) but, again, more than one mechanism seems to be at work (10, 204).

F. Tissue Electrical Resistance and Cell Swelling

Leão (217) was the first to discover the transient increase in tissue electrical impedance accompanying SD, and this was soon confirmed by others (91, 147, 421, 422). The increase was mainly in the tissue resistance (RT), while the reactive components remained essentially unaffected. The most likely explanation of increased RT was swelling of cells at the expense of interstitial space. Cell swelling was confirmed by morphological studies (181, 410, 411, 414, 417, 419, 423).

Tissue resistance would, however, be an exact index of cell volume only if cell membrane resistance was so high that the fraction of the measuring current flowing through cells could be neglected, and if the membrane resistances would not change. Analyzing impedance and phase angle at several frequencies, Ranck (316) concluded that, during SD, interstitial space shrinks, neuronal membrane resistance decreases and, a little later, glial membrane resistance increases (see also Ref. 84). The distinction of glial and neuronal membrane behavior was based on an assumed difference in membrane time constants in excess of 1,000, and a very “leaky” glial membrane (315). More recent measurements show that a sizable fraction of current imposed on the brain tissue does flow through cell membranes (84, 97, 99, 297), and neuronal membrane resistance indeed drops drastically during SD (67, 364 and sect. uH) so that an increased fraction of the current must take the transcellular route.

A more reliable index of changes in interstitial volume fraction (ISVF) is derived from the concentration of indicator substances that do not penetrate cell membranes. For practical reasons indicators are preferred that can be measured with ion-selective microelectrodes. Among them are tetramethyl- and tetraethylammonium ions (TMA+ and TEA+) and certain anions (75, 126, 284, 306). From the increase in the concentration of such indicators, the drastic shrinkage of the interstitial spaces during SD and HSD could be accurately gauged (78, 126, 160, 230, 304). In interpreting the drastic decrease in ISVF it should be remembered that it takes only a moderate cell swelling to compress most of the interstitial space. For example, where the normal ISVF occupies 13% of the tissue volume (251), a 70% decrease in ISVF (160) corresponds to only about a 10.5% expansion of the average intracellular volume.

ISVF shrinks also during moderate neuronal excitation (75), but much less than during SD or HSD (160). Neurons, especially dendrites, swell because NaCl uptake exceeds the discharge of K+ and organic anions (sect. uD), while glial cell swelling is driven by KCl uptake stimulated by the rising [K+]o (170, 173, 262).

G. Intrinsic Optical Signals

SD of activity in a frog retina in vitro was first reported by Gouras (108), who also noticed the visible “milky area” that expanded over the tissue together with the electrical signs of SD. Martins-Ferreira and Oliveira Castro (241, 298) recorded four successive phases of optical change accompanying SD and attributed them to changing light scattering. Snow et al. (364) reported the less-intense SD-related intrinsic optical signals (IOS) in hippocampal tissue slices. Recording IOS with a camera attached to a microscope permits real-time two-dimensional mapping of the spread of SD, whereas electrodes can register the voltages only from a limited number of points. In the retina, the optical signals are maximal in the inner plexiform layer (241), corresponding to the region of maximal ΔV (260). In hippocampal tissue slices, IOS are most marked in the dendritic layers, while cell body layers are relatively inert (6, 11, 266) as expected from electrical recordings (134) and current source density analysis (435).

Light scattering has been used for decades to mea-
sure changes in cell volume in cell suspensions. Cell volume increase is reliably associated with a decrease of light scattering, attributable to the dilution of scattering particles in the cytosol (3, 28, 301, 352). This presents a problem, for even though cells undoubtedly swell during SD, the main optical change associated with SD is an increase, not a decrease, in scattering (6, 11, 189, 192, 241, 364, 452). Kreisman et al. (190) found a potential source of artifact that could explain the paradox. When tissue slices are at a liquid-gas interface and the surface of the slice bulges, the angles of incidence and reflection of light change and so does the recorded signal, independently of scattering within the tissue. This, however, is not the whole explanation.

Recently, we (3, 82, 266; D. Fayuk, P. G. Aitken, G. G. Somjen, and D. A. Turner, unpublished data) compared the IOS of hippocampal tissue slices during SD and during osmotically induced cell volume changes. Two kinds of optical signals are generated in these slices, and neither is caused by the artifact described by Kreisman et al. (190). As expected, mild to moderate hypotonic cell swelling was correlated with decrease in light scattering, and hypertonic shrinkage with its increase. SD and HSD are preceded by a brief decrease of scattering, but when the SD-related $\Delta V_o$ begins, the IOS abruptly reverses polarity. The intense increase of scattering returns to baseline more slowly than does $V_o$. The IOS changes were qualitatively similar in interfaced and in submerged slices, and therefore could not be due to the change in curvature of the surface (“lensing”) of the tissue slice. The reversal from scattering decrease to scattering increase at the onset of $\Delta V_o$ during SD was recently confirmed by Tao (398), who used optical fibers in contact with the tissue to exclude surface artifacts.

When Cl$^-$ in the bathing solution is replaced by an anion that does not penetrate cell membranes, the scattering increase is abolished (242), and in its place the scattering decrease continues during and after the $\Delta V_o$ (266). The cell swelling, measured as the shrinkage of the TMA$^+$ space, was, however, not diminished by deleting Cl$^-$ (264, 266). In the absence of NaCl, swelling was probably due to the influx of NaHCO$_3$ (see sect. $iii$). With Cl$^-$ deficiency, the swelling-related scattering decrease was unmasked, while in the presence of Cl$^-$ the SD-induced scattering increase obscured it. The source of the Cl-dependent scattering increase is not known, but it could be related to swelling of mitochondria and other organelles. Bahar et al. (18) found that during SD mitochondria are powerfully depolarized, but lowering of [Cl$^-$]o suppressed the SD-related mitochondrial depolarization while it also abolished the increased scattering.

Accepting that there is another process besides cell shrinkage that can increase scattering (3, 266), it is possible to understand the sequence of IOS seen during SD in isolated retinas, defined as phases a-d by the Brazilian school (239, 241, 298, 415). Phase a is a brief, weak decrease of light scattering, followed by a sharp, large increase (phase b), then a decrease slightly below baseline (phase c), and finally another large and prolonged increase (phase d). It is the sharp scattering increase during phase b that coincides with the negative $\Delta V_o$ (239), similarly to hippocampal slices. $R_T$ is high throughout phases a, b and c, signaling cell swelling, while during phase d $R_T$ is well below baseline. It follows that phases a and c are caused by cell swelling, while phase d is caused by cell shrinkage or “undershoot” of the cell volume as it recovers from the preceding swelling. Phase b represents the superimposed SD-induced (mitochondrial?) scattering increase that is independent of cell volume.

Andrew and associates (12, 14, 287) identified another possible source for the light scattering increase caused by hypoxia combined with low glucose, or by excitotoxicity. They attribute the increased scattering to the beading of dendrites, which is a sign of irreversible injury (148). Unlike dendritic beading, the scattering increase associated with uncomplicated SD or HSD is completely reversible, and it does not lead to loss of neuronal function, provided that oxygenation is restored in time (3, 266).

To sum up, four independent sources have been suggested for the IOS of brain slices, and these are not mutually exclusive. Cell swelling is associated with a light scattering decrease. SD and (reversible) HSD are associated with a Cl$^-$-dependent scattering increase that may be due to swelling of intracellular organelles. Strong swelling of tissue slices at liquid-gas interfaces can alter reflected light when the radius of curvature of the slice surface changes. Finally, (irreversible) beading of dendritic processes can increase light scattering.

