Brief Communication

Gabapentin reduces infarct volume but does not suppress peri-infarct depolarizations

Ulrike Hoffmann^{1,3}, Jeong Hyun Lee^{1,3}, Tao Qin¹, Katharina Eikermann-Haerter¹ and Cenk Ayata^{1,2}

¹Neurovascular Research Laboratory, Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Charlestown, Massachusetts, USA; ²Stroke Service and Neuroscience Intensive Care Unit, Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Charlestown, Massachusetts, USA

Spreading depression (SD) is an intense depolarization wave implicated in brain injury. In focal ischemia, recurrent peri-infarct depolarization (PID) waves akin to SD worsen the ischemic injury by exacerbating the blood flow-metabolism mismatch. We recently showed that gabapentin suppresses SD. We, therefore, tested gabapentin on PIDs and stroke outcome. Gabapentin pretreatment (200 mg/kg, intravenously) reduced the infarct volume by 23% after transient focal ischemia in mice. However, the frequency and duration of PIDs were not suppressed when recorded for 2 hours during ischemia, suggesting that gabapentin reduces infarct volume independent of PID suppression.

Journal of Cerebral Blood Flow & Metabolism (2011) **31**, 1578–1582; doi:10.1038/jcbfm.2011.50; published online 20 April 2011

Keywords: gabapentin; peri-infarct depolarization; spreading depression; stroke

Introduction

Peri-infarct depolarizations (PIDs) are intense neuronal and glial depolarization waves akin to spreading depression (SD) originating within ischemic penumbra in experimental animals, as well as in stroke patients (Dohmen *et al*, 2008). Although SDs in normal brain do not cause injury, PIDs worsen the ischemic tissue outcome by increasing oxygen and glucose utilization and reducing tissue perfusion, thereby exacerbating the metabolic mismatch in compromised tissue (Nakamura *et al*, 2010; Shin *et al*, 2006; Strong *et al*, 2007). Therefore, suppression of PIDs may afford protection in ischemic brain. However, drugs that are known to suppress SD

acutely often have unfavorable neurologic side effects precluding their use in critically ill patients. Gabapentin is an analgesic, adjunct antiepileptic, and migraine prophylactic drug, which targets the auxiliary $\alpha_2\delta$ subunit of $\text{Ca}_v2.1$ channels to inhibit the channel and attenuate presynaptic Ca^{2+} influx and neurotransmitter release. We recently showed that gabapentin can suppress SD susceptibility after a single dose in experimental animals (Hoffmann *et al*, 2010). We, therefore, tested whether gabapentin, widely used in clinical practice with an excellent safety and tolerability profile, can also suppress PIDs to explain its previously reported protective effect in acute ischemic stroke (Williams *et al*, 2006).

Materials and methods

Institutional guidelines for animal care and use for research purposes were strictly followed, and study protocol was approved by institutional review board. A total of 59 mice (C57BL/6, male, 25 to 30 g, Charles River Laboratories, Kingston, NY, USA) were used. Gabapentin was administered as a single 200 mg/kg intravenous dose (Toronto Research Chemicals, Ontario, Canada) 60 minutes before stroke induction; 50 and 400 mg/kg were tested in smaller groups. Intravenous route was used because of the

Correspondence: Dr C Ayata, Department of Radiology, Neurovascular Research Laboratory, Massachusetts General Hospital, 149 13th Street, Room 6408, Charlestown, MA 02129, USA. E-mail: cayata@partners.org

³These two authors contributed equally to this work and listed in alphabetical order.

This work was supported by the National Institute of Health (NS061505, NS055104), Deutsche Forschungsgemeinschaft (HO 4211/1-1) and the American Heart Association (10SDG2610275). Received 5 March 2011; revised 19 March 2011; accepted 23 March 2011; published online 20 April 2011

saturable oral absorption kinetics and nonlinear bioavailability (Radulovic *et al*, 1995). Gabapentin dose and treatment protocol were chosen based on published data (Hoffmann *et al*, 2010; Radulovic *et al*, 1995; Traa *et al*, 2008). Saline was used as vehicle control. Mice were randomly assigned into vehicle and treatment groups. Ischemia induction and outcome assessments were performed in a masked manner.

