Anesthesiology
1997; 87:1470–8

Lidocaine Suppresses the Anoxic Depolarization and Reduces the Increase in the Intracellular Ca^{2+} Concentration in Gerbil Hippocampal Neurons

Keyue Liu, M.D., Naoto Adachi, M.D., Ph.D.,† Hisato Yanase, M.D., Ph.D.,‡ Kiyoshi Kataoka, M.D., Ph.D.,§ Tatsuru Arai, M.D., Ph.D. ||*

Background: **The movement of ions, particularly Ca^{2+}, across the plasma membrane of neurons is regarded as an initial element of the development of ischemic neuronal damage. Because the mechanism by which lidocaine protects neurons against ischemia is unclear, the effects of lidocaine on the ischemia-induced membrane depolarization, histologic outcome, and the change in the intracellular Ca^{2+} concentration in the gerbil hippocampus were studied.**

Methods: **The changes in the direct-current potential shift in the hippocampal CA1 area produced by transient forebrain ischemia for 4 min were compared in animals given lidocaine (0.8 μmol administered intracerebroventricularly) 10 min before ischemia and those given saline. The histologic outcome was evaluated 7 days after ischemia by assessing delayed neuronal death in hippocampal CA1 pyramidal cells in these animals. In a second study, hypoxia-induced intracellular Ca^{2+} increases were evaluated by *in vitro* microfluorometry in gerbil hippocampal slices, and the effects of lidocaine (10, 50, and 100 μM) on the Ca^{2+} accumulation were examined. In addition, the effect of lidocaine (100 μM) drug perfusion with a Ca^{2+}-free ischemia-like medium was investigated.**

Results: **The preischemic administration of lidocaine delayed the onset of the ischemia-induced membrane depolarization (anoxic depolarization) and reduced its maximal amplitude. The histologic outcome was improved by the preischemic treatment with lidocaine. The *in vitro* hypoxia-induced increase in the intracellular concentration of Ca^{2+} was suppressed by the perfusion with lidocaine-containing mediums (50 and 100 μM), regarding the initiation and the extent of the increase. The hypoxia-induced intracellular Ca^{2+} elevation in the Ca^{2+}-free condition was similar to that in the Ca^{2+}-containing condition. Perfusion with lidocaine (100 μM) inhibited this elevation in the Ca^{2+}-free condition.**

Conclusions: **Lidocaine helps protect neurons from ischemia by suppressing the direct-current potential shift, by inhibiting the release of Ca^{2+} from the intracellular Ca^{2+} stores, and by inhibiting the influx from the extracellular space. (Key words: Anesthetics, local: lidocaine. Animals: gerbils. Brain: anoxic depolarization; hippocampus; ischemia. Ions, calcium: intracellular. Measurement techniques: direct-current potential; fluorometry.)**

THE central nervous system contains no reserve supply of oxygen and only small stores of glucose or energy-rich compounds, which are essential to maintain ionic gradients across the nerve membrane. In cerebral ischemia, energy failure triggers the depolarization of the neuronal membrane because of inadequate active ion transport.[1] Various excitatory neurotransmitters are also released,[2–4] and a marked influx of Ca^{2+} into postsynaptic neurons occurs. This, in part, provokes the catastrophic enzymatic process leading to irreversible neuronal injury.[5]

Lidocaine is a local anesthetic that blocks nerve conduction.[6] In a previous study, we found that lidocaine reduced the increase in the extracellular concentration of excitatory amino acids in transient forebrain ischemia and improved the ischemia-induced neuronal damage.[7] Furthermore, this agent influences the action of ryanodine receptors, which regulate the efflux of Ca^{2+} from the endoplasmic reticulum to the cytosol.[8] The purpose of the present study was to characterize the *in vivo* effects of lidocaine on ischemia-induced alterations in the direct-current (DC) potential shift (anoxic depolarization [AD]) and histologic outcome in gerbils, and to investigate the *in vitro* effects of lidocaine on a hypoxia-induced increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$).

Materials and Methods

The study was approved by the Committee on Animal Experimentation at Ehime University School of Medi-

* Visiting Researcher of Anesthesiology and Resuscitology.

† Lecturer in Anesthesiology and Resuscitology.

‡ Lecturer in Physiology.

§ Professor in Physiology.

|| Professor in Anesthesiology and Resuscitology.

Received from the Department of Anesthesiology and Resuscitology, Ehime University School of Medicine, Shitsukawa, Ehime, Japan. Submitted for publication March 14, 1997. Accepted for publication August 7, 1997.

Address reprint requests to Dr. Adachi: Department of Anesthesiology and Resuscitology, Ehime University School of Medicine, Shitsukawa, Shigenobu-cho, Onsen-gun, Ehime 791-02, Japan.

cine, Ehime, Japan. Male Mongolian gerbils weighing 60–80 g (Seiwa Experimental Animals, Fukuoka, Japan) were housed in groups in a room controlled at 23 ± 1°C and maintained in an alternating 12-h light and 12-h dark cycle (lights on at 6:00 A.M.). Animals were deprived of food but not water at least 6 h before ischemia because of the influence of hyperglycemia on ischemic brain damage.[9] All experiments were performed under spontaneous ventilation. In experiment 1, the changes in the DC potential shift produced by transient forebrain ischemia in the hippocampal CA1 field were monitored in 16 gerbils, and delayed neuronal death 7 days after the ischemia was studied by light microscopy in these animals. Another group of ten gerbils was subjected to forebrain ischemia to determine the physiologic variables. In experiment 2, microfluorometry was used to investigate the action of lidocaine on a hypoxia-induced intracellular Ca^{2+} accumulation in gerbil hippocampal slices (ten slices for each).