H. Membrane Potential and Input Resistance of Neurons During SD and HSD

Brožek (38) sampled membrane potentials by advancing a microelectrode through cortex and registering the voltage deflections as the electrode tip penetrated cells before, during, and after the passage of a wave of SD. Average membrane voltages were less negative during SD than before it, suggesting depolarization, and more negative thereafter, indicating transient hyperpolarization following SD. Collewijn and Van Harreveld (57) were the first to record the intracellular potential ($V_i$) of a neuron long enough to follow its course through SD. They recognized that the intracellular electrode records the sum of intra- and extracellular voltage shifts and, in the case of SD, $\Delta V_o$ is too large to be ignored. After correcting $\Delta V_i$ for $\Delta V_o$ they concluded that during SD the membrane potential of neurons can briefly approach zero. Their findings
were repeatedly confirmed (67, 137, 267, 364, 373, 403) (Fig. 4), but some investigators neglected to correct for \( \Delta V_o \) and therefore underestimated the depolarization (e.g., Refs. 105, 397). It will be noticed that, unlike \( \Delta V_o \), in most cases neither the course of membrane potential \( (V_m) \) nor that of \([K^+]_o \) have a saddle shape with two maxima; rather, there is typically an initial peak followed by a lower, prolonged plateau, or else a slowly declining late phase (Figs. 2B and 4 as well as \( I_h \) in Fig. 5A). If, however, \( \Delta V_i \) is not corrected for \( \Delta V_o \), then \( \Delta V_i \) can show an artifactual “drift” in a positive direction (267).

Neuronal input resistance \( (R_{in}) \) was measured during SD and HSD by a number of teams using “sharp” intracellular electrodes (259, 265, 267, 364) or using patch-clamp electrodes in whole cell configuration (67). Snow et al. (364) reported the collapse of \( R_{in} \) to a degree where it was too small to measure. Based on current-voltage \( (I-V) \) plots obtained by depolarizing voltage ramps in whole cell recordings, Czéh et al. (67) measured average \( R_{in} \) during SD to be 34% of its control value and 21% during HSD when using Cs-gluconate pipettes, and 52% with K-gluconate pipettes (Fig. 5B). The effect varied widely, with \( R_{in} \) dropping below 10% in some cells, while others seemed not to participate in the SD of their neighbors. With sharp electrodes filled with K-acetate solution, Müller and Somjen (265) found \( R_{in} \) reduced to 11.7 ± 6.3% during HSD in similar hippocampal CA1 pyramidal neurons. The averages differed, but the ranges overlapped in the two sets of data, and neither method indicated complete “breakdown” or ionic transparency of the membrane.

III. MECHANISMS OF SPREADING DEPRESSION AND HYPOXIC SPREADING DEPRESSION-LIKE DEPOLARIZATION

A. Neurons Are Not Short of Oxygen During SD, Only During HSD

Van Harreveld’s asphyxial hypothesis was the first proposed explanation of SD (426) which, however, was quickly discarded. It ascribed SD to ischemia resulting from a...
spreading wave of vasoconstriction. The vascular responses associated with SD are, however, complex. Just before the $\Delta V_o$ vessels may constrict, but this is not always observed. The depolarization itself is associated with marked vasodilatation, which is followed by prolonged but moderate hypoperfusion (59, 102, 124, 127, 207, 210, 211, 254, 257, 308, 444, 445). Local blood flow is so abundant that hemoglobin oxygenation increases in spite of increased metabolic demand (445) and extracellular tissue oxygen tension (PO$_2$) tends to increase, especially at the onset of SD, although it may decrease later (219, 443). Most importantly, mitochondrial oxidative enzymes become oxidized during SD, in contrast to hypoxia and ischemia when mitochondrial enzymes become reduced already before the onset of HSD, and maximally during HSD (161, 191, 228, 247, 248, 248, 250, 313, 321, 331, 389).

**B. Grafstein’s Potassium Hypothesis**

The second and still most influential proposal was Grafstein’s potassium hypothesis (110). According to Grafstein (110), K$^+$ released during intense neuron firing accumulates in the restricted interstitial spaces of brain tissue, and the excess [K$^+$]$_o$ further depolarizes the very cells that released it, resulting in a vicious cycle that leads to inactivation of neuronal excitability. In the meantime, some of the accumulated K$^+$ diffuses through the interstitial spaces to neighboring cells, which then also depolarize, fire, and go through the same cycle, thus producing the slowly propagating wave of SD. At Grafstein’s request, Hodgkin derived a mathematical expression for this process (111).

The core of Grafstein’s idea survives today. There is little doubt that the rise of [K$^+$]$_o$ is a link in the chain of events causing SD. There were, however, problems with the details of the theory, as originally formulated. To the surprise of most, tetrodotoxin (TTX) did not prevent SD, even though it suppressed action potential firing (94, 181, 299, 400). Today we know, of course, that K$^+$ can be released from cells without the firing of action potentials. Yet another problem is that, at a given point in the tissue, [K$^+$]$_o$ does not start to increase ahead of the $\Delta V_o$, as it should, if K$^+$ were the agent of the propagation of SD (134, 219). As we shall see in section 1.5.1, the increase in [K$^+$]$_o$ appears to be a key to the ignition and the evolution of the SD process (162), but not necessarily to its propagation (see sect. 1.5.2). In contrast, during hypoxia there always is a slow, gradual increase in [K$^+$]$_o$ well before the start of the $\Delta V_o$ (122, 123), which may well be important in the spread of HSD (6).

**C. Van Harreveld’s Glutamate and Dual Hypotheses**

The third major proposal was van Harreveld’s glutamate hypothesis (412, 418, 420). It was van Harreveld (412) who first proposed that glutamate may be a physiologically important excitatory compound, based on three observations: 1) it was present in extracts of normal brain, 2) it caused the contraction of crustacean muscle, and 3) it induced SD when applied to the cortical surface. Circumstantial evidence seemed to favor glutamate over potassium as the agent of SD. Neither the release of glutamate nor its excitatory action was antagonized by TTX. Of glutamate, it has long been known that it causes the uptake of NaCl and water into cells (9). Finally, glutamate is released during SD (81, 155, 338, 418, 420). Opinions doubting the role of glutamate were and are, however, voiced as well (65, 77, 290, 293).

The arguments in favor of glutamate can be extended to other excitatory transmitters (283, 325, 366). Indeed, there have been reports implicating acetylcholine, at least in the retina (325, 326) but not in neocortex (218). Transmitters and high [K$^+$]$_o$ may both play a role. Van Harreveld himself had modified his views, allowing for two types of SD, one mediated by K$^+$, the other by glutamate (413). There is much evidence in favor of this dual hypothesis (162).

**D. In SD, Neurons Lead and Glial Cells Follow**

In the normal central nervous system, the resting intracellular potential recorded by sharp micropipette electrodes from glial cells (usually astrocytes) is, on average, more negative and more stable than that of neurons, whereas their input resistance ($R_{in}$) is lower. Low glial membrane resistance is mainly due to high “resting” conductance for K$^+$ while input resistance is further lowered by the electrical coupling between cells by gap junctions. Repeated electrical stimulation or seizure discharges cause [K$^+$]$_o$ to rise, and this depolarizes glial cells (reviewed in Ref. 367). In the spinal gray matter and in the neocortex K$^+$-induced glial depolarization contributes a large part of the extracellular sustained potential shifts that accompany prolonged neuron excitation (366). The prominent $\Delta V_o$ that is typical of SD has also been assumed to be generated in large part by glia, and this was one of the reasons for suggesting for a leading role of glial cells in the generation of SD (224, 237). In the hippocampal formation, the glial contribution to $\Delta V_o$ is, however, minor compared with the neuronal fraction (reviewed in Refs. 365, 371).

