Research report

Recurring episodes of spreading depression are spontaneously elicited by an intracerebral hemorrhage in the swine

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Abstract

Intracranial bleeding damages the surrounding tissue in a complex fashion that involves contamination by blood-borne products and loss of ionic homeostasis. We used electrophysiological techniques to examine the functional changes in the developing intracerebral bleed and in surrounding regions using an in vivo swine model. Intracerebral hemorrhage (ICH) was induced by collagenase injection into the primary somatosensory cortex (SI). Somatic evoked potential (SEP) elicited by electrical stimulation of the contralateral snout as well as changes in DC-coupled potential were monitored in the SI from the time of collagenase injection in order to measure the effects of ICH. The SEP decreased in amplitude within minutes of the intracerebral injection. Its short-latency component was abolished within the first hour after collagenase injection without any sign of recovery for the duration of the experiment. As the SEP started decreasing in amplitude, we observed spontaneous, recurring episodes of cortical spreading depression (SD) as early as 20 min post-injection. The timing of SDs in SI is consistent with our interpretation that SDs were initially generated at multiple sites adjacent to the lesion core and propagated into the surrounding area. With time, SD became less frequent near the injection site, shifting to more distant electrodes in the surrounding area. Our results indicate that ICH leads to the reduction in SEP amplitude and induces spontaneous episodes of SD. Loss of ionic homeostasis is most likely the physiological basis for the SEP change and for the induction of SD. Recurring SD spontaneously generated in experimental ICH needs further study in humans with ICH.

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1. Introduction

Intracerebral hemorrhage (ICH) occurs in 15–20% of stroke patients and in brain injury [8,26]. We have initiated a series of studies to characterize the functional changes produced by collagenase-induced ICH since the pathophysiology of hemorrhagic stroke is still poorly understood in spite of its clinical importance. The temporal progression of acute ICH has been characterized in the rat using the collagenase model of hemorrhagic stroke [16,23,24].

In order to gain insights into the sequela of ICH in the human brain, we adapted the collagenase-induced ICH model to the large gyrencephalic brain of the swine. The swine brain resembles the human brain by having a large volume and a well-developed pattern of convolutions in the cortex. Collagenase was injected into the rostrum area of the primary somatosensory cortex (SI). The swine snout is somatotopically represented over the large R1 area which is doughnut shaped with the sulcus naris in the middle of the coronal gyrus [5,20]. This somatotopic organization enables us to study the effect of collagenase-induced hemorrhage on cortical neurons by recording the somatic evoked potential (SEP) from the area of the cortex receiving direct thalamocortical projections from the stimulated location of the snout before, during and after collagenase injection into the projection area.

In a preliminary study [17], we observed cortical spreading depression (SD) while monitoring the DC-potential changes in the hemorrhagic core as a function of
time after collagenase injection. We now characterize the development of SD, which is distinguished by large changes in the extracellular concentrations of major ions [12]. Originally described by Leão [14], SD is generated when the extracellular potassium concentration increases from the resting level of about 3.5 mM to above 10 mM [12]. SD is described by temporary loss of neuronal activity in the cortical area undergoing this phenomenon with a slow propagation of the affected area from the initiation site toward the surrounding cortical areas [11,13]. SD has been associated with epilepsy [25], stroke [18], migraine [13] and traumatic brain injury [19]. We therefore hypothesized that SD should be generated initially in the core of the hemorrhagic injury and propagate to the surrounding areas. As the hematoma develops and ionic homeostasis is lost in the core area, adjacent tissue loses the ability to spontaneously generate SD, and SD ceases. The dysfunctional cortical area should gradually move outward from the ICH core, generating SD farther from the ICH core over time.

2. Materials and methods

2.1. Surgical procedure

The animal protocol for the present study was approved by the University of New Mexico School of Medicine Animal Research Committee. All of the experiments were conducted in the Magnetophysiology Laboratory, Veterans Affairs Medical Center, Albuquerque, NM.

