Calcium Ion Transients in Peri-Infarct Depolarizations May Deteriorate Ion Homeostasis and Expand Infarction in Focal Cerebral Ischemia in Cats

Kouichi Ohta, MD; Rudolf Graf, PhD; Gerd Rosner, PhD; Wolf-Dieter Heiss, MD

Background and Purpose—Harmful effects of peri-infarct depolarizations (PIDs) may depend on recurrent Ca\textsuperscript{2+} influx. Thus far, few studies have documented the relevance of PIDs in gyrencephalic animals, and the progressive nature of this process has not been investigated over extended periods. We therefore studied in prolonged focal ischemia in cats spatial and temporal profiles of extracellular calcium ([Ca\textsuperscript{2+}]) shifts in relation to direct current (DC) potential, nitric oxide (NO) concentration and regional cerebral blood flow alterations, and final pathological outcome.

Methods—In halothane-anesthetized cats receiving either vehicle (n=12) or MK-801 treatment (5 mg/kg IV; n=10), the left middle cerebral artery was permanently occluded. Laser-Doppler probes, ion-selective microelectrodes, and NO electrodes measured simultaneously regional cerebral blood flow, DC potential, electrocorticogram, [Ca\textsuperscript{2+}]\textsubscript{i}, and NO concentrations in ectosylvian and suprasylvian gyri of the left cerebral cortex.

Results—Persistent depolarization immediately after middle cerebral artery occlusion occurred in 10 ectosylvian and 4 suprasylvian gyri of vehicle-treated animals and in 9 ectosylvian and 3 suprasylvian gyri of MK-801–treated animals. PIDs associated with transient decreases of [Ca\textsuperscript{2+}]\textsubscript{i} were detected in suprasylvian gyri of only 4 vehicle-treated animals, of which 3 developed recurrent PIDs. Electrocorticogram was suppressed during PIDs, and electrocorticogram recovery worsened in a stepwise manner with consecutive depolarizations. PID duration increased slightly with ongoing ischemia and evolved to persistent depolarization at a final stage. NO transients were not detected during PID, and regional cerebral blood flow transients were not pronounced. Infarction was larger with initial persistent depolarization than with PID and was smallest in MK-801–treated animals.

Conclusions—PID is not a common finding in peri-infarct zones in cats, and it is suppressed by the N-methyl-D-aspartate antagonist MK-801. However, if repeated PIDs are generated, they result in a stepwise, progressive breakdown of neuronal function and ion homeostasis, probably contributing to the growth of infarction in focal cerebral ischemia. Recurrent Ca\textsuperscript{2+} influx is a mechanism that presumably contributes to this process. (Stroke. 2001;32:535-543.)

Key Words: calcium ■ cerebral infarction ■ ischemia ■ MK-801 ■ nitric oxide ■ spreading cortical depression ■ cats

A cardinal feature of focal cerebral ischemia is the presence of perifocal regions with moderate reduction of regional cerebral blood flow (rCBF). In a sequential multi-tracer positron emission tomography study, we demonstrated that penumbral tissue progressively deteriorates in the course of the first day after middle cerebral artery (MCA) occlusion in cats.\textsuperscript{1} In the surroundings of an ischemic focus that is exempted from persistent cortical depolarization, transient peri-infarct depolarization (PID) may occur repeatedly.\textsuperscript{2,3} In an early study of focal cerebral ischemia in baboons, Harris and coworkers\textsuperscript{4} saw transient changes in extracellular Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]) concomitant with extracellular K\textsuperscript{+} changes resembling spreading depression in the normal brain.\textsuperscript{5} Since their report, it has been hypothesized that PID provides a possible mechanism of Ca\textsuperscript{2+} influx in the peri-infarct cortical tissue and that the gradual recruitment of damaged cells in the penumbra can be caused by Ca\textsuperscript{2+} transients accompanying recurrent PIDs.\textsuperscript{6} More recently, [Ca\textsuperscript{2+}]\textsubscript{i} shifts accompanying PIDs in rat focal ischemia were reported.\textsuperscript{7} In fact, repetitive PIDs in the peri-infarct area have been related to enlargement of infarct volume in focal cerebral ischemia of rats\textsuperscript{8,9} and cats,\textsuperscript{10} but the progressive nature of this process has never been documented in prolonged studies.