The membrane potential of “idle cells,” later proven to be neuroglia, was recorded during SD for the first time by Karahashi and Goldring (165), followed by Higashida et al. (138). As expected, the depolarization of glial cells more or less mirrored the $\Delta V_o$ of the cortical surface. Later Higashida et al. (137) and Sugaya et al. (391) compared neuronal and glial recordings and came to contrasting conclusions. Higashida et al. (137) found that neurons
were more strongly depolarized than glial cells. According to Mori et al. (260–262), Müller (glial) cells in retina take up K\(^+\) during SD; therefore, they cannot be the source of the rise of [K\(^+\)]\(o\) and their membrane behaves as a potassium electrode. In contrast, Sugaya et al. (391) reported that depolarization started earlier and was more profound in cortical glial cells than in neurons. They also found that not all neurons depolarized during SD, while the response of glial cells was uniform. These observations and the lack of effect of TTX led them to believe that glial cells produce SD and neurons merely follow their lead. Our recordings from a limited number of glial cells show responses that were milder than those of neurons (66, 267, 376). As in the retina (261), the membrane potential of hippocampal glial cells decreased as expected for a “passive” K\(^+\)-permeable membrane with the rise of [K\(^+\)]\(o\) and \(R_{in}\) decreased only slightly. These data agree with those of Higashida et al. (137). Yet, similarly to Sugaya et al. (391), we (67) also found a few neurons that refused to participate in the SD, even though the simultaneously recorded \(\Delta V_o\) signaled that SD did occur in the remainder of the population.

Interest in the role of neuroglia in SD was rekindled with the discovery of the waves of elevated intracellular calcium activity in glial cell cultures (62, 88, 103). When a local stimulus, for example glutamate or NMDA, raises intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(i\)) in a cluster of glial cells, other cells that are linked through gap junctions follow suit, and the wave of [Ca\(^{2+}\)]\(i\) increase is spreading at a slow velocity reminiscent of the propagation of SD (62, 89, 103, 269, 270). Similarly spreading calcium waves have also been recorded in hippocampal slices (69, 70), retina (83), and organ cultures (198). Nedergaard (240) has proposed a primary role to the calcium waves in the generation of SD. Basarsky et al. (29) have shown, however, that SD can occur in the absence of the intracellular calcium waves, when calcium is deleted from the bathing medium. It follows that Ca\(^{2+}\) influx, whether into glial cells or neurons, is not required for SD generation or propagation (see also sect. iiiF).

There are other reasons to doubt a leading role for glial cells in generating SD or HSD. The metabolic poisons fluoroacetate and fluorocitrate incapacitate glial cells hours before they affect neurons (201). Yet these toxins do not prevent SD, but rather facilitate its onset (202, 203). This supports the idea that, instead of instigating SD, glial cells inhibit it, as suggested already by Mori et al. (262) and Gardner-Medwin (96).

Glial protection against SD is achieved in part by stabilization of extracellular ion levels, especially [K\(^+\)]\(o\) (reviewed in Refs. 27, 280, 368). Computer simulation makes this contention plausible (see sect. iiiJ). Ion regulation is a joint function of neuroglia and the capillary endothelium which forms the blood-brain barrier (36, 47, 280). Additionally, astrocytes prevent overflow of transmitters into interstitial fluid (344).

In summary, glial cells do play a passive role in the total SD response (437). They depolarize, because their membrane potential is determined by the rise of [K\(^+\)]\(o\) and they swell because they take up KCl. The timing of glial depolarization follows \(\Delta V_o\) closely because both are determined by the aggregate behavior of the neuron population. In contrast, the onset of the depolarization can vary widely among individual neurons because it is determined mainly by the activation of specific membrane conductances. As we shall see in section iiiJ, the SD process is ignited when neuron dendritic persistent inward currents begin to exceed persistent outward currents, and for some neurons this moment may precede while in others it may lag behind the group average. Despite individual variability, it is neurons that initiate the SD process.

E. Role of Sodium Channels and of Glutamate-Controlled Channels

As already mentioned in section iiiA, TTX in amounts sufficient to abolish action potentials postpones or reduces but does not prevent SD (94, 181, 260, 353, 391, 400). Inhibition by TTX is stronger against HSD than against SD (5, 267, 447), and in a minority of identically treated slices, TTX actually prevented HSD (5). Other drugs that act on voltage-gated Na\(^+\) channels, such as diphenylhydantoin (phenytoin) and local anesthetics, slow the propagation of SD in retina, raise its threshold, and sometimes block it completely (50, 51, 181).

The rapid, large decline of [Na\(^+\)]\(o\) (128, 187) leaves little doubt that there is an intense inward surge of this ion during SD. The question is whether the influx of Na\(^+\) is required for the generation of SD. In the isolated retina SD is slowed in a concentration-dependent manner, and eventually stopped entirely, if Na\(^+\) is substituted by choline or TMA\(^+\) (216, 243). Remarkably, the substitution of Tris\(^+\) for Na\(^+\) had no effect on the circling SD in this preparation (236). In isolated hippocampus, substituting Na\(^+\) by N-methyl-D-glucamine (NMDG\(^+\)) suppressed the \(\Delta V_o\) of HSD (268). It follows that, ordinarily, the depolarization is indeed mediated mainly if not exclusively by Na\(^+\) influx, and Ca\(^{2+}\) in the amounts it is normally present in extracellular fluid cannot take its place (see also sect. iiiF).

One must ask, What pathway do Na\(^+\) take when voltage-gated Na\(^+\) channels are blocked by TTX? A clue is provided by the fact that in both SD and HSD the depolarization approaches zero voltage without ever moving into the positive range (57, 267), and the SD-related whole cell current reverses at a slightly negative level (67) (see sect. iiH). This points to a mixed ion conductance rather
than one exclusively selective for Na\(^+\). A nonselective conductance could also explain the intense outflow of K\(^+\). In theory, such a mixed flux of ions could occur through perforations that are not normally present, or at least are not normally open. Alternatively, the mixed conductance could be provided by the opening of transmitter-controlled channels. Like the SD-related current, glutamate-controlled current reverses near zero membrane potential (200). Finally, it could be the result of the simultaneous activation of inward and outward currents.

The glutamate hypothesis could be tested, once selective agonists and antagonists of glutamate receptors became available. Agonists of all three major ionotropic glutamate receptors, quisqualate, kainate, and NMDA, were effective in inducing SD (212). Antagonists of NMDA receptors inhibited SD (17, 107, 132, 188, 197, 233–235, 259), but the same agents were ineffective against HSD (1, 132, 209, 234, 409, 448). Antagonists of quisqualate and kainate receptors were without effect on either SD or HSD (17, 134, 208, 209). To reconcile the seeming discrepancy between the universal effectiveness of glutamate agonists versus the selectivity of NMDA antagonists, it was suggested that quisqualate and kainate provoke SD indirectly, by stimulating glutamate release, and the released glutamate then activates NMDA receptors (338, 353).

The observations just quoted suggested that activation of NMDA receptors is required for the generation of SD but not of HSD. There are, however, problems with this proposition. The amount of aspartate and glutamate spilled into interstitial space during normoxic SD is quite small compared with the huge amounts released during HSD (32, 33, 81, 338). Moreover, not all trials with NMDA antagonists were equally successful. A dose of an antagonist that successfully blocked the propagation of SD did not necessarily suppress SD at the site of stimulation (235). Also, the selectivity of higher doses of the dissociative anesthetics, such as ketamine, kynurenate, or MK-801, is suspect (58, 346). For example, the dose of kynurenate that blocked glutamate-evoked SD failed to prevent SD provoked by high K\(^+\), except when the dose was raised to very high levels (212). Lauritzen et al. (208) pointed out that this difference between glutamate-evoked and K\(^+\)-evoked SD supports van Harreveld’s (413) advocacy of two kinds of SD, only one of which is dependent on glutamate. In urethane-anesthetized rats, the highly selective competitive NMDA antagonist CPP blocked only the late component of the SD-related \(\Delta V_o\) and it did not prevent the propagation of the SD wave (Fig. 6 and Ref. 134). And, unlike the complete failure of other anti-NMDA drugs in suppressing HSD (132, 233), in hippocampal slices both CPP and the non-NMDA glutamate antagonist 6,7-dinitroquinoxaline-2,3-(1H,4H)-dione (DNQX) did postpone the onset of HSD and reduced the amplitude of the \(\Delta V_o\) (159, 268, 448).