Experiment 1: In Vivo *Experiments on the DC Potential Shift and on Histologic Outcome*

Animals were anesthetized and maintained with 2% halothane and 98% oxygen. Through a ventral middle cervical incision, both common carotid arteries were exposed and separated carefully from adjacent nerves and tissues. Silk threads (4.0) were looped around them. After the animal was placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) in the prone position, the skull was exposed and a small burr hole was drilled in the left hemisphere 2 mm anterior and 2 mm lateral to the bregma to insert a thermocouple needle probe (TN-800; Unique Medical Corp., Tokyo, Japan). The tip of the thermocouple needle probe was positioned about 2 mm below the brain surface. An identical probe was inserted into the rectum. Brain and rectal temperatures were carefully maintained at 37–38°C with a heating lamp (Koehler-type illumination lamp; Olympus, Tokyo, Japan). Two additional burr holes were drilled — one in the left hemisphere (0.5 mm posterior and 2 mm lateral to the bregma) to administer drugs and the other in the right hemisphere (2 mm posterior and 2 mm lateral to the bregma) to measure the DC potential shift.

The electrode consisted of a glass micropipette with a tip diameter of about 4 μm, which was filled with 3 M KCl with an Ag/AgCl electrode in the barrel. This local electrode was implanted in the right hippocampal CA1 field (2 mm posterior and 2 mm lateral to the bregma, and 2 mm below the brain surface) through the burr hole previously described. The remote electrode (Ag/AgCl) was inserted into the subcutaneous portion of the neck. The DC potential shift was monitored between these electrodes with a model AB-621G DC amplifier (Nihon Kohden, Tokyo, Japan). After a stabilization period of 60 min, animals were assigned to one of two groups. In one, lidocaine (0.8 μmol in 10 μl saline) was administered intracerebroventricularly through a 27-gauge needle inserted into one burr hole. Control animals were given 10 μl saline.

Transient forebrain ischemia was achieved by pulling the threads around the bilateral common carotid arteries with 8-g weights. This was done 10 min after lidocaine or saline administration while maintaining the brain and rectal temperatures at 37.5 ± 0.2°C.[10] After the 4-min period of ischemia, the threads were cut to restore the blood flow. Rectal and brain temperatures were maintained at 37–38°C under halothane anesthesia for 90 min after the reflow. The difference in the DC potential shift was compared by analyzing its onset latency (from the start of ischemia to the sudden depolarization), amplitude, recovery time of depolarization to half-maximal amplitude, and duration at half-maximal amplitude (fig. 1). The thermocouple probes were then gently removed. After all surgical incisions were carefully sutured, the animal was removed from the stereotaxic apparatus. The animal was brought to its cage in a room maintained at constant temperature and allowed access to food and water *ad libitum*.

Seven days later, the animals were anesthetized with an intraperitoneal injection of sodium pentobarbital. The brains were perfused with heparinized saline and fixed with 10% buffered formalin. After dehydration with graded concentrations of alcohol solutions, the brains were embedded in paraffin. Brain slices, 5 μm thick, were stained with hematoxylin and eosin. The number of pyramidal cells in the hippocampal CA1 field per constant area (1 × 1 mm) was counted in healthy animals, and then the number of preserved neurons in the experimental animals was counted in the corresponding area from the coronal section (1.5 mm posterior to bregma). Then the average of percentages of necrotic cells on both sides was obtained for each animal.

Another group of ten gerbils was prepared to determine the physiologic variables that may influence the extent of the neuronal damage in ischemia. The animal was anesthetized with 2% halothane and 98% oxygen. Five animals were given lidocaine intracerebroventricularly, and the remaining five animals were given saline

