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EFFECT OF LIDOCAINE ON DYNAMIC CHANGES IN CORTICAL REDUCED NICOTINAMIDE ADENINE DINUCLEOTIDE FLUORESCENCE DURING TRANSIENT FOCAL CEREBRAL ISCHEMIA IN RATS ☆

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Abstract—Rats were subjected to 90 min of focal ischemia by occluding the left middle cerebral and both common carotid arteries. The dynamic changes in the formation of brain ischemic areas were analyzed by measuring the direct current (DC) potential and reduced nicotinamide adenine dinucleotide (NADH) fluorescence with ultraviolet irradiation. In the lidocaine group ($n = 10$), 30 min before ischemia, an intravenous bolus (1.5 mg/kg) of lidocaine was administered, followed by a continuous infusion (2 mg/kg/h) for 150 min. In the control group ($n = 10$), an equivalent amount of saline was administered. Following the initiation of ischemia, an area of high-intensity NADH fluorescence rapidly developed in the middle cerebral artery territory in both groups and the DC potential in this area showed ischemic depolarization. An increase in NADH fluorescence closely correlated with the DC depolarization. The blood flow in the marginal zone of both groups showed a similar decrease. Five minutes after the onset of ischemia, the area of high-intensity NADH fluorescence was significantly smaller in the lidocaine group (67% of the control; $P = 0.01$). This was likely due to the suppression of ischemic depolarization by blockage of voltage-dependent sodium channels with lidocaine. Although lidocaine administration did not attenuate the number of peri-infarct depolarizations during ischemia, the high-intensity area and infarct volume were significantly smaller in the lidocaine group both at the end of ischemia (78% of the control; $P = 0.046$) and 24 h later ($P = 0.02$). A logistic regression analysis demonstrated a relationship between the duration of ischemic depolarization and histologic damage and revealed that lidocaine administration did not attenuate neuronal damage when the duration of depolarization was identical. These findings indicate that the mechanism by which lidocaine decreases infarct volume is primarily through a reduction of the brain area undergoing NADH fluorescence increases which closely correlates with depolarization.

Key words: hypoxia, infarction, lidocaine, NADH fluorescence, DC potential, depolarization.

INTRODUCTION

Cerebral infarction is a potential consequence of brain ischemia, which may be induced by neurologic or cardiac surgery. Lidocaine may protect neurons during brain ischemia; a number of studies have suggested that lidocaine has neuroprotective effects. In two randomized, double-blinded, prospective clinical studies, continuous administration of lidocaine significantly ameliorated postoperative cognitive dysfunction in patients undergoing cardiac surgery for left heart valve procedures (Mitchell et al., 1999) or coronary artery bypass surgery with cardiopulmonary bypass (Wang et al., 2002). In animal models of ischemia, administration of lidocaine has been shown to ameliorate cognitive dysfunction (Popp et al., 2011) and reduce infarct volume (Shokunbi et al., 1990; Lei et al., 2001, 2004). Sodium entry and the loss of membrane potential comprise some of the first steps in the development of ischemic neuronal damage. The primary neuroprotective mechanism of lidocaine may be related to blockade of voltage-dependent sodium channels, which results in the suppression of cellular energy requirements (Fried et al., 1995; Raley-Susman et al., 2001; Seyfried et al., 2005) and delayed ischemic depolarization (Ayad et al., 1994; Liu et al., 1997). The mechanism by which lidocaine decreases infarction after focal ischemia is not fully understood but may be associated with suppression of the size of the area of ischemic depolarization, an attenuation of the peri-infarct depolarizations (PIDs), and/or a reduction of neurologic damage during the ischemic depolarization.

Nicotinamide adenine dinucleotide/reduced nicotinamide adenine dinucleotide (NAD^+/NADH) is an electron carrier in the mitochondrial respiratory chain. Under conditions of ischemia, NADH accumulates in mitochondria due to the increase in anaerobic glycolysis and an inhibition of mitochondrial oxidative phosphorylation. NADH fluorescence has been used in the past to identify the redox state of mitochondria (Eng

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Abbreviations: ANOVA, analysis of variance; CBF, cerebral blood flow; CCA, common carotid artery; DC, direct current; MCA, middle cerebral artery; NAD^+, nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; PID, peri-infarct depolarization.

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et al., 1989; Takeda et al., 2004) due to the unique behavior of NADH which, when excited with ultraviolet light (366 nm), the reduced form (NADH) fluoresces (460 nm emission), while the oxidized form (NAD^+) does not. Because membrane depolarization is accompanied by an increase in energy demand that leads to the accumulation of NADH in mitochondria, dynamic changes on the cortical surface can be visualized by NADH fluorescence (Strong et al., 1996; Higuchi et al., 2002; Sasaki et al., 2009).

The purpose of this study is to elucidate the effect of lidocaine on dynamic changes in areas of ischemic depolarization, the propagation of PIDs, and the relationship between ischemic depolarization and neurologic damage. We used simultaneous NADH fluorescence images and two-point direct current (DC) potential measurements to provide both temporal and spatial observations during 90 min of focal ischemia.

