Factors Influencing the Frequency of Fluorescence Transients as Markers of Peri-Infarct Depolarizations in Focal Cerebral Ischemia

A.J. Strong, DM; S.E. Smith, PhD; D.J. Whittington, BSc; B.S. Meldrum, DSc; A.A. Parsons, PhD; J. Krupinski, MD; A.J. Hunter, PhD; S. Patel, PhD

Background and Purpose—Peri-infarct depolarizations (PIDs) that occur in ischemic boundary zones of the cerebral cortex of experimental animals have been shown to promote rather than simply to indicate the evolution of the lesion and are especially prominent in the rat. To study the influence of one factor, species, on PID incidence, we compared the frequency of PIDs in a primate species, the squirrel monkey, with that in the cat after middle cerebral artery occlusion. Plasma glucose was reviewed as a possible cause of interexperiment variability in the cat experiments.

Methods—In open-skull experiments under chloralose anesthesia, changes in cortical fluorescence believed to indicate NADH/NAD$^+$ redox state, as markers of PIDs, were recorded by serial imaging of the cortical surface in vivo for 4 hours after middle cerebral artery occlusion.

Results—Fluorescence transients occurred in squirrel monkeys at a frequency (mean ± SD) of 0.7 ± 0.8 hours$^{-1}$ (n = 5), which was not significantly less than in that observed in cats (1.3 ± 1.6 hours$^{-1}$, n = 8). Data from the cat experiments indicated a relationship between number of transients (dependent) and plasma glucose, with a striking increase in PID frequency in association with values of mean postocclusion plasma glucose <4.1 mmol/L (Mann-Whitney U = 15.0, P = 0.034); this observation agrees well with other published findings.

Conclusions—Transient changes in fluorescence strongly suggestive of peri-infarct depolarizations, either transient or terminal, occur and propagate in the ischemic cerebral cortex of a nonhuman primate. The results also suggest that the relationship of frequency of peri-infarct depolarizations with plasma glucose requires further examination, to confirm the finding and to determine a safe lower limit for a target range for control of plasma glucose if insulin is used in the management of patients with cerebral ischemia. (Stroke. 2000;31:214-222.)

Key Words: spreading cortical depression ■ NADH ■ middle cerebral artery occlusion ■ depolarization ■ hypoglycemia

The occurrence of spontaneous depolarizations in the cortical ischemic boundary zone or penumbra associated with occlusion of the middle cerebral artery (MCAO) has been amply documented since the early descriptions in baboons$^1$ and cats.$^2$ That such peri-infarct depolarizations (PIDs) might increase ischemic damage (rather than serving only to mark its progression) was first, to the best of our knowledge, suggested in 1983$^3$; mechanisms whereby progression might occur as a consequence of recurrent PIDs have also been described.$^4$–$^6$ However, only relatively recently has it been demonstrated that there is a clear relationship of infarct size with numbers of PIDs$^7$–$^9$ and that PID number can determine infarct size, rather than the reverse.$^{10}$

Given this relationship, reliable knowledge of the factors that determine the frequency of PIDs clearly becomes important. Certain of these factors are well recognized: as exam-
primate brain, the present experiments were designed to examine and compare the frequencies of occurrence of fluorescence transients in the cat and in a nonhuman primate, the squirrel monkey. During analysis of the data, in a search for sources of interexperiment variability in PID frequency, we found evidence that PID frequency may be highly sensitive to quite modest reductions in plasma glucose. There is considerable published evidence to support and explain this finding, which, if confirmed, would have important implications for the management of plasma glucose levels in patients with acute traumatic or ischemic brain injury.

Materials and Methods

Animals and Housing

Adult squirrel monkeys (Saimiri sciureus) weighing 0.9 to 1.4 kg were housed in one group, and adult colony-bred male cats weighing 2.8 to 4.0 kg were housed in groups of 3 to 9 in UK Home Office-approved caging. The environment was maintained at 19°C to 22°C and a relative humidity of 55±3%, respectively, with a 14-hour/10-hour light/dark cycle (light on from 6 AM to 8 PM). Food and water were available ad libitum, and food was withdrawn 16 hours before surgery.