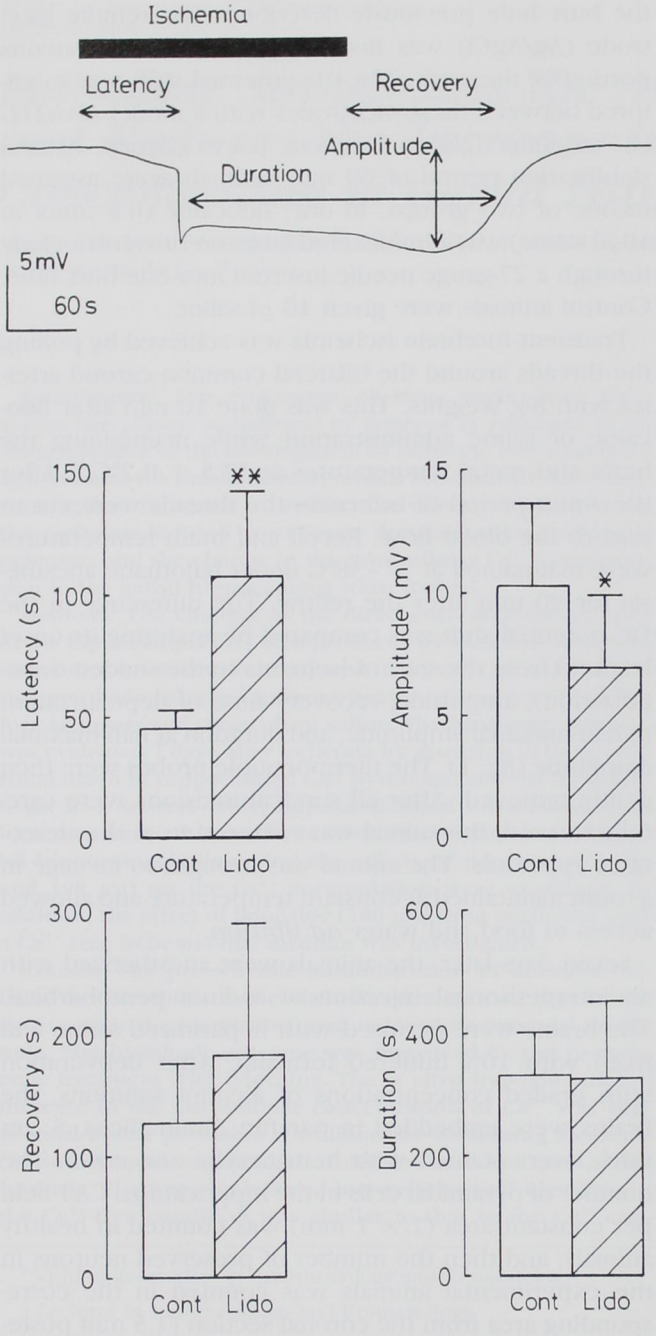

Fig. 1. Effects of lidocaine administered intracerebroventricularly 10 min before ischemia on the anoxic depolarization (AD). The onset latency, amplitude, recovery time, and duration of AD were analyzed. Each column represents the mean ± SD for seven to nine animals. Cont = saline-injected control group; Lido = lidocaine (0.8 μmol)-injected group. *$P < 0.05$, **$P < 0.01$ compared with the respective values in the control group.

while they were in the prone position using a stereotaxic apparatus. With the animal in the supine position, the animal was prepared for forebrain ischemia. Then, after making an incision in the abdomen, the abdominal aorta was exposed, and a 24-gauge Teflon catheter was inserted into the abdominal aorta to monitor the blood pressure using a model AP-641G blood pressure amplifier (Nihon Kohden, Tokyo, Japan). Ten minutes after the drug was administered, forebrain ischemia for 4 min was induced by clamping the bilateral carotid arteries. Fifteen minutes after reperfusion, the blood sample was collected through the catheter to analyze serum glucose, electrolytes, and arterial blood gas levels according to routine laboratory procedures (blood glucose testing system by electrode, MPG01; Daikin, Osaka, Japan; ABL505 Radiometer; Copenhagen, Denmark). The rectal temperature was maintained at 37–38°C.

Experiment 2: Measurement of $[Ca^{2+}]_i$

Gerbils were anesthetized with ether and then decapitated. The hippocampi were removed rapidly and placed in an ice-cold physiologic medium (124 mM NaCl; 5 mM KCl; 2 mM $CaCl_2$; 2 mM $MgCl_2$; 1.25 mM NaH_2PO_4; 26 mM $NaHCO_3$; 10 mM glucose). Hippocampal transverse slices approximately 300 μm thick were cut with a vibrating slicer (DTK-1000; Dosaka Co., Kyoto, Japan). Three to five slices were obtained from each hippocampus and incubated in the physiologic medium equilibrated with a 95% oxygen and 5% carbon dioxide gas mixture for 1 h at 26°C. The slices were preloaded with a fluorescence indicator, rhod-2 acetoxymethyl ester (Dojin, Kumamoto, Japan), which was diluted to 20 μM in the physiologic medium and equilibrated with a 95% oxygen and 5% carbon dioxide gas mixture for 45 min at 26°C. After loading, the slices were further incubated in the physiologic medium for at least 30 min at 26°C.

The $[Ca^{2+}]_i$ levels were measured using an inverted fluorescence microscope, a high-performance video camera, and an image processor system. A low-magnification objective lens (×4) and a side illumination system were used to visualize the fluorescent image of the slice. The slice was transferred to a flow-through chamber (volume ~0.2 ml) mounted on the fluorescence microscope equipped with a heat plate stage (IMT2; Olympus) and superfused at 3 ml/min with the appropriate medium at 37°C. The temperature of the medium in the chamber was monitored using a thermocouple needle probe (0.4-mm diameter; TN-800; Unique

Medical Corp.) and a thermocouple meter (TME-300; Unique Medical Corp.). The slice was excited with 550 nm light produced by an ultraviolet lamp (100 W; Osram, Munich, Germany), filtered by an interference filter (550 nm; band width, <16 nm) and conducted to the slice through an optic fiber (5-mm diameter). The fluorescence signals (>580 nm) were captured on a silicon intensified target camera (C2400-8; Hamamatsu Photonics, Hamamatsu, Japan) and processed using an image processor (Argus-100; Hamamatsu Photonics).

Before $[Ca^{2+}]_i$ was measured, the slice loaded with rhod-2 was excited with 550 nm light, and the picture (on a television monitor) was examined to confirm that the dye was uniformly distributed throughout the slice.

Slices were evenly divided into the following four experimental groups. After the slice was placed into the chamber, the slice was perfused with the normoxic medium (a physiologic medium equilibrated with a 95% oxygen and 5% carbon dioxide gas mixture) for 15 min, and the $[Ca^{2+}]_i$ in the preischemic state was measured. *In vitro* hypoxia was induced by switching the normoxic medium to a glucose-free hypoxic medium equilibrated with a 95% nitrogen and 5% carbon dioxide gas mixture. The fluorescence intensity was measured in the image after the induction of *in vitro* hypoxia. Then the numerical values (pixels) were divided by the value of the corresponding element that had been taken before the measurement. Thus the ratio of $[Ca^{2+}]_i$ was obtained every 10 s.