The present study sought to follow this process in a gyrencephalic animal, the cat, up to a terminal point of functional impairment and structural damage by using a multiparametric approach that included in particular a detailed analysis of transmembrane Ca\textsuperscript{2+} shifts as putative mechanisms of infarct expansion occurring concurrently with PIDs in the ischemic penumbra. Additionally, the N-methyl-D-aspartate (NMDA) antagonist MK-801 has been used in a...
second experimental group as a means to interfere with these mechanisms, as has been shown in rat models of focal ischemia.8,9 Since few and mostly indirect PID recordings exist in gyrencephalic animals,10,11 we chose to occlude the MCA in cats as a focal ischemia model. For the in vivo multiple electrode measurements of electrocorticogram (ECoG), DC potential, [Ca\textsuperscript{2+}]o, and nitric oxide (NO) concentration were performed in relation to the topographical gradient of rCBF, the focal ischemia, and the final outcome of the ischemic insult, which was histopathologically evaluated.

### Materials and Methods

#### Animal Preparation

Twenty-two adult cats of both sexes weighing 2.7 to 4.8 kg were used. The study was approved by the local animal care committee and the Regierungspresident of Cologne and was in compliance with the German laws for animal protection. Generalized anesthesia was induced by 25 mg/kg IM ketamine hydrochloride. The left femoral vein and artery were catheterized to administer drugs and to measure arterial blood pressure, arterial blood gases, hematocrit, and plasma glucose concentration. The animals were tracheostomized and artificially ventilated with a 70% NO/30% O\textsubscript{2} gas mixture. Generalized anesthesia was maintained during surgery by 0.8% to 1.5% and during experimental recordings by 0.6% to 0.8% halothane inhalation. The animals were immobilized with 0.2 mg/kg IV pancuronium bromide and 5 mg/kg per hour IV gallamine triethiodide, and artificial ventilation was controlled to keep arterial and expiratory gases within normal physiological ranges. Deep body temperature was kept at 37.0°C with a heating blanket feedback controlled by a rectal temperature probe.

The experimental setup was similar to that used in our previous study on transient focal ischemia, and details have been provided in that report.12 In brief, a transorbital route was used for MCA occlusion that followed implantation of an occluding device and sealing of the orbita. Simultaneous measurements were performed in 2 cortical sites on the left cerebral hemisphere. Their stereotaxic coordinates were 8 mm anterior/15 mm lateral in the ectosylvian gyrus and 4 mm anterior/8 mm lateral in the suprasylvian gyrus.13 The ectosylvian gyrus is located proximally and the suprasylvian gyrus more distally in the territory of the MCA. Burr holes of 3-mm diameter were drilled into the skull above the recording sites, and the dura was removed under microscopic control. In each site, an ion-selective microelectrode and an NO electrode were adjacently inserted 1.0 mm deep into the cortex with a micromanipulator, and a thermocouple for measurement of regional brain temperature and a laser-Doppler probe (tip diameter, 800 μm; Moor Instruments) for measurement of rCBF were placed on the cortical surface. The burr holes were filled with absorbable gelatin sponge (Gelfoam) containing cerebrospinal fluid and totally covered with dental cement. Brain temperature was maintained at 37.0°C with the use of heating lamps above the animal’s head that were feedback controlled by the thermocouple on the ectosylvian gyrus.

#### Electrodes

A double-barreled glass micropipette14 with a tip diameter of approximately 3 μm consisted of a reference barrel filled with 150 mmol/L NaCl and an ion-selective barrel filled with Ca\textsuperscript{2+} ionophore (Fluka) and 150 mmol/L CaCl\textsubscript{2}. This microelectrode recorded ECoG and DC potential on the reference channel (low-pass filters: 30 and 0.1 Hz, respectively) and [Ca\textsuperscript{2+}]o. To avoid polarization, the ECoG and DC potentials were recorded against a calomel electrode15 placed on the nasion. Calibration at 37.0°C was performed in 0.03- to 3.0-mmol/L CaCl\textsubscript{2} solutions.