Surgical Preparation and MCAO

In a protocol approved by the Home Office, halothane was used to induce anesthesia in cats and squirrel monkeys (4% in a mixture of 70% N₂O and 30% O₂), and each animal was then initially allowed to breathe halothane (2%) spontaneously via a face mask. Rectal temperature was maintained at 37°C (36.5°C to 38°C) with a heating blanket (Harvard Apparatus). The left femoral vein was cannulated for fluid administration, and the left femoral artery was cannulated for the continuous monitoring of arterial blood pressure and for repeated blood sampling for serial measurements of arterial blood glucose concentration, PaO₂, PaCO₂, [HCO₃⁻], and pH. Arterial blood (200 μL) was collected from the left femoral artery of the animals for immediate analysis at 30-minute intervals during the experiment by use of an IL1304 analyzer (Instrumentation Laboratories). Blood glucose was measured electrochemically with Exactech strips (Medisense, Coleshill). A tracheostomy was performed, and the animal was then intubated and ventilated mechanically with pancuronium (bolus: 0.02 mg · kg⁻¹ · h⁻¹) for neuromuscular blockade. Induction anesthesia was replaced with intravenous anesthesia with chloralose (100 mg · kg⁻¹ in 2.5% w/vol borax in saline with supplemental doses of 5 to 10 mg · kg⁻¹ to maintain mean arterial blood pressure at 80 to 130 mm Hg). Intravenous infusion of 0.9% saline (Baxter) was maintained at a rate of 4.5 mL · kg⁻¹ · h⁻¹ for 0.8 hours

The main trunk of the right middle cerebral artery (MCA) was electrocauterized and divided to ensure occlusion. All experiments were terminated by perfusion-fixation 4 hours after MCAO.

Image Acquisition

The experimental system closely resembled that described previously and comprises in summary (1) fluorescence excitation (25-W xenon source) at 370 nm delivered via 2 liquid light guides and oblique epi-illumination, (2) intensified charge-coupled device camera (Prostab), and (3) a frame-grabbing system (Synoptics Ltd) within a personal computer host, programmed with appropriate image-acquisition sequences. Sequences of between 20 and 130 images were acquired at a rate of 1 image every 12 seconds, before and after MCAO. A fresh baseline image was used for each sequence; thus, in any sequence, before or after MCAO, only changes in fluorescence occurring during that sequence were routinely measured or displayed. On termination of a sequence, a fresh one was commenced, usually immediately, but after intervals of ~5 minutes on 3 or 4 occasions in each of 6 of the experiments (continuous in the remaining 2). Thus, sampling was essentially continuous for 95% of occlusion time.

Image Processing

Image sequences were reviewed in pseudocolor as differences from baseline, both to detect transients and to exclude artifacts. Fluorescence transients taken to mark a PID were defined as a rapid (36±12 seconds) increase in cortical fluorescence and were classified as “originating” when a new increase in fluorescence was seen to arise on a gyrus or, often, to radiate from a short, narrow band of cortex adjacent to a sulcus (Figure 1). Alternatively, transients were designated as “propagating” when a preceding fluorescence transient had been observed on an adjacent gyrus and the time of appearance on the new gyrus was compatible with a propagation rate around the walls of the sulcus of 1 to 5 mm/min (Figure 1).

Statistics

Rectal and paraffin pool temperatures, blood pressure, glucose, gases, and pH recorded during the experiment were analyzed by MANOVA. Significance of any relationship between fluorescence transient incidence and mean ischemic plasma glucose was tested by a number of approaches, as described in Results.

Results

Physiological Variables

In cats, PaCO₂ (33.8±3.9 to 33.4±5.1 mm Hg, mean±SD), arterial pH (7.35±0.5 to 7.34±0.3), and HCO₃⁻ (18.7±3.1 to 18.2±2.2 mmol/L) changed very little during the experiments, although there was a trend toward reduction in mean arterial pressure in cats, from 77±24 to 60±17 mm Hg at 4 hours after occlusion (despite supplemental infusions of colloid volume expander). Mean maximum and minimum brain surface temperatures in cats were 37.0±0.5°C and 36.3±1.1°C, respectively. All variables remained stable in squirrel monkeys. Plasma glucose data in cats are described below.