These observations force the following conclusions. Neither neuron firing nor synaptic transmission is required for SD generation, nor is the activation of NMDA receptors an absolute requirement for the generation of SD, and even less for HSD. Nonetheless, glutamate and aspartate, as well as some TTX-sensitive Na\(^+\) channels, do play a role (see sect. III).
F. Role of Calcium Channels

$[\text{Ca}^{2+}]_o$ sinks to very low levels during both SD and HSD, and the time course of its decline more or less mirrors the rise of $[\text{K}^-]_o$ and parallels the decline of $[\text{Na}^+]_o$ (78, 124, 128, 134, 159, 187, 281), raising the question whether $\text{Ca}^{2+}$ current contributes to the depolarization. Blocking voltage-gated $\text{Ca}^{2+}$ channels by adding Ni$^{2+}$ or Co$^{2+}$ to the bathing fluid substantially reduces the amplitudes of $\Delta V_o$, $[\text{K}^-]_o$ increase, and $[\text{Ca}^{2+}]_o$ decrease, and it prevents the propagation, but not the initiation, of normoxic SD (159). These divalent cations have, however, actions besides blocking $\text{Ca}^{2+}$ channels (15, 140). More importantly, removing calcium from the extracellular fluid does not prevent SD or HSD, and it may even favor its onset (24, 29, 93, 255, 453). In contrast, substituting Na$^+$ by a membrane-impermeant cation does suppress SD as well as HSD (243, 268), demonstrating that the $\text{Ca}^{2+}$ present in the extracellular medium are not capable of supporting SD. This does not mean that the flow of $\text{Ca}^{2+}$ into cells during SD does not have important consequences, only that Na$^+$ carry the bulk of the charge necessary for the depolarization.

While $[\text{Ca}^{2+}]_o$ drops by $\sim$1 mM during HSD (159), at 37°C $[\text{Ca}^{2+}]_i$ increases by $<0.2$ μM (438). Simple arithmetic indicates that, even taking account of the different volume fractions of interstitium and cytosol, much of the $\text{Ca}^{2+}$ that enters must be buffered and/or sequestered. Yet even if $\text{Ca}^{2+}$ is removed from the extracellular medium, $[\text{Ca}^{2+}]_i$ rises to about the same extent, indicating release from intracellular stores (456). This seeming paradox suggests that, when the cytosol is flooded by influx of huge amounts of $\text{Ca}^{2+}$, buffers take up most of it, but in the absence of external supply, under the influence of HSD, some stores release their content into the cytosol. Increased mitochondrial permeability could cause such release (18).

G. Behavior of Chloride

Together with Na$^+$, Cl$^-$ also disappears from interstitial fluid during SD, although not in 1:1 proportion. Phillips and Nicholson (306) compared the movements of a series of anions of varying ion radius during SD and came to the conclusion that the limit for the size of the channel or "pore" that admits anions during SD lies between 6 and 11.2 Å (see also Ref. 240). Until recently, it seemed that cell swelling was dependent on Cl$^-$ influx (419, 424). Müller (264) has now examined the effects of the chloride transport inhibitors furosemide, DIDS, and DNDS on HSD and found only minor changes in the magnitude of the $\Delta V_o$ and in the onset time of the depolarization. More surprisingly, substituting methyl-sulfate or gluconate for Cl$^-$ in the bath did not prevent cell swelling during HSD (measured as the shrinkage of TMA$^+$ space) (264, 266). As mentioned in section nG, removal of extracellular chloride suppressed the HSD-related light-scattering increase (242) and unmasked the decrease in light scattering that is caused by cell swelling (266). Normally, with Cl$^-$ abundant in extracellular fluid, it almost certainly is the main anion entering cells during cell swelling (423, 424). Which anions accompany Na$^+$ when Cl$^-$ is absent is less clear. Bicarbonate is the likely candidate because it is the second most abundant anion in extracellular fluid, and its molecular size is smaller than the limit estimated by Phillips and Nicholson for the SD-induced anion flux (240, 264, 306).

H. Role of Potassium Channels

Last but by no means least, we must ask what is the role of voltage-gated K$^+$ channels. In the isolated retina, the broad-spectrum K$^+$ channel blocker TEA$^+$ slowed the propagation of SD (93, 243, 342). We (5) tested the effect on HSD of TEA as well as 4-aminopyridine (4-AP), which inhibits the inward rectifier and A-type channels only (141). Both TEA and 4-AP shortened the delay from oxygen withdrawal to the onset of HSD, probably because blocking K$^+$ channels enhances the excitability of neurons. However, even though HSD started earlier, the amplitude of the $\Delta V_o$ and of the increase of $[\text{K}^-]_o$ were consistently and substantially depressed by TEA but not by 4-AP. We concluded that some but not all of the K$^+$ leaving cells flows through TEA-sensitive channels (5). ATP-sensitive K$^+$ channels probably carry some of the K$^+$ released during HSD (448).

I. Not One “SD Channel,” But the Cooperation of Several Generates the Depolarization

The trials with channel blocking drugs were inspired by a search for a specific ion current that could explain the precipitous decrease of membrane resistance and depolarization of neurons. Diverse selective antagonists partially depressed or delayed SD or HSD, but none completely prevented them. One might conjecture, therefore, that during SD pathological pathways open which normally are absent or dormant. We have rejected this conclusion after finding that simultaneously blocking all known major inward currents with a cocktail of CPP, DNXQ, TTX, and Ni$^{2+}$ reliably prevented HSD (265). Administered separately, each ingredient in this cocktail delays the onset of SD or HSD, but even three of the four combined could not reliably prevent it (159, 268); it takes all four inhibitors to achieve consistent protection. It seems that, normally, several ion channels cooperate in generating HSD or SD but, if some are incapacitated, one of the channels alone is sufficient to mediate a slowed
version of the process, albeit not always in every member of the neurons in the population. Once SD has been initiated, the membrane potential will in the end reach the usual depolarized level. It is important to remember that the extracellular voltage shift $\Delta V_o$ can be depressed if fewer than the usual number of neurons participate in the SD, even though those that do depolarize fully.

The voltage to which the membrane is moved during SD is not determined by the number of channels available, but by the feedback that governs the process (268).

J. Solving the Puzzle by Computer Simulation

Several mathematical models of SD have been published (41, 111, 282, 318, 320, 351, 407, 408). These computations were more relevant to the propagation of SD than with its initiation. SD propagation is the topic of section 11L.