Juvenile farm swine, weighing 4–12 kg, 3–5 weeks old, were initially anesthetized with sodium thiopental, 25–30 mg/kg intrathoracically. Sodium thiopental was used since SD can be elicited under a barbiturate anesthesia [1,2,11] but it is suppressed by anesthetics such as ketamine, which blocks N-methyl-D-aspartate (NMDA) receptors and prevents SDs [9]. Following 1% lidocaine application the trachea was intubated for mechanical respiration of room air, and the femoral artery was cannulated to monitor physiological blood gases and for continuous isotonic fluid infusion. The cephalic vein was cannulated in the upper forelimb and the anesthesia was then administered at a rate of 9 mg/kg/h i.v. during the remaining surgery. The animal was then placed in a head-holder. The scalp area was infiltrated with 1% lidocaine so that an incision could be made to expose the dorsal surface of the skull. The SI cortical surface was exposed bilaterally by removing the bone and dura. The animal was then transported into a magnetically and electrically shielded room [21]. A warm water pad was used to maintain body temperature at 37°C. Blood pressure, electrocardiography (ECG), blood pH, pCO₂, and pO₂ were monitored and maintained within normal physiological limits. Anesthesia was decreased to 1.5–3 mg/kg/h during EEG recording and sodium tubocurarine (0.3 mg/kg/h i.v.) was administered. The effectiveness of anesthesia was monitored continuously with ECG and EEG.

2.2. Recording procedure

Electrical potential recordings were made with either a 6-channel array (n=4) or 12-channel array (n=4) of glass micropipettes (tip diameter 5–10 μm, filled with 2 M NaCl). A Ag–AgCl electrode wire contacted the NaCl fluid and was connected with the amplifier. The glass electrodes were positioned between plexiglass plates and held secure with plastic screws. The six electrodes in the 6-channel array were positioned linearly 1 mm apart. The 12-channel array was comprised of four electrodes positioned 2 mm apart along three columns (separated by 5 mm between electrodes 1–4 and 5–8 and 7 mm between electrodes 5–8 and 9–12). Electrode 9 was not functioning in all the experiments while electrode 8 did not work during one of four experiments. Thus these cases were excluded in the statistical analysis.

After stereotaxically implanting the recording electrode array 3 mm ventral to the cortex, a layer of 4% agarose (type I-A, Sigma Chemical) was poured over the cortical surface to minimize cortical movement. A Ag–AgCl reference electrode was placed in the epidural space of the frontal brain region. Baseline level of spontaneous activity and evoked responses in the intact SI cortex were collected for 20–30 min prior to collagenase injection. An area of the snout contralateral to the recorded SI cortex was electrically stimulated (2 mA, 100 μs) at 0.5 Hz using a bipolar pair of electrodes. The evoked responses (bandpass=0.1 Hz to 1 KHz) were averaged over 50 epochs. The stimulation site was moved until the short latency component of the SEP was maximized in the recording electrodes adjacent to the collagenase injection pipette.

In one group of animals (n=8), 625 U of collagenase (type XI, Sigma Chemical) in 5 μl sterile saline+5 μl heparin (10,000 U/ml) was delivered using an 80-μm outer diameter glass pipette over 16 min with a microinfusion pump (Harvard Instruments). Five microliters of saline were placed in the tip of the injection pipette prefacing the collagenase injectate to insure that baseline data were not influenced by collagenase leaking into the brain tissue environment. Heparin was added to the carrier, following the protocol of Del Bigio et al. [6]. The occurrence of SD was monitored continuously from the time of collagenase injection for up to 6 h with a DC-coupled recording of the potential within the SI. SEPs within the SI cortex were monitored periodically using the same electrodes.

A separate group of animals (n=3) received a 1250 U collagenase injection in 10 μl of saline/heparin. The rate of SEP attenuation was compared between the 625 U and 1250 U of collagenase injection, using the 6-channel electrode array.
3. Results

3.1. Effects of collagenase on SEP

Histological examination of the affected cerebral hemisphere revealed that an intracerebral injection of collagenase produced a hemorrhagic mass primarily in white matter of the SI cortex. Fig. 1 displays a representative example of the hemorrhage 5.5 h after an injection of 625 U of collagenase (Fig. 1B) and another 6 h after an injection of 1250 U of collagenase (Fig. 1C). These two coronal sections were obtained along the plane indicated in the illustration of the dorsal view of the cortex (Fig. 1A). The extent of the injury was larger for injections of higher concentrations of collagenase.