Propagated Fluorescence Transients

The times required for surgical preparation and for imaging during ischemia precluded imaging for substantial periods of time before MCAO. The mean sampling duration was 11 minutes in both species, and no transients were seen.

The incidences of transients after MCAO in cats and squirrel monkeys are shown in the Table, together with their distribution in cats by gyrus and an assessment of whether the transient originated on the gyrus or had propagated from a neighboring gyrus. Transients occurred in squirrel monkeys at a frequency of 0.7±0.8 hours⁻¹ (n=5), which was not significantly less than that observed in cats (1.3±1.6 hours⁻¹);
There was great variability in the incidences, both in cats and in squirrel monkeys. In cats, the range was from 1 to 20 in 4 hours; in 1 monkey, 8 PIDs were recorded over 4 hours (Figure 2); in another, none were observed. In cats, it was possible to describe the initial location and subsequent propagation of fluorescence transients by reference to the convenient gyral anatomy and established topography of infarction and penumbra in the MCAO stroke model. In the 8 cat experiments, an aggregate of 10 new transients occurred on the marginal gyrus, 18 on the suprasylvian gyrus, and 17 on the ectosylvian gyrus (11 in experiment 7, within a relatively short period between 70 and 140 minutes after MCAO, when plasma glucose was in the range of 3.4 to 3.8 mmol/L). In the great majority, the direction of propagation was centrifugal from the core, or circumferential on the gyrus of origin, but 7 transients were seen to spread toward the core area (Figure 1).

In squirrel monkeys, the cortical topography cannot be defined as readily as in cats, but the general onset, resolution, and propagation characteristics of the transients observed were similar to those in cats. In the experiment in which the most transients were seen, we observed propagation of a transient around the site of delayed fluorescence recovery from a previous transient (Figure 2); it was evident that this area subsequently repolarized, because it was invaded later by a third transient (Figure 2, f through h).

**Plasma Glucose**

In cats, plasma glucose was measured at MCAO and hourly thereafter. Although the values for SD for plasma glucose do not appear wide, values remained low in experiments 6 and 7, with mean values of 3.48 and 3.78 mmol/L, respectively; the highest incidence of PIDs occurred in these 2 experiments (Table; Figure 3). The relationship of PID number with plasma glucose was examined in cats (Table; Figure 3), and a possible threshold dependence of fluorescence transient number on plasma glucose was clearly evident. The relationship failed to reach statistical significance when analyzed by linear or logarithmic regression. However, in the light of existing published work suggesting that perilesion depolarizations might be promoted by reduced availability of glucose to the brain (please see Discussion), we dichotomized the experiments according to a putative mean plasma glucose threshold for increased transient frequency of 4.10 mmol/L; frequency of transients was significantly higher in experiments in which mean plasma glucose at and after MCAO was <4.10 mmol/L (Table; Figure 3) (Mann-Whitney \( U = 15.0, P = 0.034 \)).

**Discussion**

Since spontaneous transient increases in extracellular potassium were first described in the cortical ischemic penumbra after MCAO in baboons and cats, a very extensive investi-
A considerable effort has been devoted to exploring their nature. It is widely agreed that such cation transients are markers of focal neuronal depolarizations propagating across the cerebral cortex, now usually designated peri-infarct depolarizations (PIDs). Although sharing certain features (depolarization, cortical propagation, and transient increases in extracellular potassium \(^1\) and decreases in extracellular calcium \(^{17}\)) with Leão’s cortical spreading depression \(^{18}\) (CSD), PIDs differ from CSD in 3 critical aspects. First, the intense hyperemic transient of CSD \(^{19}\) is not seen in association with PIDs \(^{20}\); this is most readily attributable to proximal vascular occlusion, with incomplete compensation by collateral flow. Second, and as a consequence of diminished hyperemia, the cortical tissue PO\(_2\) transient, which is positive in CSD, is reversed to a transient decrease in PIDs. \(^{20}\) Third, whereas CSD is not associated with neuronal damage, \(^{21}\) there is a linear relationship of infarct size with number of PIDs, \(^{8}\) and PID number is the determining variable in this relationship. \(^{10}\)