The effects of lidocaine at concentrations of 10, 50, and 100 μM on the ischemia-like condition were evaluated. After the rhod-2 loading incubation, the slice was perfused with lidocaine-containing normoxic medium for 15 min after placement of the slice into the chamber, and then the medium was switched to the lidocaine-containing ischemia-like medium.

To investigate the effect of lidocaine in a condition free from extracellular Ca^{2+}, the slice was perfused with Ca^{2+}-free medium that was prepared by replacing $CaCl_2$ with $MgCl_2$ in normoxic and ischemia-like mediums. First, the slice was perfused with the Ca^{2+}-containing normoxic medium for 15 min after placement of the slice in the chamber, and then the medium was changed to the Ca^{2+}-free normoxic medium. After 5 min, the medium was switched to the Ca^{2+}-free ischemia-like medium.

Statistical Analysis

The data obtained from measuring the DC potential were analyzed by unpaired *t* tests. The data from the histologic experiments were evaluated with the Mann-Whitney test. The data from the fluorometry were analyzed using repeated two-way analysis of variance to detect differences among groups. When differences were found, the Scheffé's test was used *post hoc* to compare each value with that in the control group.

Results

The forebrain ischemia produced a slow change in the extracellular membrane potential in the early phase. Subsequently, a sudden and marked change was observed in the hippocampal CA1 area. The administration of lidocaine (0.8 μmol) produced a 140% prolongation of the onset latency of the rapid change in the DC potential shift. The maximal amplitude of the DC potential shift was suppressed to 67% of that in the control group. The recovery time and the duration at half-maximal amplitude of the depolarization revealed no significant differences between the two groups (fig. 1).

Animals in the control group regained consciousness and righting reflex within 30 min after halothane anesthesia was stopped. Similar to the control animals, the animals treated with lidocaine recovered from anesthesia within 30 min. There were no differences between the two groups in taking food and water in the 7 days between ischemia and sacrifice. No seizures were noted in either group. As shown in table 1, there were no differences in the physiologic variables between the two groups.

Histologically, most of the pyramidal cells had degenerated after 7 days in the control animals subjected to forebrain ischemia. In contrast, the intracerebroventricular administration of lidocaine (0.8 μmol) 10 min before the ischemia significantly reduced the damage (fig. 2).

When the hippocampal slices were perfused with the *in vitro* ischemia-like medium, almost no increase in the ratio of $[Ca^{2+}]_i$ was observed in the CA1 field within 240 s after the beginning of the *in vitro* ischemia. Subsequently, an acute and large increase in $[Ca^{2+}]_i$ spread throughout the CA1 field, and the ratio reached a plateau (figs. 3 and 4). When slices were perfused with the lidocaine-containing medium, the extent of the $[Ca^{2+}]_i$ increase was dose dependently inhibited, with the effects becoming significant at concentrations of 50 and 100 μM. The latencies of the onset of the increase in $[Ca^{2+}]_i$ in slices perfused with 50 and 100 μM lidocaine were also prolonged significantly, the values being 392 and 400 s (mean, n = 10), respectively.

Table 1. Physiologic Variables

	Control	Lidocaine
Mean arterial blood pressure and heart rate		
Before ischemia		
Blood pressure (mmHg)	71 ± 14	76 ± 10
Heart rate (bpm)	334 ± 24	346 ± 52
Ischemia		
Blood pressure (mmHg)	76 ± 13	76 ± 6
Heart rate (bpm)	304 ± 52	352 ± 30
Ischemia (2 min)		
Blood pressure (mmHg)	78 ± 19	75 ± 9
Heart rate (bpm)	306 ± 52	300 ± 42
Reperfusion		
Blood pressure (mmHg)	62 ± 17	74 ± 7
Heart rate (bpm)	308 ± 55	320 ± 66
Reperfusion (5 min)		
Blood pressure (mmHg)	65 ± 16	78 ± 8
Heart rate (bpm)	312 ± 57	364 ± 54
Reperfusion (10 min)		
Blood pressure (mmHg)	71 ± 9	81 ± 10
Heart rate (bpm)	342 ± 36	382 ± 55
Reperfusion (15 min)		
Blood pressure (mmHg)	71 ± 11	81 ± 12
Heart rate (bpm)	344 ± 36	378 ± 46
Arterial blood analysis		
Glucose (mg/dl)	144 ± 14	158 ± 39
*p*H	7.271 ± 0.041	7.285 ± 0.019
Pa_{CO_2} (mmHg)	36.7 ± 9.9	35.4 ± 4.0
Pa_{O_2} (mmHg)	392.5 ± 97.7	451.1 ± 51.5
HCO_3^- (mM)	16.4 ± 5.7	15.5 ± 1.4
Base excess (mM)	−9.8 ± 5.9	−10.4 ± 2.4
Na^+ (mM)	150.0 ± 3.7	150.2 ± 1.3
K^+ (mM)	3.1 ± 0.9	2.9 ± 0.7
Ca^{2+} (mM)	1.06 ± 0.13	1.03 ± 0.13

Lidocaine (0.8 μmol) was administered intracerebroventricularly under halothane anesthesia, and the mean arterial blood pressure was determined before ischemia, immediately after the start of ischemia, 2 min after the start of ischemia, immediately after reperfusion, 5 min after reperfusion, 10 min after reperfusion, and 15 min after reperfusion. Then 1 ml of blood was collected through the abdominal aorta. Each value represents the mean ± SD for five animals.