We (162, 381; and unpublished observations) used the simulation environment devised by Hines, Moore, and Carnevale (142) to test whether a neuron model incorporating realistic physiological parameters could generate SD-like depolarization. The geometry and the resting electrical properties of the model were based either on a hippocampal pyramidal cell published in the Duke-Southampton Archive of Neuronal Morphology (48, 311) or on a simpler schematic design. In either case the “cell” had a small soma with dendrites attached. The surface membrane was surrounded by restricted interstitial space, resting concentrations were set for ions both inside and outside, and changes in ion concentration caused by membrane currents were continuously calculated. The original model contained only Na$^+$ and K$^+$ but in the more recent version Cl$^-$ as well as impermeant anions were also computed, and electroneutrality in the solutions was respected. The ISVF was either fixed at 15% of the neuron intracellular volume or it was made an inverse function of osmotic cell swelling. At rest the membrane potential was controlled by Na$^+$, K$^+$ “leak” conductances, with Cl$^-$ added in the new version. Voltage-gated Hodgkin-Huxley-type rapidly inactivating Na$^+$ currents ($I_{Na,T}$) (141, 144) were present in the soma; slowly inactivating $I_{Na,P}$ (63) were present in soma as well as in dendrites. Rapidly inactivating potassium “A” currents ($I_{K,A}$) and delayed rectifier currents ($I_{K,DR}$) that do not inactivate were inserted in soma and dendritic tree (141). In addition, dendrites equipped with currents controlled by NMDA receptors ($I_{NMDA}$). In the newer, more complete version, the very tip of the apical dendrites and the basal dendrites were passive, endowed only with leak conductances. $I_{NMDA}$ depended on both [K+]o and on membrane potential because elevated [K+]o causes the release of glutamate and also enhances NMDA-controlled currents by direct action, and the Mg$^{2+}$ block of NMDA-controlled channels is voltage dependent (92, 141, 173, 309, 332, 394, 395). Changes in ion concentrations were restored by a “Skou-type” electronegatic Na$^+$-K$^+$ exchange pump transporting 3 Na$^+$ out against 2 K$^+$ into the cell (206). In addition, [K+]o was “buffered” by a “glia-endothelial” uptake function. In the original model (162), glial uptake was represented by a buffer equation, in the newer version the glia-endothelial system operated through leak conductances for K$^+$, Na$^+$, and Cl$^-$, and glial $V_m$ and glial ion concentrations were continuously computed. The cell could be stimulated by depolarizing current injected into the “soma” compartment.

When the Na$^+$-K$^+$ pump and the glia-endothelial uptake were operating optimally, injected depolarizing currents evoked the steady, repetitive firing of lifelike action potentials, which ceased promptly when the stimulus stopped as it does in neurons in healthy brains. If either the ion pump or the glial buffer were weakened, pathological behavior ensued. The mildest pathology consisted of “afterdischarge” when the slow clearing of excess [K+]o kept the soma membrane depolarized after cessation of the stimulus current. In more severe cases, the model generated recurrent bursts of action potentials resembling “clonic seizures.” And, finally, the “cell” went into long-lasting depolarization that resembled SD of live neurons (Figs. 7 and 8). When the pump and the glial buffer functions were readjusted to optimal level, the same stimulus that had triggered SD evoked only regular firing limited in duration by the stimulating current.

Activation of either $I_{Na,P}$ or $I_{NMDA}$ alone could produce SD-like depolarization, but when both were operating, SD threshold was lower, its latency shorter, and its duration longer. As in real life (134), simulated SD-like depolarization began in the dendritic tree, whence it was conducted into the soma. Conduction along dendritic processes is faster than propagation in the neuron population, in simulated as well as in real SD.

SD was ignited when persistent (slowly inactivating) inward currents in the dendritic membrane exceeded outward currents so that the total membrane current turned inward (in formal terms: $I_{Na,P} + I_{NMDA} > I_{K,DR} + I_{Na/Pump}$ (Fig. 8B). Key to reaching this ignition point of SD was the positive feedback between rising [K+]o and the resulting depolarization that in turn activated membrane conductances which then released even more K$^+$ (Fig. 9). In our model we imitated $I_{Na,P}$ and $I_{NMDA}$, but they could be replaced by any current if it 1) flows inward, 2) inactivates or desensitizes slowly or not at all, 3) is activated by depolarization or elevated [K+]o or both, 4) by depolarization it forces the (secondary) release of K$^+$ into a restricted extracellular space, and 5) the removal of K$^+$ from the interstitium does not keep pace with its release. The ignition point of SD is not a fixed threshold in one variable. Ignition is reached by confluence of several...
processes, reminiscent of the Reynolds number which defines the transition from laminar to turbulent flow.

The ignition point of the simulated SD could be raised or lowered by manipulating the parameters that govern the relevant currents, but the leak conductances of the glia-endothelial ion buffer had an especially powerful effect. High leak conductance of the glial membrane effectively prevented SD, because glial uptake of K\(^+\) restrained the rise of \([K^+]_o\).

The aggregate (net) dendritic membrane current had two inward maxima in succession, producing a saddle (or peak-and-hump) shape. Contrary to expectation, this was
not the result of the successive activation of two distinct membrane conductances, but of the complicated interplay of conductances and ion-driving forces. An initial sharp surge of inward current arose when persistent Na\(^+\) conductance \(g_{Na,P}\) and NMDA conductance \(g_{NMDA}\) were activated in rapid succession due to the rise of \([K^+]_o\) and depolarization. Then both currents were sharply re-
duced by the decrease in driving force as \([Na^+]_o\) decreased and \([Na^+]_i\) increased, even though their conductances remained high. Yet depolarization was maintained mainly because of the sustained elevation of \([K^+]_o\). The delayed hump of the saddle arose when outward K\(^+\) current was reduced and inward Na\(^+\) current enhanced as the Na\(^+\)-K\(^+\) pump began to restore ion gradients. The process was terminated when the ion pump repolarized the membrane sufficiently to deactivate all voltage-controlled ion conductances. As in real neurons, the course of \(V_m\) did not show the double maxima that were typical of the total membrane current.

In live brains, several factors can facilitate SD ignition. \(I_{Na,P}\) is augmented by hypoxia (119, 120) and by elevated \([K^+]_o\) (382). Glutamate is released not only by the K\(^+\)-induced depolarization of presynaptic terminals (283, 366) and of glia (34, 332, 394), but also by the swelling of glial cells (30, 170, 171, 173). Cell swelling restricts the volume into which K\(^+\) is released and so it amplifies the rise of \([K^+]_o\). Because acidosis inhibits and alkalosis favors SD generation while the SD itself alters pH, there is yet another possibility for strengthening the feedback. The initial alkalinization produced by the SD process is likely to promote its own regenerative evolution, while the subsequent acidification shortens its trajectory (404, 405).

K. Critique of the Model: Neglected Ions and Missing Channels

Propagation in the tissue could, of course, not be tested in a model consisting of a single neuron. Others have simulated the spread of SD among cells, and these will be the topic of section III. Our model was deficient in other ways as well, for example, the absence of calcium and Ca\(^{2+}\)-dependent K\(^+\) conductances, or of H\(^+\), secondary messengers, as well as metabolism and other biochemical functions. The purpose of the exercise was to define the minimal biophysical machinery capable of generating SD-like depolarization.

We chose to represent \(I_{Na,P}\) and \(I_{NMDA}\) because when either of these two currents is blocked, SD and HSD onset are slowed and ion fluxes appear reduced. From the literature reviewed earlier, it may be fair to conclude that \(I_{Na,P}\) dominates HSD while \(I_{NMDA}\) is the leader in SD, without either of the two having an exclusive role. Because the combination of TTX, CPP, and DNQX powerfully delayed and sometimes but not always prevented HSD, it seems that in the absence of both \(I_{Na,P}\) and \(I_{NMDA}\) there are other, as yet to be revealed inward currents that can produce a feeble SD (268). Among possible candidates are TTX-resistant Na\(^+\) current (146) or nonspecific ion currents (60, 90, 145). Channels activated by cell swelling or membrane stretch have been considered.
(375), but to date there is no evidence of their presence in central neurons (2, 379), only in glial cells (30, 172). Low extracellular osmolarity as well as low [NaCl]o do enhance synaptic transmission (152) and voltage-dependent Ca2+ currents while they depress K+ current (372), and these effects, which could facilitate SD, have not been incorporated in the model.

L. Mechanisms of the Spread of SD

SD spreads in contiguous gray matter as if it were a wave, without recourse to synaptic transmission, at a (more or less) uniform velocity of a few millimeters per minute (6, 11, 109, 216, 218, 294). The wave stops where white matter begins and at the edge of glial-fibrous scars left by previous injury or infarction (153, 431). Cytoarchitecture does matter; some areas are preferentially invaded (6, 11, 218, 279, 294). Cuts that interrupt some but not all the layers of neocortex do not stop the spread (109). In neocortex as well as in hippocampus, the leading edge of the wave is in the layers containing apical dendrites (136, 294). Intense excitation conveyed by way of fiber tracts can elicit SD at distant sites (180, 218, 432).