It is now very clear from a large volume of literature that CSD is readily elicited in the (lissencephalic) rat brain but less readily in the gyrencephalic cat or primate brains. It is, however, unclear whether the same comparison can be applied to PIDs, and this study was undertaken to confirm that PIDs do indeed occur in the ischemic primate brain and to compare the frequencies of PIDs in the cat and primate brains. The relevance of this issue to human disease states and their treatment is discussed below. We found clear evidence for PIDs in squirrel monkeys subjected to MCAO, with a frequency that is not significantly different from that in cats; possible reasons for this apparent similarity are discussed later.

An important observation was the considerable interexperimental variability in PID frequency in both species. Although it suggests a statistically significant dependence of PID frequency on mean postocclusion plasma glucose level in cats, the experiments were not originally designed to examine this issue. However, we believe both that attention should be focused on this issue because of its relevance to the proper application of one proposed treatment regimen (glucose-insulin-potassium) in clinical management of patients with stroke and that the question needs to be examined in further, specifically designed experiments.

### Detection of PIDs by Fluorescence Imaging

In considering our results, some discussion of the method we have adopted for PID detection and the interpretation of the resulting data is first necessary. CSD is associated with a transient oxidation of the NAD/H couple, resulting in depression of NADH fluorescence (emission maximum 450 nm) \(^{22}\); thus, depression of this fluorescence may be used as a surrogate marker of CSD in the normally perfused cortex. Using an imaging method for NADH fluorescence (rather than detection at a single point with a fluorometer) \(^{5,22}\) we have described 2 patterns of fluorescence change after MCAO: either sustained increases in fluorescence or transient increases (or sometimes decreases; please see below) that resolve toward baseline, sometimes with an undershoot, over periods of some 2 to 10 minutes. \(^{13}\) We interpret sustained...
fluorescence increases as most probably coinciding with terminal depolarization, ie, development of core conditions, well characterized previously by increases in extracellular potassium or negative changes in DC potential (sustained in both cases). The second pattern we observed was of multiple transient increases in fluorescence in the suprasylvian and middle/posterior marginal gyri, although on some occasions, a transient that propagates into the anterior marginal gyrus (anterior cerebral artery territory) will there reverse its polarity to a primary decrease in fluorescence. Although our method does not provide a quantitative measure of changes in NAD/NADH redox potential, the topographical pattern of transient changes is consistent both with reduction of the couple in the penumbra, where flow recruitment in response to the transient is restricted, and with oxidation, where a transient has propagated into normally perfused cortex. The reversal in polarity is closely comparable to the opposite polarities of tissue PO2 transients in CSD versus PIDs. Thus, the imaging method we used here not only marks the occurrence of a depolarization but also tracks its propagation and, from the polarity, indicates a distinction between PID (with the implicit risk of promoting tissue damage) and, in normally perfused cortex, an NADH oxidation transient that may reasonably be interpreted as CSD. Because the imaging method samples almost the entire penumbra (in cats), it is a more comprehensive sampling tool than single or dual intracortical electrodes. The longest interval between acquisition sequences was 4 minutes; because propagation rates of transients are in the range of 1 to 3 mm/min and duration at a given cortical site is 1 to 3 minutes, and because imaging continued for 95% of occlusion duration, it is unlikely that any transient that commenced during an interval between sequences would escape detection.