Tissue and Neurologic Outcome

Middle cerebral artery was occluded (fMCAO) under isoflurane anesthesia (2.5% induction, 1% maintenance, in 70% N₂O/30% O₂) for 1 hour, using an intraluminal silicone-coated 7-0 monofilament inserted into the internal carotid via the external carotid artery. Cerebral blood flow (CBF) reduction and reperfusion were monitored using a laser Doppler probe placed over the ischemic core. Mice were kept in a temperature-controlled warming chamber during and for 2 hours after fMCAO. Twenty four hours after stroke onset, mice were neurologically examined and deficit graded based on a 5-point scale: 0, normal; 1, failure to extend the forepaw fully when suspended vertically; 2, circling to the contralateral side; 3, falling or leaning over to the contralateral side; 4, no spontaneous walking and a depressed level of consciousness. Mice were then deeply anesthetized, brains harvested, and infarcts assessed using triphenyltetrazolium chloride (Sigma-Aldrich, St Louis, MO, USA) staining on 1-mm-thick coronal slices. Infarct areas at each slice level were integrated to obtain a final infarct volume. Both direct and indirect (contralateral hemisphere minus ipsilateral non-infarcted volume) infarct volumes were calculated to assess ischemic swelling (direct minus indirect infarct volume). Animals that prematurely died within 24 hours were excluded from outcome analysis (see Results). Incomplete ischemia (CBF > 20%) or reperfusion (CBF < 50%), or evidence for subarachnoid hemorrhage directly visualized during brain harvesting at 24 hours, were *a priori* criteria for exclusion, although none of the animals fulfilled these criteria for exclusion in this study.

Electrophysiologic Monitoring of peri-infarct depolarizations

The occurrence and duration of PIDs were studied in a separate group of mice under isoflurane (spontaneously breathing, 5% induction, 1% maintenance, in 70% N₂O/30% O₂) or pentobarbital (50 mg/kg, intraperitoneal) anesthesia (mechanically ventilated), and full physiologic monitoring. Blood pressure was continuously recorded (PowerLab; ADInstruments, Colorado Springs, MO, USA) and arterial blood gases and pH were measured every 30 minutes via a femoral artery catheter in spontaneously breathing (*n* = 17) or mechanically ventilated mice (*n* = 12). Rectal temperature was kept at 37°C using a thermostatic heating pad (FHC, Bowdoinham, ME, USA). Level of anesthesia was maintained throughout the experiment to eliminate cardiovascular response to tail pinch. In all groups, systemic physiologic parameters were within normal range for anesthetized mice (Table 1).

After general surgical preparation, fMCAO was performed as described above, mice were quickly placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA), and two burr holes (< 1 mm diameter) were drilled under saline cooling at (posterior and lateral from bregma, respectively): -0.5 and 1.5 mm; 2.5 and 1.5 mm. Dura was kept intact and care was taken to avoid bleeding. The steady (DC) potential and electrocorticogram were recorded with glass micropipettes filled with 200 mmol/L NaCl, inserted 300 μm below pia (Axoprobe-1A; Axon Instruments, Burlingame, CA). Ag/AgCl reference electrode was placed subcutaneously in the neck. The data were continuously recorded using a data acquisition system for off-line analysis (ADInstruments, Colorado Springs, MO). All surgical procedures were completed and electrophysiologic recordings started within 30 minutes after fMCAO onset and continued for 2 hours.