The potassium hypothesis (110) and the glutamate...
hypothesis (412) had this feature in common: both relied on the release of a substance normally stored in brain cells to explain both the initiation and the propagation of SD. Humoral mediation of SD propagation is supported by two observations. Inspired by the classical experiment of Otto Loewi (227), Martins-Ferreira et al. (244) demonstrated that the fluid in which retinas had been bathed while they were undergoing SD could induce SD in another, otherwise untreated retina. Moreover, Obrenovitch and Zilkha (292) reported that intracerebral microdialysis with a drug-free physiological solution inhibits the propagation of SD through the dialyzed area, presumably because the substance mediating propagation is diluted. The humoral agent mediating SD was not identified in these experiments, and it could have been glutamate or K\textsuperscript{+}, or any other excitant compound, singly or in combination.

Several computational models treated SD propagation as a diffusion-reaction process (41, 72, 111, 282, 407, 408). Key to these treatments was the calculation of the diffusion of K\textsuperscript{+} (or of an unspecified humoral agent) in the interstitial spaces. The release of K\textsuperscript{+} into the interstitium was assumed to depend on its accumulation, resulting in positive feedback, without specifying in detail the membrane mechanism underlying the release. Reasonable velocities of propagation have been computed in this way, even if the wave forms derived from the equations were not always lifelike.

There are, however, flaws with both the K\textsuperscript{+} and the glutamate hypothesis of SD propagation. While [K\textsuperscript{+}]\textsubscript{o} begins to increase during hypoxia much before the onset of HSD (126, 267), no such prodromal rise is observed ahead of an advancing wave of normoxic SD (134, 219) [also compare Figs. 1 and 2 in Hansen and Lauritzen (124)]. Extracellular glutamate concentration increases to many times higher level during HSD than during SD (81), yet SD and HSD propagate at about the same velocity (6). There was no correlation between glutamate release and SD in retina (77, 95). Neither dialyzed glutamate nor the inhibition of glutamate uptake facilitates the initiation or the propagation of SD (290, 293). Scheller et al. (338) detected glutamate at the site of SD initiation, but not at some distance in the path in which SD was spreading. They (338) attributed this failure to the insensitivity of the assay.

Dissatisfaction with extant proposals prompted the search for alternatives. Obrenovitch and collaborators (290, 291, 293) confronted the apparent paradox of the failure of glutamate to facilitate SD even though the activation of NMDA receptors is necessary for SD. To resolve the contradiction they proposed that 1) K\textsuperscript{+} are the agents of SD propagation, but 2) high [K\textsuperscript{+}]\textsubscript{o} achieves its effect by depolarizing NMDA receptors and thus relieving the Mg\textsuperscript{2+} block of the NMDA receptor, without need for the excessive spilling of glutamate.

An alternative theoretical solution proposes that the agent mediating SD spreads by way of intercellular junctions instead of diffusion through interstitial spaces. Reid et al. (319) raised this possibility while commenting on the ambiguous role of neuroglia. As a “potassium sponge,” glia guards against the eruption of SD (96, 262) (see sect. m, D and J), but once SD erupted, glial tissue could advance its spread by broadcasting K\textsuperscript{+} through intercellular gap junctions. Observing that the rise of [K\textsuperscript{+}]\textsubscript{o} coincides with \(\Delta V\), but precedes by several seconds the decrease in [Na\textsuperscript{+}]\textsubscript{o} and [Ca\textsuperscript{2+}]\textsubscript{o}, Lehmenkühler (219) agreed that K\textsuperscript{+} are being propelled by way of the quasi-syncytial network of glial cells. This, then, is a variant of Grafstein’s (110) hypothesis: K\textsuperscript{+} would be the mediator of SD propagation, but, instead of diffusing through the interstitial spaces, it would move by way of cytoplasmic bridges among glial cells. The idea was supported by the observation that drugs which close gap junctions, such as heptanol, octanol, and halothane, block SD propagation in the isolated retina (272, 273), hippocampal organ cultures (198), and brain tissue (203, 307, 317, 337). It has been known for some time that tissue acidosis, which tends to close gap junctions (61, 328, 385), also inhibits SD (25, 401, 405). We (203) have attributed the interdiction of SD propagation by heptanol and octanol to the closing of gap junctional connections among neurons rather than glial cells, because the selective glial poisons fluorocacetate and fluorocitrate failed to suppress SD or prevent its spread (202, 203).

Another set of observations also pointed to SD being spread through intercellular junctions linking neurons (133, 134, 136, 375). In the brain of urethane-anesthetized rats, an oncoming wave of propagating SD is heralded by a brief burst of extracellular “population spikes.” Unlike seizure discharges that ride on a negative shift of the extracellular potential and are accompanied by a steady elevation of [K\textsuperscript{+}]\textsubscript{o} (104, 378, 380), the pre-SD spike bursts erupt before the onset of the \(\Delta V\), when [K\textsuperscript{+}]\textsubscript{o} is still normal. The large amplitude of these extracellular compound action potentials indicates “lock-step” firing (378) of many neurons, and the synchronization extends over longer distances than could be spanned by ephaptic interaction. To explain the long-distance synchrony, Herreras and co-workers (133, 134) proposed that the opening of previously closed interneuronal gap junctions precedes the advancing wave of the depolarization.

SD propagation mediated by gap junctions has recently been tested in computer simulation by Shapiro (350, 351). His model consists of a row of single-compartment “cells” connected by gap junctions and surrounded by an interstitial space. It incorporates a formidable array of ion channels and transporters and provides for the calculation of an equally impressive number of variables. Not only ion concentration changes due to electrodifussion across cell membranes and through gap junctions, but also osmotic water flow, and hence cell swelling, have
been computed. SD was initiated by raising \([K^+]_o\) to 50 mM, imitating the common laboratory practice of injecting KCl into brain tissue. In this model SD did not propagate if gap junctions were closed, nor if cells were not allowed to swell.

Just as the K+ and glutamate hypotheses, the idea of gap junctions has problems. Gap junctions among neurons are more numerous in very young infant animals than older ones (164, 455), yet the inclination for generating SD increases with age. This is not a fatal flaw, because gap junctions are found among pyramidal neurons in hippocampus of mature animals as well (13, 19, 231). In addition, the postulate is for normally closed junctions to open during SD. The pharmacological evidence is weakened, however, by the fact that agents such as heptanol, halothane, and acidity inhibit not just gap junctions but a wide range of membrane functions (203, 277, 310, 383). In the retina, low concentrations of heptanol and octanol accelerated the propagation of SD; only higher concentrations inhibited it (245).

In summary, the mechanism of SD propagation could, but need not, be identical to that of SD initiation. There are four competing hypotheses to explain SD propagation, two of which are based on the interstitial diffusion of a humoral agent, either K+ or glutamate, and the two others postulating mediation through gap junctions among either glial cells or neurons. There is no conclusive evidence for or against any one of these proposals, nor are they mutually exclusive. As with SD initiation, there may be more than one path converging toward the same destination.

M. Susceptibility to SD

The reasons for the increasing susceptibility to SD with aging, and for its wide variability among different regions of the central nervous system, have not been determined, but in the light of the preceding discussion, we can at least ask what are the factors that can modulate SD ignition. Among the obvious ones is cytoarchitecture. The larger ISVF, the more released K+ and glutamate will be diluted. Glial cells are important. The presence of sheer numbers, and the state of their membrane transport systems which regulate extracellular K+ and excitatory amino acids (27, 280, 344) and probably also pH (329), powerfully affect the likelihood of SD. Finally, the maturity and distribution of transmitter systems and ion channels in neuron membranes are likely determinants of SD generation.