When slices were perfused with Ca^{2+}-free *in vitro* ischemia-like medium, an increase in $[Ca^{2+}]_i$ was observed in the CA1 field similar to that seen in the Ca^{2+}-containing condition (fig. 5). The mean value of the latencies of the increase in $[Ca^{2+}]_i$ was 236 s (mean, n = 10). Perfusion with lidocaine (100 μM)-containing medium produced a gradual and moderate increase in $[Ca^{2+}]_i$. The latency at the beginning of the increase in $[Ca^{2+}]_i$ was markedly prolonged to 384 s (mean, n = 10) by 100 μM lidocaine, and the ratio of fluorescence intensity after 600 s was depressed to 65% of that in the Ca^{2+}-free control.

Discussion

We have shown the improvement of the ischemia-induced neuronal damage by lidocaine: prolongation of the onset of the AD and suppression of its amplitude. In addition, inhibition of a hypoxia-induced increase in $[Ca^{2+}]_i$ by the lidocaine was observed.

The measurement of the DC potential shift between an extracellularly placed electrode in the brain and a remote electrode closely reflects the movement of Na^+, K^+, Cl^-, and Ca^{2+} across the membrane.[1] In cerebral ischemia, the depletion of adenosine 5′-triphosphate in the central nervous system and glutamate efflux from both neurons and glia progress together[11]; then the sudden depolarization of the neuronal membrane occurs with a huge Ca^{2+} influx into the neurons.[5] The latter phenomenon causes further adenosine 5′-triphosphate depletion and glutamate efflux. In the present experiment, the intracerebroventricular administration of lidocaine prolonged the onset of the AD and protected the neurons. One previous study showed that the increase in the extracellular concentration of excitatory amino acids such as glutamate and aspartate provoked by ischemia was depressed by the perfusion of this agent through a microdialysis probe.[7] Because these excit-

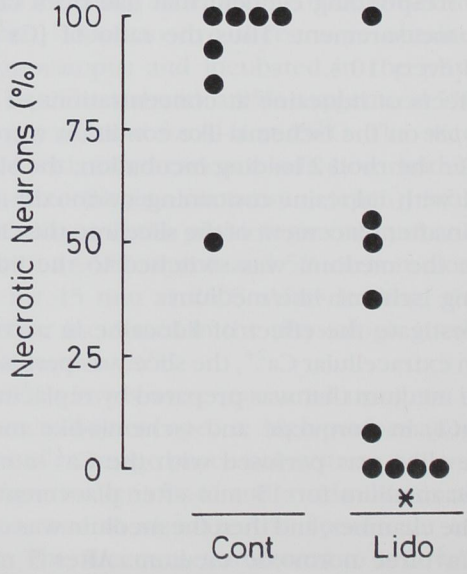

Fig. 2. Effects of lidocaine administered intracerebroventricularly 10 min before ischemia on the delayed neuronal death of CA1 pyramidal cells. CA1 pyramidal cells were examined 7 days after 4 min of ischemia, and the percentage of degenerated pyramidal cells (ordinate) was determined. Values obtained from individual animals are shown. Cont = saline-injected control group; Lido = lidocaine (0.8 μmol)-injected group. *$P < 0.01$ compared with the control group.