Statistical Analysis

The systemic and electrophysiologic data, as well as the stroke outcome, were compared between saline and

Table 1 Systemic physiology and peri-infarct depolarizations

	Isoflurane		Pentobarbital	
	Saline (n = 10)	Gabapentin (n = 9)	Saline (n = 6)	Gabapentin (n = 6)
PID frequency (/h) ^a	2.3 ± 0.3	2.8 ± 0.4	2.7 ± 1.0	3.2 ± 0.7
Average PID duration (sec)	66 ± 13	49 ± 4	55 ± 9	105 ± 48
Cumulative PID duration (sec/2 hours)	291 ± 42	306 ± 13	147 ± 59	248 ± 90
Average PID amplitude (mV)	24 ± 1	25 ± 1	25 ± 2	25 ± 3
pH	7.35 ± 0.02	7.33 ± 0.01	7.36 ± 0.02	7.35 ± 0.04
pCO ₂ (mm Hg)	35 ± 2	37 ± 2	32 ± 4	34 ± 6
pO ₂ (mm Hg) ^a	127 ± 5	138 ± 3	170 ± 13	154 ± 9
Blood pressure (mm Hg) ^b	78 ± 2	76 ± 2	90 ± 3	79 ± 7

PID, peri-infarct depolarization.

Saline (0.1 ml, i.v.) and gabapentin (200 mg/kg, i.v.) were administered 1 h before filament occlusion of middle cerebral artery.

^a*P* < 0.05, pentobarbital versus isoflurane.

^b*P* < 0.05, saline versus gabapentin.

Figure 1 Gabapentin improves ischemic outcome but does not suppress peri-infarct depolarizations (PIDs) after middle cerebral artery occlusion. Gabapentin (200 mg/kg, i.v., 60 minutes before ischemia) reduced infarct volume when assessed 24 hours after 60 minutes transient fMCAO (**A**). Infarct reduction was similar between cortical and subcortical tissues and apparent in most coronal slice levels (**B**). Neurologic deficits, measured using a 5-point scale (see Materials and Methods), also tended to be milder in gabapentin-treated mice ($P = 0.067$) (**C**), whereas the cerebral blood flow (CBF) reduction or reperfusion in ischemic core did not differ between the groups as measured by laser Doppler flowmetry (**D**). (**E**) Representative 2 hours extracellular steady (DC) potential recordings showing recurrent spontaneous PIDs in saline- or gabapentin-treated mice (200 mg/kg, i.v., 60 minutes before ischemia). (**F**) Graphs showing the time of occurrence of each PID in saline and gabapentin groups. Each horizontal line indicates the onset and end of recordings in individual experiments, whereas each symbol indicates a PID. (**G**) Pooled cumulative PID occurrence in all experiments as a function of time show that PID occurrence rate tended to increase during the second hour of recording in both groups. Gabapentin did not alter PID occurrence when compared with saline-treated mice. Cumulative PID numbers were corrected to reflect the small difference in the number of mice in each group. * $P < 0.05$ gabapentin versus saline.

gabapentin groups using *t*-test or Mann–Whitney test. Mortality was compared using χ^2 test. Data were expressed as mean $\pm$ standard error for systemic physiology, electrophysiology and number of PIDs, or as median (25 to 75% range) for neurologic score. $P < 0.05$ was considered statistically significant.

Results

Gabapentin reduced the infarct volume by 23% compared with saline-treated mice (indirect infarct 56 ± 3 versus 73 ± 4 mm³; $n = 8$ and 9 , respectively; $P = 0.005$; Figures 1A and 1B). Infarct reduction was similar in cortex and subcortical tissues, and present at all coronal levels. The ischemic swelling volume did not differ between groups (8 ± 3 versus 9 ± 2 mm³). Neurologic deficits tended to be milder as well in the gabapentin-treated group (1.5 (1.0 to 2.0) versus 2.0 (2.0 to 2.0), gabapentin and saline, respectively; $P = 0.067$; Figure 1C), whereas mortality did not significantly differ from the controls (1 out of 9 versus 3 out of 12, in gabapentin and saline groups, respectively; $P = 0.42$). Cerebral blood flow in the ischemic core also did not differ between the groups during ischemia and reperfusion (Figure 1D).