An NO electrode (ISO-NOP200, World Precision Instruments)16-19 was kept at a constant potential of +0.85 V against the Ag/AgCl reference electrode, thus determining the electrode specificity to NO among other gases in the tissue.20 The NO electrode showed no cross sensitivity to gases such as N\textsubscript{2}, O\textsubscript{2}, and CO\textsubscript{2}. Calibration was performed before each experiment by in vitro chemical generation of NO with 50 μmol/L NaNO\textsubscript{2} (E. Merck), 0.1 mol/L H\textsubscript{2}SO\textsubscript{4}, and 0.1 mol/L KI solutions at 37.0°C. The lower detection limit was 0.5 mmol/L NO.

#### Experimental Protocol

The variables (eg, blood pressure, brain temperature, rCBF, ECoG, DC potential, [Ca\textsuperscript{2+}], NO concentration) were continuously recorded throughout the experiment with a PC-based data acquisition system (DABAS). Baseline recordings were obtained until the parameters became stable. Thereafter, 12 cats in the vehicle treatment group received intravenous vehicle injections (20 mL Ringer’s solution injected intravenously in 10 minutes). In the MK-801
treatment group, 10 cats received the NMDA receptor blocker MK-801 (5 mg/kg in 20 mL Ringer’s solution injected intravenously in 10 minutes) (Research Biochemicals International). Thirty minutes after either treatment, the left MCA was occluded permanently. Eighteen hours after induction of ischemia, animals were perfused with 4% paraformaldehyde solution, and brains were removed. After paraffin embedding, 7-μm-thick coronal sections of the brain were cut at distances of 2 mm and stained with hematoxylin-eosin. Infarction in each slice was determined microscopically, and the area was measured with the use of the public domain NIH Image program (developed at the US National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/).

Statistical Analysis

Data are presented as mean±SD. Cortical DC potential (mV) and tissue NO concentration (nmol/L) are described as changes from baseline levels, and a negative value indicates a decrease from baseline. rCBF was calculated as percentage of baseline. The significance of differences at P<0.05 was tested between sequential measurements, between groups, or between treatments by ANOVA and multiple post hoc comparisons (Fisher’s protected least significance difference) (Statistica, StatSoft Inc), unless otherwise stated in the text.

Results

In both experimental groups, physiological variables before treatment were within normal ranges (Table 1) and remained almost at the same levels after MK-801 treatment, as they did after 2 hours of MCA occlusion. Even at 12 hours, major alterations of physiological variables were not observed. PaCO₂ increased slightly in both groups, as did hematocrit and blood glucose.

A set of cortical recordings in ectosylvian gyrus and suprasylvian gyrus obtained in parallel from the same vehicle-treated animal documents the contrast between quickly and a slowly progressing pathophysiological processes in different zones of focal ischemia (Figure 1). In the ectosylvian gyrus (Figure 1A), occlusion of the MCA produced an immediate reduction in rCBF to approximately 5% of baseline, producing regional ischemia, and initiated a steep cortical depolarization with a marked reduction in ECoG amplitude. This initial persistent depolarization was accompanied by a characteristic initial transient increase in [Ca²⁺], followed by a steep decline and a temporary rise of NO, as described earlier in a study of transient focal ischemia in the same model. With ongoing occlusion, severe ischemia persisted. Gradual recovery of the DC potential and a gradual decline of NO were observed during this phase. In the suprasylvian gyrus of the same animal (Figure 1B), ischemia was less severe than in the ectosylvian gyrus. rCBF at 30
minutes was reduced to 59.1% of baseline. A brief reduction of the ECoG amplitude immediately after occlusion was followed by an increase above control levels; the DC potential showed a minor deviation (<2 mV) from baseline, while \([\text{Ca}^{2+}]_o\), was not altered. These initial changes were totally different from those in the neighboring ectosylvian gyrus, and the most striking contrast emerged approximately 1 hour after occlusion. The DC potential exhibited a transient spreading depression–like PID, with duration of a few minutes and complete recovery to baseline. Such PIDs appeared repeatedly and were paralleled by transient changes in \([\text{Ca}^{2+}]_o\), and, in an irregular fashion, by transient alterations of rCBF. NO decreased gradually after MCA occlusion, but transient NO alterations corresponding to PIDs were not observed. After the 14th PID episode, at approximately 8 hours after onset of ischemia, the DC potential evolved into persistent depolarization (a in Figure 1B). This time point preceded a marked drop in rCBF (b in Figure 1B) by approximately 50 minutes. Thus, the transition from transient to persistent depolarization did not result from a sudden worsening of ischemia but rather caused a secondary rCBF decrease in the peri-infarct zone.