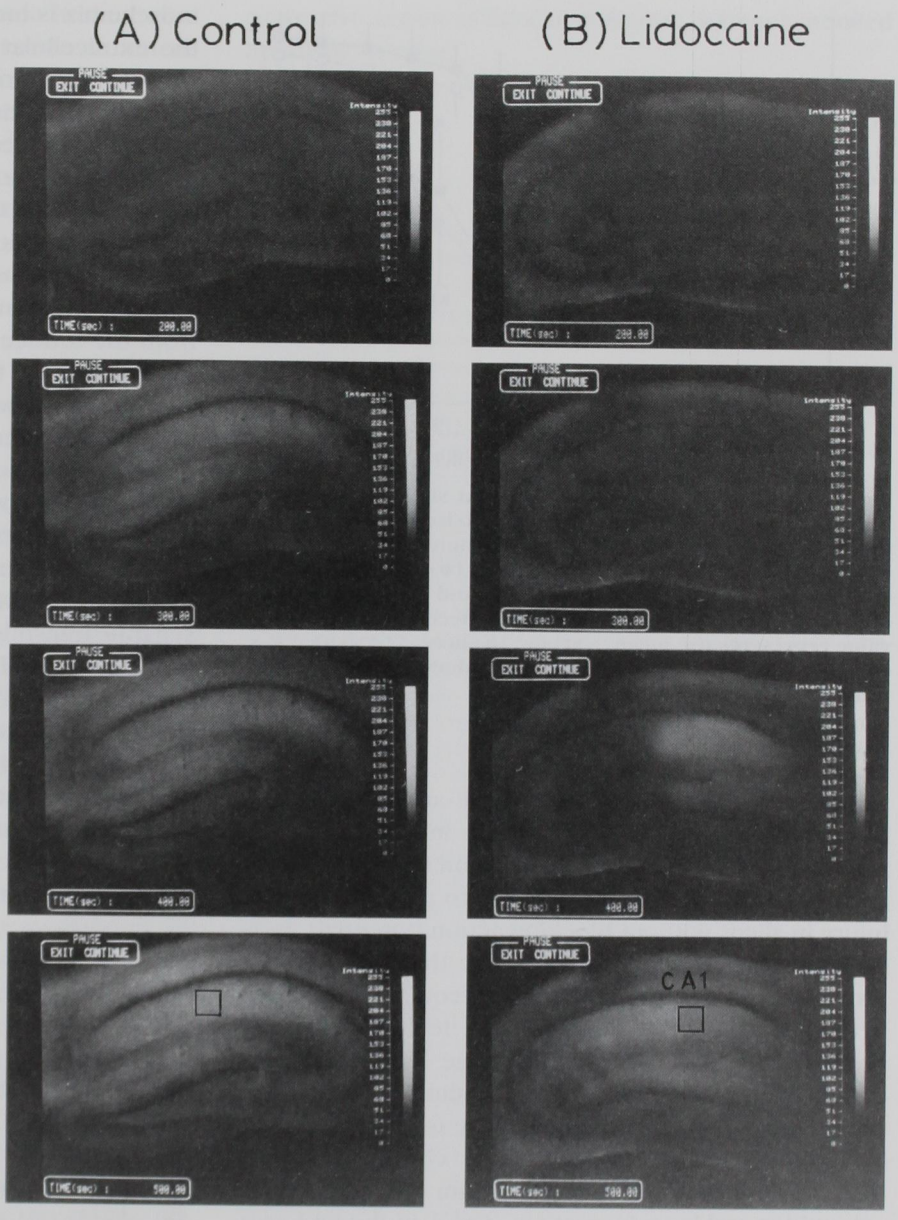

Fig. 3. Photographs showing $[Ca^{2+}]_i$ elevation induced by *in vitro* ischemia-like conditions in gerbil hippocampal slices. (*A*) the standard ischemia-like medium, (*B*) lidocaine (100 μM)-containing ischemia-like medium. *Open rectangles* represent measured areas.

atory neurotransmitters are released on the onset of the AD, the present preischemic administration of lidocaine may contribute to the histologic improvement by delaying cytosolic glutamate release.

In the histologic observation 7 days after the ischemia, transient forebrain ischemia for 4 min at a brain temperature of 37.5°C produced severe damage in the hippocampal CA1 region in control animals. This result corresponds to that in the previous report that transient forebrain ischemia for 3 min in normothermic conditions consistently induced severe damage in the CA1 field in gerbils.[10] The intracerebroventricular administration of lidocaine (0.8 μmol) 10 min before the ischemia markedly ameliorated this injury. The CA1 area is innervated by glutamatergic fibers,[12] which cause neuronal death in ischemia by their excess release of glutamate,[13] mainly by reversing the Na^+-cotransport system in a Ca^{2+}-independent manner.[14] Because the blockade of Na^+ channels by tetrodotoxin has been shown to reduce Ca^{2+}-independent excitatory amino acid release,[15] the action of lidocaine by blocking Na^+ channels may take part in the inhibition of the release of excitatory

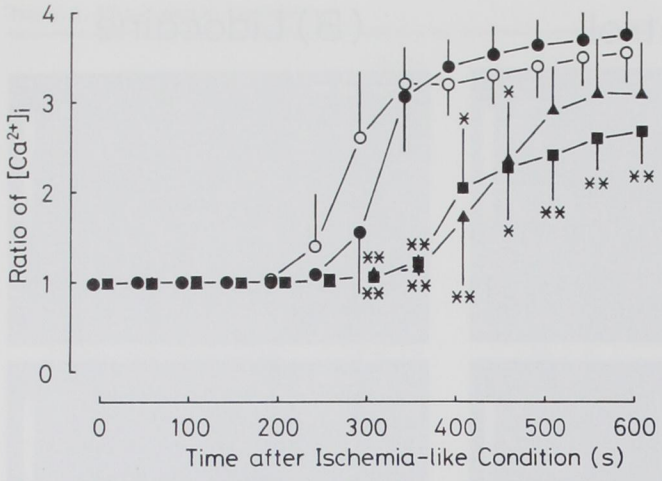

Fig. 4. Changes in the ratio of $[Ca^{2+}]_i$ in slices of the gerbil hippocampal CA1 field exposed *in vitro* to ischemia-like conditions. The standard ischemia-like medium (○); 10 μM lidocaine-containing, ischemia-like medium (●); 50 μM lidocaine-containing, ischemia-like medium (▲); and 100 μM lidocaine-containing, ischemia-like medium (■) effects are shown. Each value represents the mean ± SD for 10 slices. *$P < 0.01$, **$P < 0.001$ compared with the respective values in the standard ischemia-like group.

amino acids. The diminution of the amplitude of the AD, which represents the decrease in the ion fluxes, may also be the result of the inhibition of the glutamate release. Lidocaine has been shown to reduce ischemic injury in slices without blocking action potentials, indicating that the amount of lidocaine that needs to improve the damage is less than that required to achieve neural blockade.[16-18] Furthermore, tetrodotoxin suppresses the AD by preventing of the Na^+ load in the early stage of ischemia.[19] These findings indicate that the inhibition of initial events in the ischemic cascade by blocking voltage-dependent Na^+ channels reduces the damage provoked by downstream events.

A sudden and large increase in $[Ca^{2+}]_i$ in the CA1 area was observed in the *in vitro* experiment. This finding corresponds well with the selective vulnerability in this area, because the elevation of $[Ca^{2+}]_i$ in postsynaptic neurons after an excess release of glutamate is regarded as a crucial factor in the development of neuronal damage on ischemic attack.[20] Because the administration of lidocaine dose dependently prevented the increase in $[Ca^{2+}]_i$ on the extent and the onset, the improvement of the damage by lidocaine in the *in vivo* experiment may be the result of these inhibitory effects as well as the blockade of Na^+ channels.