Despite smaller infarct volumes, the frequency and temporal pattern of PIDs during the 2 hours recording period, and their average and cumulative durations were not reduced by 200 mg/kg gabapentin (Figures 1E–G; Table 1). Additional experiments using a lower or a higher dose also did not suppress PIDs (50 and 400 mg/kg, $n = 5$ and 2 , respectively; data not shown). Because isoflurane is known to suppress SD susceptibility, we also tested gabapentin under pentobarbital anesthesia. Although we found higher PID frequencies compared with isoflurane anesthesia, there was again no difference between the treatment groups (Table 1). Electrographic epileptiform discharges were rarely observed under our experimental conditions, and their incidence or duration did not appear to differ between treatment groups (not shown).

Discussion

Our data show that a single pre-ischemic dose of gabapentin reduces infarct volume in an established stroke model in mice, as previously shown in rats (Williams *et al*, 2006). This neuroprotective effect was not accompanied by electrophysiologic PID suppression in acutely ischemic cortex, suggesting alternative mechanisms by which gabapentin prevents infarct expansion. One such mechanism may be suppression of postischemic seizure activity (Traa *et al*, 2008; Williams *et al*, 2006). Although we could not electrophysiologically show this because of general anesthesia, suppression of convulsive or non-convulsive seizures after reperfusion might have contributed to gabapentin's neuroprotective efficacy. Notably, after the initial flurry of PIDs during ischemia, a second phase of PIDs does occur peaking around 12 hours after reperfusion and coinciding with infarct growth (Hartings *et al*, 2003). Therefore, it is also possible that these delayed PIDs are more

susceptible to and were suppressed by gabapentin to explain the infarct reduction, although we find this unlikely after a single pre-ischemic dose of gabapentin with a relatively short plasma half-life (Radulovic *et al*, 1995).

Several factors may have contributed to the lack of efficacy of gabapentin on PIDs. First, elevated extracellular [K⁺] (e.g., ischemic penumbra) diminishes the efficacy of drugs to inhibit SD (Petzold *et al*, 2005; Shin *et al*, 2006). Our recordings were indeed obtained from hypoperfused tissue because the duration of PIDs at the stated coordinates was almost doubled compared with SDs in normal mouse cortex (Eikermann-Haerter *et al*, 2009). Therefore, under penumbra conditions, gabapentin may have lost its efficacy to suppress SD. Second, inhalational anesthetics are known to suppress SD (Kudo *et al*, 2008) and may have abrogated the PID suppression by gabapentin. However, this is unlikely because we obtained similar results under barbiturate anesthesia. Third, the dose and timing of gabapentin administration was also unlikely to be a factor, because lower or higher doses of gabapentin also appeared to be ineffective on PIDs in our pilot studies, and because similar treatment protocols have been efficacious in previous rodent studies of pain and epilepsy as well as SD (Hoffmann *et al*, 2010; Hunter *et al*, 1997; Welty *et al*, 1993). A notable shortcoming of single point measurements using glass micropipettes is that they lack spatial information, and might have missed an effect of gabapentin on the duration of PIDs restricted to a specific zone in penumbra. In addition, we cannot rule out an effect of gabapentin on the neurovascular coupling and the hemodynamic response to PIDs, as our CBF measurements using LDF were limited to ischemic core and aimed only to confirm successful occlusion and reperfusion in our study.

It should be noted that gabapentin shows only modest efficacy on SD compared with some other clinically used drugs known to suppress SD, such as migraine prophylactic drugs (Ayata *et al*, 2006; Hoffmann *et al*, 2010). Therefore, PID suppression may still be a relevant neuroprotective mechanism that can be targeted by this class of drugs. Nevertheless, gabapentin's rapid onset of action on SD and favorable safety and tolerability profile warrant further investigation of its neuroprotective efficacy and mechanisms in the ischemic stroke, as well as in other brain injury states in neurocritical care.

Disclosure/conflict of interest

The authors declare no conflict of interest.