SEPs elicited by electrical stimulation of the snout decreased in amplitude within minutes after the start of 1250 U of collagenase injection into the SI projection area of the snout (Fig. 2A). These SEPs were recorded with electrode 2 whose location in the 6-electrode array is depicted in Fig. 3A. The negative polarity of the SEP short-latency component was due to the fact that the electrode was positioned 3 mm below the cortical surface. In all collagenase-injected animals, the SEP was nearly abolished by 60 min after the start of collagenase injection (Fig. 2B). A significantly more rapid drop in the amplitude of the SEP short-latency component was measured during the initial 60 min post-collagenase injection in animals receiving 1250 U collagenase as compared to animals injected with 625 U collagenase, \( P<0.004 \) (Fig. 2C).

3.2. Temporal, spatial characteristics of SD

Spontaneous, recurring episodes of SD were detected in brain tissue of collagenase-injected animals. Fig. 3 depicts representative SDs recorded with the 6-channel electrode array along the anterior–posterior direction after an injection of 625 U of collagenase into the SI indicated by an ‘X’ in the inset (Fig. 3A). The initial episode of SDs was detected within 24 min from the start of collagenase injection (Fig. 3B, left). During the early period, SDs were seen at first near the injection site and then at more distant electrode locations. The latencies of the SDs at these electrode sites did not indicate a uniform propagation from a single focus, but rather suggested that SD appeared to have originated from multiple foci adjacent to the injection site. The SDs tended to propagate in both directions during the early period. Approximately 90 min after collagenase injection (late period), SDs became less frequent or disappeared altogether near the injection site, while SDs were still seen at more distant electrodes (Fig. 3B, right). Electrodes 3 and 4, adjacent to the lesion core, showed a decrease in SD episodes during the late period as compared to the early period, whereas SDs continued to occur in electrodes 1 and 6 during the late period.

These key temporal and spatial characteristics were quantified by examining the SDs in all the recordings across four animals. Fig. 3C shows the percentage of SDs seen at each electrode site relative to the total number of SDs seen at all the electrode sites during that particular period: early, mid- and late. Of the 58 total SDs observed in all animals during the early period, 19 SDs (33%) were recorded in electrodes 3 and 4 (1 mm away from the injection site). Twenty SDs (34%) were recorded in electrodes 2 and 5 (2 mm away from the injection site) and 19 SDs (33%) were detected in electrodes 1 and 6 (3 mm away from the injection site). Sixty-three SDs were observed during the mid-period: 14 SDs (22%) at 1 mm, 24 SDs (38%) at 2 mm, and 25 SDs (40%) recorded at 3 mm away from the injection site. During the late period, 37 total SDs were recorded in the four animals: five SDs (14%) at 1 mm, 15 SDs (40%) at 2 mm, and 17 SDs (46%) at 3 mm from the site of injection.

Maximal duration of each period was 24 min in the early period, 42 min in the mid-period, and 74 min in the late period. During the early period SD was seen equally often at all electrode sites, indicating that SD was being generated near the injection site and propagating to outer electrode locations. The proportion of SD detected near the injection site decreased relative to those at the periphery as time progressed. This interaction between the time periods and electrode distance from the injection site was statistically significant \( (F=3.82, \text{df}=4/27, P<0.01) \).

A more extensive mapping of SDs was conducted with the 12-channel electrode array in the region of the SI cortex surrounding the injection site. These results corroborated the 6-channel electrode data (Fig. 4). The ‘X’ in Fig. 4A represents the collagenase injection site, and the numbers indicate the placement of electrodes in SI. Fig. 4B represents SDs recorded during the early period (left) and late period (right) following collagenase injection. SDs were seen with this electrode array as early as 17 min. The timing of SDs at the different electrode sites indicates that SDs were recorded at multiple loci near the injection site and propagated to all the surrounding areas during the early period. This is in contrast with the assumption of a single focus, which leads to inconsistent velocities in the gyral portion of the SI. With time the extracellular negativity during SDs produced near the injection site

In the control group \( (n=3) \), animals received a 5-μl saline injection and electrical potential recordings were monitored using the 12-channel electrode array. No significant differences in SEP recordings were apparent following intracerebral saline injection as compared to baseline signals and no SD was detected (data not shown).