Table 2 summarizes the occurrence of different types of tissue depolarization after MCA occlusion. In the ectosylvian gyrus of the vehicle treatment group, the chances of having initial persistent depolarization were 10:12, in contrast to the suprasylvian gyrus with much lower chances of 4:12 \((P<0.05; \chi^2)\). In the MK-801 treatment group, a similar occurrence of initial persistent depolarization was found, indicating that MK-801 does not affect its initiation in the 2 gyri. PIDs occurred in only 4 of 12 suprasylvian gyri of the vehicle treatment group. These 4 were among those 8 suprasylvian gyri that did not show initial persistent depolarization. In 3 suprasylvian gyri among these 4, PID appeared repeatedly for 10 to 22 times at a rate of 0.1 to 1.9 episodes per hour. In the fourth case PID appeared only once. In the MK-801 treatment group, PID was totally absent in both ectosylvian and suprasylvian gyri. Even in 7 suprasylvian gyri lacking initial persistent depolarization, transient depolarization of cortical tissue was not observed. Thus, MK-801

Table 2. Occurrence of Initial Persistent Depolarization and of Spreading Depression–Like Transient Depolarization (PID) in the 2 Treatment Groups

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Gyrus</th>
<th>Initial Persistent Depolarization</th>
<th>PID</th>
</tr>
</thead>
<tbody>
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<td>Present</td>
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<td>12</td>
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<td>SG</td>
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<td>8*</td>
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<tr>
<td>MK-801</td>
<td>10</td>
<td>EG</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SG</td>
<td>3*</td>
<td>7*</td>
</tr>
</tbody>
</table>

EG indicates ectosylvian gyrus; SG, suprasylvian gyrus.
*Significant difference between gyri in either vehicle or MK-801 group \((P<0.05; \chi^2)\).
†Significant difference between treatment groups \((P<0.05; \chi^2)\).
§All cases of PID occurred in suprasylvian gyri lacking initial persistent depolarization.

Figure 2. Changes in multiple parameters in ectosylvian gyri with initial persistent depolarization of the vehicle treatment group \((n=10)\) and the MK-801 treatment group \((n=9)\) as a function of time after MCA occlusion. Mean±SD values are plotted every half hour for 12 hours. A negative value for NO concentration indicates a decrease from baseline. *\(P<0.05\), †\(P<0.005\), §\(P<0.0005\), significantly different from vehicle treatment; a\(P<0.05\), b\(P<0.005\), c\(P<0.0005\), significantly different from baseline.
effectively suppressed PID in the suprasylvian gyrus ($P<0.05$, $\chi^2$ test).