The elevation of the cytosolic concentration of Ca^{2+} in ischemia is induced by both the influx of Ca^{2+} from the extracellular space and the release of Ca^{2+} from intracellular stores such as the endoplasmic reticulum and mitochondria. In this study, $[Ca^{2+}]_i$ in the Ca^{2+}-free ischemia-like medium increased in a manner similar to that in the Ca^{2+}-containing condition, and the ratio also attained nearly the same value after 600 s. This may indicate that most of the increase in $[Ca^{2+}]_i$ is from intracellular sources. However, it is unlikely that the increased amount of cytosolic Ca^{2+} is provided entirely from the intracellular sources. In the present study, it is not clear that the extracellular concentration of Ca^{2+} in the microenvironment of the slices decreased by switching the medium to the Ca^{2+}-free medium. In our previous study on recordings of extracellular DC and evoked field potentials combined with measurement of $[Ca^{2+}]$, field excitatory postsynaptic potential (EPSP) disappeared 4 min after switching the normoxic medium to Ca^{2+}-free normoxic medium, indicating that synaptic transmission was depressed by removing extracellular Ca^{2+}.[21] This suggests that the extracellular concentration of Ca^{2+} is reduced to negligible levels at this point on the electrophysiologic aspect. Although the *in vitro* hypoxia was induced 5 min after the onset of perfusion with Ca^{2+}-free normoxic medium in the present experiment, $[Ca^{2+}]_i$ in the Ca^{2+}-free ischemia-like medium increased in a manner similar to that observed in the Ca^{2+}-containing condition. This similarity

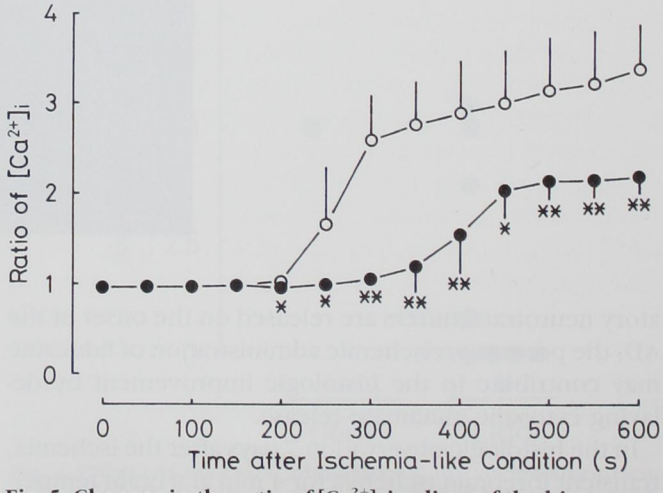

Fig. 5. Changes in the ratio of $[Ca^{2+}]_i$ in slices of the hippocampal CA1 field that underwent Ca^{2+}-free *in vitro* ischemia-like conditions. The Ca^{2+}-free, *in vitro* ischemia-like medium (○); and 100 μM lidocaine-containing, Ca^{2+}-free, *in vitro* ischemia-like medium (●) effects are shown. Each value represents the mean ± SD for ten slices. *$P < 0.01$, **$P < 0.001$ compared with the respective values in the Ca^{2+}-free ischemia-like group.

may be caused by an insufficient decrease in the extracellular concentration of Ca^{2+} in the microenvironment of the slices, whereas field EPSP is inhibited at this concentration. The Ca^{2+} influx is generally presumed to be caused through voltage-gated Ca^{2+} channels and through agonist-gated Ca^{2+} channels, mainly N-methyl-D-aspartate-gated Ca^{2+} channels. Because voltage-gated Ca^{2+} channels are quickly inactivated during a maintained depolarization,[22] the major component of Ca^{2+} influx induced by *in vitro* hypoxia may be through N-methyl-D-aspartate-gated Ca^{2+} channels.

Two mechanisms of the release of Ca^{2+} from the endoplasmic reticulum to cytosol have been clarified.[23,24] One is the release through ryanodine receptors, which exist on the membrane of the endoplasmic reticulum. When Ca^{2+} flows into the cytosol from the extracellular space through mainly N-methyl-D-aspartate-gated Ca^{2+} channels in an ischemic event, the increase in cytosolic Ca^{2+} stimulates ryanodine receptors, thereby releasing Ca^{2+} from intracellular Ca^{2+} stores (Ca^{2+}-induced Ca^{2+} release). The other mechanism involves the inositol 1,4,5-triphosphate (IP_3) receptors on the endoplasmic reticulum. Stimulation of postsynaptic metabotropic glutamate receptors enhances the activation of phospholipase C, resulting in the facilitation of phosphatidylinositol turnover. Thus IP_3 receptor-linked Ca^{2+} channels open in response to the increase in the intracellular concentration of IP_3, which causes the release of Ca^{2+} (IP_3-induced Ca^{2+} release). However, the mechanism by which lidocaine reduces the release of Ca^{2+} from the endoplasmic reticulum was not made clear in the present study. There is a report that IP_3 receptors are highly localized in pyramidal cells in the hippocampal CA1 area and Purkinje cells in the cerebellum,[25] both of which are vulnerable to ischemia. In contrast, ryanodine receptors, which cause Ca^{2+}-induced Ca^{2+} release, are widely distributed in the whole brain.[26] In another study, lidocaine was shown to facilitate the affinity for ryanodine and the rate of ryanodine association with its binding site in a nonischemic condition.[8] For these reasons, lidocaine seems to inhibit the IP_3 receptor-mediated Ca^{2+} release in ischemia.

In this study, lidocaine prevented the increase in $[Ca^{2+}]_i$ during ischemia, probably as a result of an inhibition of the release of Ca^{2+} from the intracellular Ca^{2+} stores and the inhibition of the influx from the extracellular space. The reduction of $[Ca^{2+}]_i$ in the early stage of ischemia plays an important role in preventing the cascade leading to neuronal damage, and the present findings contribute to our understanding of the neuroprotective action of lidocaine against ischemia-induced neuronal damage.