At the conclusion of the experiment the animal was euthanized by thiopental overdose and the brain was fixed in formalin for histological assessment. Statistical analysis of SEP data was conducted using a two-tailed paired \( t \)-test, and the two-way analysis of variance test for the SD data.
Fig. 1. (A) Illustration of the cortical surface of the doughnut-shaped coronal gyrus that is somatotopically organized to represent the sensory projections of the contralateral half of the swine snout. The dotted line indicates the coronal dissection plane relative to the site of collagenase injection. (B) Coronal section of a 625 U collagenase-induced hematoma in the swine primary somatosensory (SI) gyrus, at the site of injection. (C) Coronal section of a 1250 U collagenase-induced hematoma in the SI gyrus at the site of injection. Both these tissue sections were taken from single plane electrode array experiments.
the site of collagenase injection, while electrodes positioned medial to the sulcus naris continued to detect substantial SD episodes during the late period.

These features illustrated in Fig. 4B were quantified by an analysis of the entire data set across four animals (Fig. 4C). As in Fig. 3C, the graph in Fig. 4C indicates the percentage of SDs detected in a particular group of electrodes related to SDs recorded at all electrode sites for each period. Of the 110 total SDs observed in all animals during the early period, 22 SDs (20%) were recorded in region x (electrodes 3 and 11) which were the electrodes in the anterior/posterior plane of the injection site. Forty-one SDs (37%) were recorded in the $-y$ region, which includes all other electrodes lateral to the sulcus naris (electrodes 4, 7, 8, 12), and 47 SDs (43%) were recorded in the $+y$ region, which included all electrodes medial to the sulcus naris (electrodes 1, 2, 5, 6, 9, 10). Forty-eight SDs were observed during the mid-period: six SDs (12%) in region x, 19 SDs (40%) in the $-y$ electrodes, and 23 SDs (48%) recorded in region $+y$. During the late period, 86 total SDs were recorded in the four animals: eight SDs (9%) in region x, 29 SDs (34%) in the $-y$ region, and 49 SDs (57%) in the $+y$ region.

The maximum duration of the early period was 34 min, middle period was 34 min, and late period was 1 h and 21 min. Similar to the 6-electrode data, there was a significant interaction between the time periods and electrode location relative to the sulcus naris ($F = 3.26; df = 4/27; P < 0.03$). In summary, these data suggest that SDs were generated initially at multiple foci adjacent to the ICH core and subsequently further away from the injury core.

4. Discussion

The collagenase-induced ICH has not been characterized in the swine. The swine brain is gyrencephalic, containing a well-developed pattern of convolutions in the cortex. Since there are large differences in characteristics of the brain between the rat and the human, it is essential to obtain understanding of the sequela of an ICH in a species whose brain resembles the human brain. We believe that the porcine brain is an important non-primate model for understanding hemorrhagic stroke in human patients. The present study is the first of a series of studies being planned for characterizing the sequela of an ICH during the acute and chronic phases not only with EEG, but also with magnetoencephalography (MEG) and magnetic resonance imaging (MRI).

This study has shown two important features of ICH during the acute phase in the swine. First, the SEP amplitude is diminished following collagenase injection in a dose-dependent fashion with a time course that is consistent with the biochemical, histological and neuroimaging results from the previous rat studies [3,6,23,24]. An intracerebral injection of bacterial collagenase produces a
Fig. 3. (A) Arrangement of the injection pipette, ‘X’, and the six glass electrodes in a single plane array on the SI cortex. (B) Representative profile of spreading depression (SD) propagation in each electrode following collagenase injection during the early (left) and late (right) periods of SD in this electrode array. (C) Graph summarizes the percent of SD detected in each electrode relative to the total number of SDs seen in all the electrodes during that period. The interaction between the time periods and electrode distance from the injection site was statistically significant ($P<0.01$).

Fig. 4. (A) Arrangement of the injection pipette, ‘X’, and the 12 glass electrodes in a triple plane array on the SI cortex. (B) Representative profile of SD propagation in each electrode following collagenase injection during the early and late periods of SD in this electrode array. (C) There was a significant interaction between the time periods and electrode location relative to the sulcus naris ($P<0.03$). This graph summarizes the percent of SD detected in region $x$ (electrodes 3 and 11), electrodes positioned lateral to the sulcus naris, $−y$ region (electrodes 4, 7, 8, 12), and electrodes located medial to the sulcus naris, $+y$ region (electrodes 1, 2, 5, 6, 9, 10). Values are expressed as percent SDs relative to the total number of SDs seen in all the electrodes during that period.
dose-dependent, reproducible bleed at the site of injection as well as significant alterations in brain edema, electrolyte imbalance and hematoma size in the rat [24]. Although we have not characterized the development of the hematoma in the swine model, the time course of SEP change is consistent with previous MRI results in the rat. Rapid growth of the lesion occurred during the initial 60 min of injury and by 2 h, MRI data showed signs of early edema related to the hematoma [3,6]. The accumulation of red blood cells along white matter bundles of the affected area was seen within 30 min of injection [6]. At 2 h post-collagenase/heparin injection, histological examination revealed an almost contiguous bleed with swollen glial cells [6].