The immunity from SD of the brains of newborns (see sect. iiB) may have to do with the large volume of interstitial spaces in the newborn (222), favoring dilution of released K+ and glutamate. Immaturity of persistent sodium and NMDA or GABA receptor controlled channels could also be important (31, 229, 253, 334, 392), but the precise relationship of the maturation of channels to SD has not yet been specifically addressed. The maturation of glial cells may be expected to restrain rather than to augment SD (193, 286).

Tight packing of cellular elements is a likely factor in making the hippocampus, and especially the CA1 region, inclined to generate SD and HSD (112, 251). The relative scarcity of glial elements in this region could be another factor (112).

IV. SPREADING DEPRESSION AND HYPOXIC SPREADING DEPRESSION-LIKE DEPOLARIZATION IN HUMAN PATHOPHYSIOLOGY

A. Migraine, Concussion, and Seizure Disorders

The central nervous systems of numerous classes of animals can produce SD, and this could mean that it has been phylogenetically conserved because it is in some way useful (42). However, it is also possible that SD, like seizures, is a malfunction, a hazard inherent in the complex organization of the brain. Indeed, there are four clinical conditions in which SD is suspected to play a role: migraine, concussion, postictal depression, and hypoxia/ischemia.

Leão and Morison (218) were the first to suggest that SD may be the cause of the scintillating scotoma typical of classical migraine. The idea was picked up by Milner (258), who pointed out the similarity between the velocity of propagation of SD and the rate estimated by Lashley (205) for the march of the postulated brain process causing the scotoma during his own migraine. The evidence for a link between SD and migraine is as yet indirect. Detailed discussion of the various points of view is outside the scope of this article but may be found in a volume (220), and in other reviews, essays, and research papers (16, 49, 98, 124, 296, 354, 442). Rats do not seem to get a headache while undergoing SD (42, 176). This makes it unlikely that SD is causing the pain, but it does not refute its role in causing the scotoma of migraine.

In practice it may be difficult to distinguish concussion from cerebral contusion (436), yet a connection of SD to uncomplicated concussion suggests itself. One of the traditional ways to elicit SD in laboratory experiments is mechanical insult to the exposed brain or isolated tissue (108, 319, 327). It is easy to imagine that a blow to the head could trigger SD in many areas of the brain, including subcortical nuclei at the same time and so render the victim unconscious (124, 156, 166, 295). In the absence of structural damage or bleeding, brain function can return after a knockout in about the same time as it takes for cerebral tissue to recover from SD. Electrophysiological confirmation of this scenario is as yet lacking.
For reasons already mentioned (see sect. iiiL), the brief burst of firing that commonly precedes the depolarization at the leading edge of a wave of propagating SD must be distinguished from true seizure discharges. Nonetheless, true tonic-clonic seizures can also be followed by SD, and this has led to the suggestion that postictal depression may be due to SD-like depolarization (429). More often than not, however, neurons become hyperpolarized after termination of tonic-clonic discharges, not depolarized. Whether a seizure is followed by SD is decided by the behavior of \([K^+]_o\). As long as \([K^+]_o\) does not exceed a “ceiling” level of \(\sim 8–12 \text{ mM}\) (130, 131), SD will not occur, but if the regulation of \([K^+]_o\) is overwhelmed and the ceiling is breached, SD can ensue (162). This is especially probable in status epilepticus, when recurrent intensive convulsions are interrupted by periods of muscle relaxation and coma (314, 436). Again, this is a probable proposal, yet to be tested.

The pathological condition in which an SD-like state is almost certainly important is severe acute hypoxia or, more generally, sudden energy failure of brain tissue. This is the topic of sections ivB through ivE.

B. Comparing SD and HSD

Differences between SD and HSD have been emphasized from time to time (41, 339, 399). No doubt, the total syndromes of cerebral hypoxia and ischemia include changes that are absent in uncomplicated normoxic SD. Depolarization in SD is self-limiting, but in HSD, \(V_m\) and excitability recover only if oxygen is restored soon after the onset of depolarization. Oxidative energy is required for the restoration of ion gradients (124, 226, 438). As we have already seen (sect. iiiA), even though tissue PO\(_2\) can decrease during SD, neuronal mitochondria receive sufficient O\(_2\) to give an oxidation response, whereas in hypoxic brain, mitochondrial enzymes become reduced (161, 228, 247, 250, 321, 331, 389). It has also been pointed out that cells release inorganic phosphate during HSD, but not during SD (339, 340). This can also be attributed to the shortfall in oxidative energy and the consequent breakdown of high-energy phosphates (285, 340). Lactic acid production accounts for the acidosis of hypoxic brain that begins much before the onset of HSD (124, 129, 359). It is important to remember that even in this condition the moment of depolarization is marked by a sharp, transient alkaline shift (124, 184, 401), just as in normoxic SD.

Withdrawal of both O\(_2\) and glucose from brain slices has been called “ischemia in vitro.” Unlike hypoxic SD of brain tissue slices at a gas-liquid interface, in brain slices submerged under flowing artificial cerebrospinal fluid, the depolarization caused by “ischemia” is indeed “terminal,” i.e., irreversible (312, 397). This is because returning a solution containing O\(_2\) and glucose to a previously “ischemic” submerged slice does not immediately restore energy metabolism, for two reasons. Even at high PO\(_2\), aqueous media contain but little dissolved oxygen, while interfaced slices can be rapidly flooded with abundant O\(_2\) at the termination of hypoxia. In addition, glucose diffuses only slowly into the tissue slice so that washing it with glucose-containing solution does not immediately make it available to cells.

Another difference between SD and HSD is the timing of synaptic failure, which occurs minutes before the onset of HSD, whereas in normoxic SD synapses continue to function until depolarization inactivates ion channels. Both presynaptic and postsynaptic mechanisms contribute to the early synaptic block during hypoxia (reviewed in Refs. 370, 377), and these have nothing to do with the HSD that follows later.

Finally, there is the difference in the pharmacology of the two conditions (sect. iiiE). NMDA antagonist drugs are more effective against SD than against HSD, while TTX postpones HSD more powerfully than SD (208, 338, 376, 399).

Every one of these differences concern events that precede or lead up to the depolarization, but none speaks to the mechanism of the depolarization itself. Here the similarities are overwhelming. The waveform of the \(\Delta V_o\) is essentially identical in SD and HSD, provided that oxygenation is restored shortly after the onset of HSD (see sect. iiiC); so is the IOS that accompanies the voltage shift (see sect. iiiG); both the \(\Delta V_o\) and the IOS propagate at similar velocities in the tissue during SD and HSD (see sect. iiiB) (6); ion concentrations change in identical fashion (see sect. iiiD and E); interstitial space shrinks to the same degree (160); and the reduction in membrane potential and input resistance of neurons and glial cells are indistinguishable in the two processes (see sect. iiiH).

C. SD, HSD, and Neuron Survival

If oxygen is lacking, all cells of mammals die eventually, but certain neuron populations succumb much earlier than do other cells. The problem of selective vulnerability has preoccupied pathologists for many decades (343, 393, 433). In the hippocampus those neurons that develop HSD early during oxygen deprivation are the ones that are most sensitive to injury by hypoxia (21, 192, 287, 374).

It is not the depolarization itself that damages neurons. If calcium is removed from the bathing solution before oxygen is withdrawn, neurons recover function following a period of hypoxia that otherwise would have caused irreversible damage. However, low \([Ca^{2+}]_o\) does not prevent HSD; on the contrary, it hastens HSD onset (24, 323, 396, 451). Nor is it the calcium itself that kills...
cells. If oxygen is restored soon after the onset of HSD, function can be regained even though large amounts of Ca\(^{2+}\) have already flown into cells. It appears that [Ca\(^{2+}\)]\(_i\) must remain elevated for a critical length of time to catalyze the reactions that result in cell injury (74, 226, 263, 357, 358). The release of glutamate has been blamed for hypoxic cell injury, but it may not be the culprit (288, 289).