**Changes in Ectosylvian Gyri With Initial Persistent Depolarization**

Ectosylvian gyri exhibiting initial persistent depolarization are assumed to belong to the most densely ischemic tissue in the cerebral cortex.\textsuperscript{11} Severe ischemia was verified by a rCBF reduction to $<20\%$ of baseline during early MCA occlusion (Figure 2A). After 6 hours of ischemia, rCBF in the MK-801 treatment group recovered to some extent, with mean values rising to $>20\%$. These values were higher than those in the vehicle treatment group ($P<0.0005$ to $P<0.005$). NO decreased over hours (Figure 2B), but significant differences were not observed between the 2 groups. The DC potential in the vehicle treatment group decreased rapidly, reaching $-17.9\pm9.0$ mV at 30 minutes after MCA occlusion (Figure 2C), and increased thereafter gradually over the 12-hour observation period. Initial depolarization in the MK-801 treatment group at 30 minutes was less severe ($-13.0\pm9.0$ mV) than in the vehicle treatment group ($P<0.0005$), and this difference remained significant until 3 hours after occlusion. Thereafter, depolarization in both groups became similar, indicating that MK-801 treatment did not inhibit initial persistent depolarization but rather delayed the progression of depolarization in cortical tissue. During the first 3.5 hours of MCA occlusion, the decline of $[\text{Ca}^{2+}]_o$ in the MK-801 treatment group was gradual compared with the vehicle treatment group (Figure 2D). In the vehicle treatment group, $[\text{Ca}^{2+}]_o$ dropped as low as $0.71\pm0.42$ mmol/L 30 minutes after MCA occlusion ($P<0.0005$) and reached its lowest level at 1.5 hours. In the MK-801 group, $[\text{Ca}^{2+}]_o$, changes at 30 minutes after occlusion were not yet significant. At 1 hour, $[\text{Ca}^{2+}]_o$, was significantly lowered to $0.91\pm0.38$ mmol/L ($P<0.05$), followed by a gradual further decrease, and it remained at significantly higher levels compared with vehicle treatment until 3.5 hours after occlusion ($P<0.005$ to $P=0.05$).

A correlate of this contrast in functional outcome between the 2 treatment groups may be seen in the difference regarding final morphological damage (Figure 3). Areas of infarction in equidistant coronal brain sections were smaller in animals with MK-801 treatment ($n=8$) than in those with vehicle treatment ($n=12$) ($P<0.01$ to $P=0.05$), indicating that MK-801 administered 30 minutes before MCA occlusion had a profound protective effect against ischemic tissue damage.

**Changes in Suprasylvian Gyri With Peri-Infarct Depolarizations**

As shown in Table 2, PIDs developed in 4 of 8 suprasylvian gyri lacking initial persistent depolarization. We considered it particularly interesting to compare the 2 sets of recordings with and without PID. In suprasylvian gyri exhibiting PID, mean rCBF (Figure 4A) remained somewhat lower for the first hour after occlusion, recovered thereafter to higher values ($P<0.05$), and decreased again in the later stage after approximately 9 hours of occlusion, reaching the same values as those in gyri without PID. DC potential and $[\text{Ca}^{2+}]_o$, decreased gradually over 12 hours in both types of suprasylvian gyri, and there were no significant differences between the 2 sets of recordings (Figure 4C and 4D). The changes in DC potential and $[\text{Ca}^{2+}]_o$, were, however, smaller than those in the ectsosylvian gyri with initial persistent depolarization (Figure 2C and 2D). The 2 sets of recordings differed most remarkably regarding changes in NO concentration (Figure 4B). In suprasylvian gyri without PID, NO concentration began to decrease 3.5 hours after occlusion and stayed very low ($P<0.0005$ to $P=0.05$). This level of NO was lower than the NO concentration in ectsosylvian gyri exhibiting initial persistent depolarization (Figure 2B). On the other hand, suprasylvian gyri with PIDs displayed a rather gradual decrease, with NO levels remaining well above those obtained in gyri without PID ($P<0.0005$ to $P=0.05$). In summary, the order of NO levels during 12 hours of ischemia were (1) ectsosylvian gyri with initial persistent depolarization, (2) suprasylvian gyri with PID, and (3) suprasylvian gyri without initial persistent depolarization and PID.