The reduction in SEP amplitude can be explained by the loss of ionic homeostasis in the white matter and overlying gray matter of the swine SI within and surrounding the injection site of collagenase. The extravasation of blood-borne macromolecules and loss of oxygen supply should lead to changes in the cellular metabolism and consequently to the depolarization of the membrane potentials of neurons and glial cells. The depolarization should alter the kinetics of the voltage-sensitive channels, for example by inactivating the sodium channels, and should decrease the amplitude and frequency of the action potentials in the thalamocortical fibers and also the amplitudes of the cortical responses. Thus, we interpret the time course of reduction in SEP amplitude to reflect the temporal dynamics of the developing hematoma. This interpretation will be verified by conducting a continuous measurement of the developing mass lesion with MRI in an acute collagenase ICH preparation.

Secondly, our finding of spontaneous SDs in the cortex undergoing an ICH is, to the best of our knowledge, novel. SDs are known to occur in a cortical tissue with focal ischemia [18], but there has been no study reporting SD in an ICH model. In our model, there was a significant characteristic change in the pattern of SD onset and propagation with time after the collagenase injection. Within minutes after the injection, multiple electrodes at the injury site and in the surrounding tissue detected recurring episodes of SDs. With time, recovery to the baseline DC potential level became more prolonged and incomplete in certain electrodes, especially those proximal to the injury site. During the later period, the rate of SD generation near the injection site decreased as compared to the early SD period and the locations of SD shifted to electrodes positioned farther from the hemorrhagic core.

The 12-electrode array data indicated that the sulcus naris dividing the SI cortex influences the temporal and spatial occurrence of SD as the hemorrhage developed in tissue lateral to this sulcus. The percentage of SDs seen in region \( x \) decreased with time from the early to late periods relative to the total number of SDs seen at all electrode sites. A similar trend was seen in the other electrodes positioned lateral to the sulcus naris (-y). In contrast, electrodes located medial to this sulcus (+y) showed a proportional increase in the amount of SD propagation detected during the late period (Fig. 4C).

We did not determine the propagation velocity of SD in our preparation since the path of propagation may be quite complex in an intact cortex. The velocity can be measured, however, when SD is forced to propagate linearly along a cortical strip [1,2]. In such a case the SD initiated by an electrical stimulation of the cortex was found to propagate uniformly in a lissencephalic species (rabbit) [1], but non-uniformly in a gyrencephalic species (swine) [2]. The propagation velocity was 1–2 mm/min within the sulcus as compared to 6–8 mm/min in the gyrus. These results suggest that the SD initiated by collagenase may be different in gyrencephalic species such as the human as compared to lissencephalic species such as the rabbit and rat. The results of Bowyer et al. [2] need to be tested in our collagenase model.

Our observation of SD during the acute phase of an ICH is interesting since some investigators have proposed SD to play a role in upregulating the expression of neuroprotective genes in ischemia [10,15] and in influencing infarct volume size [4,27]. It should be possible to evaluate the role of SD in determining the size of hematoma by measuring the size with T2-weighted MRI and by detecting the occurrence of SD with diffusion-weighted imaging which is sensitive to changes in extracellular volume fraction that is reduced during SDs. The size of a hematoma can be compared under a barbiturate anesthesia which does not prevent the occurrence of SD as in our present study and in others [7,22], and under an anesthetic such as ketamine which blocks N-methyl-D-aspartate (NMDA) receptors [9] and prevents SDs.

The existence of spontaneous SD in our ICH model has possible clinical significance as episodes of SD may be occurring spontaneously in stroke patients with an ICH during the acute phase. The pathophysiology of hemorrhage has gained renewed interest because ICH may develop in patients with ischemic stroke after thrombolytic therapy. As we characterize the sequela of the ICH in the swine model, it should be possible to use non-invasive techniques such as EEG/MEG and MRI to provide better understanding of stroke.

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