It follows that any treatment that postpones HSD (in the presence of normal calcium) should extend the time limit of revivability (Wiederbelebnungszeit, Ref. 300). There is ample evidence that this is indeed so. Such interventions include, in addition to a wide array of drugs, low temperature, acidity, and hypertonicity (22–24, 53, 80, 115, 116, 143, 151, 288, 440, 447).

It could be objected that during normoxic SD neurons gain just as much Ca\(^{2+}\) as during HSD, yet multiple repeated bouts of SD are tolerated by healthy brain tissue without evidence of lasting damage (134, 275). The key is, again, time. Normoxic SD episodes are harmless because the severe depolarization and the associated increase in [Ca\(^{2+}\)]\(_i\) lasts only 45–90 s. It has been pointed out that SD that is innocuous for healthy brain tissue can cause damage in cells with compromised energy supply (100, 101). But, even in well-fed and well-oxygenated tissue, if neurons are forced to remain depolarized for extended periods, they do not regain function afterward (135, 169, 335). Like the neuron injury caused by HSD, the loss of function caused by lengthy depolarization is prevented if external Ca\(^{2+}\) is removed, and it can also be mitigated by blockade of NMDA receptors (158).

The cell swelling that occurs during hypoxia has also been blamed for injury of neurons (333, 441). Swelling per se is, however, relatively well tolerated by brain tissue. As already pointed out (sect. iiF), the maximal shrinkage of ISVF measured during SD or HSD corresponds to an increase in average cell volume of only \(~10.5\%\) (160). Properly oxygenated hippocampal slices recover normal function after being forced to swell in strongly hypotonic bathing fluid for surprisingly long periods of time (but not forever), even though severe hypotonia induces repeated waves of SD (52, 150, 152). However, swelling does aggravate the injury caused by hypoxia (302). The danger in edema of brain in situ is, however, not so much the swelling of cells but the increase in the volume of the brain as a whole, which raises intracranial pressure, obliterates blood vessels, and obstructs the outflow of cerebrospinal fluid.

In conclusion, early onset of HSD-induced Ca\(^{2+}\) influx makes hippocampal CA1 pyramidal neurons and some neocortical neurons selectively vulnerable to hypoxic-ischemic cell injury. Any treatment that postpones HSD or prevents Ca\(^{2+}\) uptake extends the period of revivability (but not indefinitely). HSD is important but probably not the only factor determining selective vulnerability.

D. SD and Hypoxia Tolerance

Several reports have suggested that brief, nonlethal hypoxia or ischemia confers to brain tissue a degree of relative tolerance of subsequent more prolonged oxygen deprivation (303, 305, 348). This is in spite of the apparent shortening of the latency of HSD during repeated brief hypoxic episodes (439). More recently it is reported that previous normoxic SD also imparts a measure of “cross-tolerance” against subsequent cerebral hypoxia or ischemia (168, 174, 246, 450) and excitotoxic injury (186). In contrast, the SD waves that emanate from an already established ischemic focus and spread into the penumbral surround apparently cause the extension of the infarct area (46, 76, 149, 154, 177, 256, 271, 317, 355, 389). However, under certain conditions, the SD-induced vasodilation apparently improved collateral blood flow and limited the perifocal extension of the infarct (43).

E. Glucose, pH, HSD, and Survival After Transient Ischemia

Clinical experience teaches not only that diabetics are at an increased risk of cerebrovascular stroke, but also that, once stroke has occurred, the eventual outcome is worse for diabetics than for other patients. The devastation has been blamed on acidosis, and experimental evidence seemed to support this assumption (129, 182, 204, 225, 274, 361). However, acidosis of the degree experienced by diabetic patients does not injure brain tissue itself, and the damage is probably done to blood vessels, or it is secondary to systemic effects (347). In fact, hyperglycemia postpones HSD (80, 122, 194, 356). Also, infarction is not correlated with the acidity of the cytosol in brain cells (276). More to the point, mild acidity of interstitial fluid, as well as high glucose-induced tissue acidosis, actually improve functional recovery from hypoxia in neuron cultures and brain tissue slices (324, 345, 347, 349, 401, 402). The emphasis here is on the word “mild,” for severe acidity undoubtedly kills cells (106, 274).

V. SUMMARY AND CONCLUSIONS

SD and the related HSD are transient but profound disturbances of brain function that can be readily provoked experimentally in most areas of central gray matter. Both SD and HSD are characterized by nearly complete neuronal depolarization, decrease of membrane resistance, and redistribution of ions across cell membranes. Glial cells are also depolarized but through a
different mechanism. The glial membrane potential is dominated by the rising \([K^+]_o\) with only minor change in membrane ion conductance, whereas in neurons, the dominant change is the activation of ion conductances.

SD and HSD differ in the prodromal events that lead to the depolarization and possibly also in the mechanism by which they spread in the tissue, but the biophysical processes of the depolarization itself are similar in the two conditions.

During SD and HSD, neurons lose \(K^+\) and organic anions, including glutamate, to the interstitial fluid. Glial cells are another likely source of glutamate. At the same time \(Na^+, Ca^{2+},\) and \(Cl^-\) flow into neurons. These exchanges are not one for one; there is a net gain of solutes and as a result cells draw water by osmosis and swell. Swelling is at the expense of interstitial space, and it is more pronounced in neuronal dendrites and probably also in glial processes than in neuron somata.

If the depolarization due to SD or to HSD is prolonged beyond a critical time, neurons become permanently unresponsive. If, however, the depolarization-induced influx of \(Ca^{2+}\) into neurons is prevented, then neurons can regain function after extended SD-like depolarization that otherwise would cause irreversible injury.

SD and HSD are accompanied by light-scattering changes in tissue that produce an IOS, which can be imaged to map the spread of SD and HSD. The initial signal is a brief decrease of scattering attributable to the dilution of cytosol due to cell swelling but, coincident with the main phase of depolarization, the polarity of the IOS reverses and light scattering increases suddenly and dramatically. The scattering increase is chloride dependent and may be related to the swelling of mitochondria and other organelles.

The cellular depolarization generates an extracellular potential shift, \(\Delta V_o\), the main phase of which is strongly negative and has two maxima, an early sharp peak followed either by a "hump" or a lower plateau. Only the late phase of the \(\Delta V_o\) is dependent on the activation of NMDA receptors.

In computer simulation, either a \(I_{Na,P}\) or an \(I_{NMDA}\) could generate SD-like depolarization. When both \(I_{Na,P}\) and \(I_{NMDA}\) were present, the depolarization began faster and lasted longer. The simulated SD ignited when total persistent dendritic membrane current turned inward and, once triggered, it ran an all-or-none course. To reach SD ignition, \([K^+]_o\) had to rise beyond a critical level. Optimally functioning simulated glia-endothelial system limited the rise of \([K^+]_o\) and so prevented SD.

Other computer models have successfully simulated the propagation of SD, using either the accumulation of \(K^+\) in interstitial space or the opening of gap junctions between neurons as the agent of spread.

In conclusion, SD and HSD can be generated by any one or a combination of several ion channels that is (1) either voltage or \([K^+]_o\) dependent, or both; (2) inactivates (desensitizes) slowly or not at all; and (3) produces an inward current and also releases \(K^+\) into a restricted interstitial space.

The final level to which the membrane potential is depolarized is not determined by the number of ion channels available, but by the feedback that governs the process.

There is no need to postulate special, pathological "SD channel" or "pore" to explain SD or HSD.

The normal stability of brain function depends on the efficient regulation keeping ion concentrations, especially that of \(K^+\), within physiologically tolerated limits.

Address for reprint requests and other correspondence: G. G. Somjen, Dept. of Cell Biology, Box 3709, Duke University Medical Center, Durham, NC 27710 (E-mail: g.somjen@cellbio.duke.edu).

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