Repetitive PIDs in the suprasylvian gyri were analyzed in more detail by zooming in on individual events. As shown in the first of 2 original recordings in Figure 5 (the first PID episode presented in Figure 1B), the ECoG amplitude began to decrease at time point 1, and the DC potential started to fall gradually, followed by a small increase in $[\text{Ca}^{2+}]_o$. Thereafter, the DC potential dropped steeply in approximately 10 seconds to $-23$ mV, a sudden decrease in $[\text{Ca}^{2+}]_o$, was initiated, and the ECoG amplitude was markedly reduced. After some recovery, the DC potential shifted even further before it increased rapidly (time point 2), accompanied by an accelerated increase in $[\text{Ca}^{2+}]_o$. The DC potential reached baseline levels (time point 3) earlier than the other variables, followed by a modest overshoot and a gradual decline to baseline. At this latest time point, the $[\text{Ca}^{2+}]_o$, increase was slowed down, and, compared with the DC potential, it recovered to baseline levels with some delay. ECoG recovery started later than that of the other variables, and ECoG amplitude did not reach levels observed before the PID episode. During progression of the experiment, the duration of individual repetitive PID episodes became slightly longer, as displayed in the second recording (seventh PID episode presented in Figure 1B) and shown in the regression analysis for 3 animals.
calculated for repetitive PIDs between time points 1 and 3. The duration of repeated PIDs was mostly within 4 minutes, and even though it was prolonged in the later course of ischemia, it remained below 6 minutes in all animals. The mean durations were 84, 108±11, 132±32, and 194±43 seconds in 4 animals exhibiting PIDs. Regression analysis of ECoG recovery between repetitive PIDs revealed a progressive, stepwise worsening of functional recovery during prolonged ischemia until it finally flattened at the time when the DC potential evolved into persistent depolarization. Transient rCBF changes during PID were sometimes missing or quite variable among episodes, with a first decrease (approximately 46.1% less than pre-episode level) usually followed by a small increase (approximately 33.1% more than pre-episode level) and thereafter by recovery to baseline.

Effects of the different types of cortical depolarization on final pathological outcome were analyzed in cats of the vehicle treatment group (Figure 6). According to presence or absence of initial persistent depolarization and PID in the suprasylvian gyrus, infarct size was largest in animals showing initial persistent depolarization, second largest in animals exhibiting PID, and smallest in those showing neither initial persistent nor transient depolarization. Differences between these 3 categorized groups of animals were statistically significant (P<0.01 to P=0.05).

Discussion

As shown in our study, 2 distinctive types of tissue depolarization exist in cerebral cortex suffering from permanent focal cerebral ischemia. These 2 types are different with regard to mechanism, site, and time of generation. The first type is initial persistent depolarization,12 which appears immediately after the initiation of MCA occlusion and persists as long as the ischemia lasts. It is obviously generated by marked reduction in rCBF and is evoked exclusively in tissue exhibiting severe cerebral ischemia with residual rCBF of <20% of baseline. It occurs not only in proximal but also in more distal regions of the MCA territory. The second type is PID, ie, transient spreading depression–like peri-infarct depolarization, which emerges later and repeatedly in the course of focal ischemia. This type of depolarization appears in cortex where rCBF reduction is moderate and persistent depolarization is not present. The appearance of PID is not necessarily preceded by a worsening of ischemia, indicating that it is not directly driven by rCBF reduction.

Similar to spreading depression, PID has been shown to propagate into peri-infarct.21–23 As observed in the present experiments, it shares many characteristics of spreading depression as it appears in the normal cortex after mechanical, chemical, or electric stimulation.5 Alterations of [Ca2+]o...
parallel PID in the present experiments consist of 4 phases: a small increase followed by an abrupt decrease, a phase with rapid recovery, and finally a slower recovery to baseline. This \( \text{Ca}^{2+} \) transient resembles that described during spreading depression. The mean duration of PID in the present experiments, which ranges from 84 to 194 seconds, is similar to that of spreading depression (3 minutes), except for a gradual elongation as PID appears repeatedly. An increase in duration of PIDs in the later course of focal ischemia has also been reported in rats. The ECoG is suppressed during spreading depression in normal tissue and in PID, but the gradual, stepwise reduction of ECoG amplitude after subsequent PID episodes hints at a progressive decomposition of the cortical neuronal network in peri-infarct zones driven by each single spreading depression–like depolarization. Suppressive effects of MK-801 have been found regarding both PIDs and spreading depression under physiological conditions. Spreading depression in normal tissue is always accompanied by a brief but marked hyperperfusion, which is elicited later than the peak of the concurrent negative DC potential shift and may be followed by a period of hypoperfusion. \( r \text{CBF} \) responses during PID are less pronounced. They may be restricted to brief shallow hyperperfusion or hypoperfusion, or they may be completely absent (see examples in Figure 1B). PID seems to occur less frequently in cat than in rat focal cerebral ischemia. In the present experiments, PID was observed in only 4 of 10 sites lacking initial persistent depolarization (8 suprasylvian and 2 ectosylvian gyri; see Table 2). In rats, PID was usually observed in 100% of focal ischemia experiments.
may have been caused in part by the anesthetic used, i.e., halothane, which has been shown in cats to reduce the probability of PID induction compared with other anesthetics such as α-chloralose.16 However, since most of the rat studies have been performed under comparable halothane concentrations, we think that the frequency or rate of PID induction is another example of interspecies differences, ranging from 0.1 to 1.9 episodes per hour in cats and from 2.5 to 9 episodes per hour in rats.3,8,21,27 A similar difference between cats and rats seems to exist for the induction rate of spreading depression evoked by high K+ in the normal brain.32 To further clarify the in vivo role of anesthetics in the suppression of PIDs, comparison with recordings in awake animals would obviously be advantageous but do not yet exist.