References

- Ayata C, Jin H, Kudo C, Dalkara T, Moskowitz MA (2006) Suppression of cortical spreading depression in migraine prophylaxis. *Ann Neurol* 59:652–661

- Dohmen C, Sakowitz OW, Fabricius M, Bosche B, Reithmeier T, Ernestus RI, Brinker G, Dreier JP, Woitzik J, Strong AJ, Graf R (2008) Spreading depolarizations occur in human ischemic stroke with high incidence. *Ann Neurol* 63:720–8
- Eikermann-Haerter K, Dilekoz E, Kudo C, Savitz SI, Waeber C, Baum MJ, Ferrari MD, van den Maagdenberg AM, Moskowitz MA, Ayata C (2009) Genetic and hormonal factors modulate spreading depression and transient hemiparesis in mouse models of familial hemiplegic migraine type 1. *J Clin Invest* 119:99–109
- Hartings JA, Rolli ML, Lu XC, Tortella FC (2003) Delayed secondary phase of peri-infarct depolarizations after focal cerebral ischemia: relation to infarct growth and neuroprotection. *J Neurosci* 23:11602–10
- Hoffmann U, Dilekoz E, Kudo C, Ayata C (2010) Gabapentin suppresses cortical spreading depression susceptibility. *J Cereb Blood Flow Metab* 30:1588–92
- Hunter JC, Gogas KR, Hedley LR, Jacobson LO, Kassotakis L, Thompson J, Fontana DJ (1997) The effect of novel anti-epileptic drugs in rat experimental models of acute and chronic pain. *Eur J Pharmacol* 324:153–60
- Kudo C, Nozari A, Moskowitz MA, Ayata C (2008) The impact of anesthetics and hyperoxia on cortical spreading depression. *Exp Neurol* 212:201–6
- Nakamura H, Strong AJ, Dohmen C, Sakowitz OW, Vollmar S, Sue M, Kracht L, Hashemi P, Bhatia R, Yoshimine T, Dreier JP, Dunn AK, Graf R (2010) Spreading depolarizations cycle around and enlarge focal ischaemic brain lesions. *Brain* 133:1994–2006
- Petzold GC, Windmuller O, Haack S, Major S, Buchheim K, Megow D, Gabriel S, Lehmann TN, Drenckhahn C, Peters O, Meierkord H, Heinemann U, Dirnagl U, Dreier JP (2005) Increased extracellular K⁺ concentration reduces the efficacy of N-methyl-D-aspartate receptor antagonists to block spreading depression-like depolarizations and spreading ischemia. *Stroke* 36:1270–7
- Radulovic LL, Turck D, von Hodenberg A, Vollmer KO, McNally WP, DeHart PD, Hanson BJ, Bockbrader HN, Chang T (1995) Disposition of gabapentin (neurontin) in mice, rats, dogs, and monkeys. *Drug Metab Dispos* 23:441–8
- Shin HK, Dunn AK, Jones PB, Boas DA, Moskowitz MA, Ayata C (2006) Vasoconstrictive neurovascular coupling during focal ischemic depolarizations. *J Cereb Blood Flow Metab* 26:1018–30
- Strong AJ, Anderson PJ, Watts HR, Virley DJ, Lloyd A, Irving EA, Nagafuji T, Ninomiya M, Nakamura H, Dunn AK, Graf R (2007) Peri-infarct depolarizations lead to loss of perfusion in ischaemic gyrencephalic cerebral cortex. *Brain* 130:995–1008
- Traa BS, Mulholland JD, Kadam SD, Johnston MV, Comi AM (2008) Gabapentin neuroprotection and seizure suppression in immature mouse brain ischemia. *Pediatr Res* 64:81–5
- Welty DF, Schielke GP, Vartanian MG, Taylor CP (1993) Gabapentin anticonvulsant action in rats: disequilibrium with peak drug concentrations in plasma and brain microdialysate. *Epilepsy Res* 16:175–81
- Williams AJ, Bautista CC, Chen RW, Dave JR, Lu XC, Tortella FC, Hartings JA (2006) Evaluation of gabapentin and ethosuximide for treatment of acute nonconvulsive seizures following ischemic brain injury in rats. *J Pharmacol Exp Ther* 318:947–55