References

1. Hansen AJ: Effect of anoxia on ion distribution in the brain. Physiol Rev 1985; 65:101–48

2. Benveniste H, Drejer J, Schousboe A, Diemer NH: Elevation of the extracellular concentrations of glutamate and aspartate in the rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. J Neurochem 1984; 43:1369–74

3. Phebus LA, Clemens JA: Effects of transient, global, cerebral ischemia on striatal extracellular dopamine, serotonin and their metabolites. Life Sci 1989; 44:1335–42

4. Adachi N, Oishi R, Saeki K: Changes in the metabolism of histamine and monoamines after occlusion of the middle cerebral artery in rats. J Neurochem 1991; 57:61–6

5. Mitani A, Takeyasu S, Yanase H, Nakamura Y, Kataoka K: Changes in intracellular Ca^{2+} and energy levels during in vitro ischemia in the gerbil hippocampal slice. J Neurochem 1994; 62:626–34

6. Ritchie JM, Greengard AG: On the mode of action of local anesthetics. Annu Rev Pharmacol 1966; 6:405–30

7. Fujitani T, Adachi N, Miyazaki H, Liu K, Nakamura Y, Kataoka K, Arai T: Lidocaine protects hippocampal neurons against ischemic damage by preventing increase of extracellular excitatory amino acids: a microdialysis study in Mongolian gerbils. Neurosci Lett 1994; 179:91–4

8. Martin C, Ashley R, Shoshan-Barmatz V: The effect of local anesthetics on the ryanodine receptor/Ca^{2+} release channel of brain microsomal membranes. FEBS Lett 1993; 328:77–81

9. Ginsberg MD, Welsh FA, Budd WW: Deleterious effects of glucose pretreatment on recovery from diffuse cerebral ischemia in cat. I. Local cerebral blood flow and glucose utilization. Stroke 1980; 11:347–54

10. Mitani A, Andou Y, Masuda S, Kataoka K: Transient forebrain ischemia of three-minute duration consistently induces severe neuronal damage in field CA1 of the hippocampus in the normothermic gerbil. Neurosci Lett 1991; 131:171–4

11. Mitani A, Andou Y, Matsuda S, Arai T, Sakanaka M, Kataoka K: Origin of ischemia-induced glutamate efflux in the CA1 field of the gerbil hippocampus: An in vivo brain microdialysis study. J Neurochem 1994; 63:2152–64

12. Storm-Mathisen J: Glutamate in hippocampal pathways, Glutamate as a Neuro-transmitter. Edited by G diChiara, GL Gessa. New York, Raven Press, 1981, pp 43–55

13. Jørgensen MB, Diemer NH: Selective neuron loss after cerebral ischemia in the rat: Possible role of transmitter glutamate. Acta Neurol Scand 1982; 66:536–46

14. Sánchez-Prieto J, González P: Occlusion of a large Ca^{2+}-independent release of glutamate during anoxia in isolated nerve terminals (synaptosomes). J Neurochem 1988; 50:1322–4

15. Lysko PG, Webb CL, Yue TL, Gu JL, Feuerstein G: Neuroprotective effects of tetrodotoxin as a Na^+ channel modulator and glutamate release inhibitor in cultured rat cerebellar neurons and in gerbil global brain ischemia. Stroke 1994; 25:2476–82

16. Weber ML, Taylor CP: Damage from oxygen and glucose deprivation in hippocampal slices is prevented by tetrodotoxin, lidocaine

and phenytoin without blockade of action potentials. Brain Res 1994; 664:167–77

17. Taylor CP, Burke SP, Weber ML: Hippocampal slices: glutamate overflow and cellular damage from ischemia are reduced by sodium-channel blockade. J Neurosci Meth 1995; 59:121–8

18. Fried E, Amorim P, Chambers G, Cottrell JE, Kass IS: The importance of sodium for anoxic transmission damage in rat hippocampal slices: Mechanisms of protection by lidocaine. J Physiol 1995; 489:557–65

19. Xie Y, Dengler K, Zacharias E, Wilffert B, Tegtmeier F: Effects of the sodium channel blocker tetrodotoxin (TTX) on cellular ion homeostasis in rat brain subjected to complete ischemia. Brain Res 1994; 652:216–24

20. Rothman SM, Olney JW: Excitatory and NMDA receptor. Trends Neurosci 1987; 10:299–302

21. Mitani A, Yanase H, Sakai K, Wake Y, Kataoka K: Origin of intracellular Ca^{2+} elevation induced by in vitro ischemia-like condition in hippocampal slices. Brain Res 1993; 601:103–10

22. Ozawa S, Tsuzuki K, Iino M, Ogura A, Kudo Y: Three types of voltage-dependent calcium current in cultured rat hippocampal neurons. Brain Res 1989; 495:329–36

23. Tsien RW, Tsien RY: Calcium channels, stores and oscillations. Annu Rev Cell Biol 1990; 6:715–60

24. Henzi V, MacDermott AB: Characteristics and function of Ca^{2+}- and inositol 1,4,5-triphosphate-releasable stores of Ca^{2+} in neurons. Neuroscience 1992; 46:251–73

25. Nakanishi S, Maeda N, Mikoshiba K: immunohistochemical localization of an inositol 1,4,5-triphosphate receptor, P_{400}, in neuronal tissue: Studies in developing and adult mouse brain. J Neurosci 1991; 11:2075–86

26. Padua RA, Wan WH, Nagy JI, Geiger JD: [^{3}H]ryanodine binding sites in rat brain demonstrated by membrane binding and autoradiography. Brain Res 1991; 542:135–40