Transient depolarization is a challenge for the ion homeostasis of cerebral tissue since depolarization is associated with Na+ influx and K+ efflux, and a decline in [Ca2+], indicates massive Ca2+ influx into neurons.23 Ca2+ influx should be provoked by depolarization of the cell membrane because many Ca2+ channels in the membrane are voltage-gated. Every time such disturbance in the balance between intracellular and extracellular ion concentrations is generated by transient depolarization, the balance must be restored by energy-dependent and thus energy-consuming mechanisms. The slowdown of the recovery phase of transient depolarization, resulting in a stepwise elongation of the duration of PIDs in the course of the present experiments, may therefore testify to gradual impairment of ion transport capacity, which can be caused by, for example, reduced glucose concentration in the tissue.24 This progressive deterioration ends finally in a persistent depolarization without recovery, indicating permanent disorder of ion homeostasis. During a single passage of spreading depression, increased consumption of oxygen and glucose33–35 has been demonstrated. In the normal brain, the metabolic workload due to spreading depression is coupled to an increase in rCBF and oxygen supply, and spreading depression is possibly a rather harmless event without major pathological changes.36 However, in the penumbral region of focal ischemia, the constraints of blood circulation prevent the adequate delivery of oxygen, resulting in transient episodes of relative tissue hypoxia during passage of transient depolarization.37 These hypoxic episodes may cause suppression of protein synthesis, gradual deterioration of energy metabolism, and finally progression of infarction into the penumbra.38

Increases in intracellular Ca2+ will activate constitutive NO synthase in neurons,39 which might explain the raised NO level in suprasylvian gyri with PID compared with those without. Higher levels of NO, a vasodilator, provide some reason for rCBF remaining higher in suprasylvian gyri with PIDs. Despite better perfusion, however, infarction was larger in animals exhibiting PIDs in the suprasylvian gyrus than in animals without. This finding suggests that transient depolarization is one important source for expansion of tissue injury into the periphery of ischemic foci. In consequence, reduced infarction due to MK-801 treatment is attributable to the suppression of transient depolarization by MK-801. In the ischemic core of animals treated with MK-801, progression of initial persistent depolarization and Ca2+ influx were delayed for 3 hours, and rCBF was kept at higher levels than in the vehicle treatment group. However, the final degree of depolarization and [Ca2+], reduction achieved in the ischemic core at 4 hours was as large as that of the vehicle treatment group. Nevertheless, infarction was smaller after MK-801 treatment. We assume that this is because of treatment effects in the mildly ischemic tissue in the periphery of MCA territory attributable to suppression of PIDs by MK-801. Therefore, even though our results provide only indirect evidence, we believe that MK-801 treatment was not able to rescue ischemic core regions but rather prevented the expansion of cerebral infarction into the periphery of focal ischemia by inhibiting PID induction.

In conclusion, marked and persistent reduction in rCBF below <20% of baseline will evoke immediate persistent depolarization in the cerebral cortex, producing the core of infarction. In regions with milder ischemia surrounding the ischemic core, transient depolarizations may be evoked repeatedly starting 1 hour after MCA occlusion or later. These transient depolarizations produce transient influx of Ca2+ and Na+, jeopardize cortical ion homeostasis and neuronal function, and have the potential to expand ischemic infarcts.

References


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