EXPERIMENTAL PROCEDURES

Animals

All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Research Control Committee of Okayama University Medical School. All efforts were made to minimize the number of animals used and their suffering. Twenty-two male Sprague Dawley rats (Charles River Laboratories Japan, Yokahama, Japan), weighing 304 ± 23 g, were used as a model of transient focal ischemia in this study. The animals were deprived of food for 8 h prior to the experiments.

Experimental groups

Animals were randomly assigned to two groups: the lidocaine group (n = 10) and the control group (n = 10). An additional two rats were placed in a separate sham operation group. In the lidocaine group, a bolus dose of lidocaine (1.5 mg/kg, at a concentration of 1 mg/mL in saline) was administered intravenously 30 min before the initiation of ischemia. The bolus was followed by a continuous infusion of lidocaine at 2 mg/kg/h, which was continued until 30 min after reperfusion. In the control group, an equivalent amount of saline was administered as sham treatment.

General procedures

Anesthesia was induced with a mixture of 4% isoflurane in oxygen. After oral tracheal intubation, anesthesia was maintained by artificial ventilation (SN-480-7, Shinano Manufacturing, Tokyo, Japan) with 2% isoflurane in 40% oxygen balanced with nitrogen. A polyethylene catheter (PE50) was placed in the right femoral artery for continuous mean arterial blood pressure monitoring and blood sampling and another was placed in the right femoral vein for the administration of saline or lidocaine. Arterial blood gases were measured (i-STAT 300F, Abbott Point of Care, Princeton, NJ, USA) before ischemia and maintained within the normal range. After the animal was placed in a stereotaxic apparatus (Narishige, Tokyo, Japan), an incision was made on the scalp. The parietal-temporal bone was exposed and the temporal muscles were partially resected. Then, a large cranial window (12 × 9 mm) was made in the left parietal-temporal bone for the observation of NADH fluorescence. The dura was left in place. To monitor the cortical depolarization associated with the initiation of focal ischemia, two borosilicate glass DC electrodes (World Precision Instruments, Sarasota, FL, USA), tip diameter < 5 μm, were placed through dural incisions in the cranial window and lowered to a depth of 750 μm below the cortical surface. The positions of the electrodes were 2 mm posterior to the bregma and 2 and 4 mm lateral to the sagittal line, respectively. To measure regional cerebral blood flow (CBF), a laser Doppler flow probe (OmegaFlo FLO-C1, Omegawave, Tokyo, Japan) was placed adjacent to the distal DC electrode. Rectal temperature was monitored and maintained at 37.0 ± 0.5 °C using a heated water blanket. Target brain surface temperature was maintained at 37 °C in accordance with rectal temperature. To control the brain surface temperature, a gentle flow of warm saline was perfused over the brain surface (Kaplan et al., 1991). Since a pilot study determined that the brain surface temperature was within 1.0 ± 0.5 °C below the drip temperature, the drip temperature was measured and maintained at 38.0 ± 0.1 °C (brain surface temperature, 37.0 ± 0.5 °C).

The surgical preparation for the initiation of focal cerebral ischemia was performed as described by Buchan and colleagues (Buchan et al., 1992). The common carotid arteries (CCAs) were exposed bilaterally and loose ligatures were placed around them. The left middle cerebral artery (MCA) was accessed by piercing the dura inside the cranial window 0–1 mm above the rhinal fissure. Ischemia was initiated by tightening ligatures around the bilateral CCAs and by occluding the left MCA via lifting the vessel to 0–1 mm above the rhinal fissure with an 80-μm stainless-steel hook attached to a micromanipulator. Reperfusion was initiated after 90 min of focal ischemia. The adequacy of circulation after relief of middle cerebral and CCA occlusion was assessed by direct visual observation with a surgical microscope and by observing the increase in flow with a laser Doppler flowmeter. After reperfusion, the removed fragment of the parietal-temporal bone was replaced and all the wounds were closed. The rats were extubated and returned to their cages with free access to food and water. Despite partial removal of the parietal-temporal bone, rats were still able to eat and drink. The rats were allowed to survive for 24 h after the onset of ischemia. An additional two rats underwent a sham operation in which the hook was placed in contact with a portion of the MCA and removed without lifting the artery.

Technique for cortical NADH fluorescence imaging

The technique used for cortical NADH fluorescence imaging has previously been described in detail (Hashimoto et al., 2000; Higuchi et al., 2002; Sasaki et al., 2009). Briefly, to excite NADH in the cortical tissues, the cortical surface was illuminated using a 200-W Xenon lamp (Hamamatsu Photonics, Hamamatsu, Japan) equipped with a 365-nm bandpass filter (Asahi Spectra, Tokyo, Japan). Images of NADH fluorescence were obtained using a charge-coupled device camera (ST-9XE, Santa Barbara Instrument Group, Santa Barbara, CA, USA) with a 460-nm bandpass filter. Images (170 × 170 pixels, width × height) were captured every 15 s. Fluorescence intensity values for each pixel in each NADH fluorescence image were divided by those of the control image obtained before the initiation of focal ischemia and the percent change (80–120%) in NADH fluorescence was expressed in each pixel using a 256 gray-scale.

Histologic evaluation

Twenty-four hours after the onset of ischemia, all animals were anesthetized with 4% isoflurane. After inserting a cannula into the ascending aorta, each animal was perfused with heparinized physiologic saline (20 units/mL) and 6%