Occurrence and Topography of Fluorescence Transients

No transients were seen during the time available in these experiments for observation before MCAO. In an unpublished review of 32 earlier experiments with the same anesthetic method and ion-elective electrodes (often at 2 sites) for detection of depolarizations, we found a mean incidence of 0.4 events/hour per experiment before MCAO. However, in the majority of those experiments, intracortical hydrogen polarography electrodes (diameter 125 μm) were in use (the focal cortical trauma associated with needle or electrode insertion is a classic method for the induction of CSD). The question arises as to whether CSD might have

**Figure 2.** Varying propagation of recurrent fluorescence transients in a squirrel monkey 190 minutes after MCAO. a, Raw gray-level fluorescence image of the exposed, paraffin-protected cerebral cortex (370 nm excitation, 445 to 465 nm emission filter), which was subtracted from later images in the sequence. Scale bar=5 mm. b, Difference image showing a central focus of protracted fluorescence increase after propagation of a fluorescence transient 21 minutes after start of sequence. c through e, Images at 32 to 34 minutes from start, showing propagation of a subsequent transient around, but not within, the area of residual fluorescence in b. f through h, A subsequent transient at 54 to 56 minutes in the sequence invades the entire area of cortex.

**Figure 3.** Scatter diagram comparing total number of fluorescence transients with mean plasma glucose (at and for 4 hours) after MCAO in 8 cats. Linear and logarithmic regression analysis \(r=0.606, P>0.05\) failed to demonstrate a significant relationship between transient frequency and plasma glucose, but with a putative plasma glucose threshold set at 4.10 mmol/L, frequency of transients was significantly higher below this level (Mann-Whitney \(U=15.0, P=0.034\)).
been induced in the present studies by possible trauma during creation of the preparation; however, scrupulous attention was paid to craniotomy technique in these experiments. In particular, exposure of the MCA, with opening of the adjacent arachnoid, secured significant drainage of cerebrospinal fluid and hence allowed the brain to fall away from the dura, conferring additional protection.

The incidence of fluorescence transients in cats after MCAO in the present series was 1.3 events per hour; our review of an earlier series \( (n=32) \) yielded a value of 1.26 per hour. Much of the aggregate number of fluorescence transients seen on the ecstosylvian gyrus in the present work is accounted for by experiment 7, and in the remaining experiments, we attribute the low transient incidence on this gyrus to early terminal depolarization, undetected at the time of occlusion (because of the need for visible light during and immediately after the surgical MCAO procedure).

**Factors Affecting the Frequency of Transients**

The frequency of transients was very variable in these experiments, and brief mention must first be made of factors that are already recognized as influencing PID frequency. The incidences of potassium-evoked CSDs in the normally perfused cat brain\(^{24}\) and of PIDs in cats (with MCAO) are appreciably reduced by halothane,\(^{11}\) which may be due in part to the capacity of this agent to uncouple glial gap junctions.\(^{25}\)

We therefore restricted any use of halothane after initial induction of anesthesia to rare, transient supplementation of chloralose, at a maximum inspired concentration of 0.75%. Pool temperature was rigorously controlled in the present cat experiments.\(^5,26\) Despite our considerable efforts to achieve uniform conditions, PID frequency varied widely between individual animals in a species (eg, Table, cats); the experience of Gill et al\(^6\) with rats undergoing MCAO was similar to ours, and we do not believe that the above factors were responsible for our interexperiment variability. Perhaps the best-established and most obvious (but extrinsic) influences on PID frequency are EAAAs.\(^6-8\)

**Plasma Glucose**

We found a statistically significant dependence of fluorescence transient frequency on plasma glucose level, and there is already considerable evidence that supports and explains this finding. Nedergaard and Astrup\(^4\) showed that the rate of PIDs in the penumbra after MCAO in rats was \( 3.8 \pm 1.8 \) at “normoglycemia” \( (9.3 \text{ mmol/L}) \) and \( 0.3 \pm 0.4 \) at hyperglycemia \( (32.5 \text{ mmol/L}) \), and they observed increased glucose phosphorylation in the same region; they suggested that tissue glycopenia was likely to be present, and the high level of plasma glucose required to reduce PID frequency is worthy of note. The present results suggest that the same principle may apply in cats, but at levels of plasma glucose likely to be encountered in clinical practice (especially if an attempt is made to control hyperglycemia to reduce brain acidosis). Given that ATP yield from anaerobic utilization of glucose is one nineteenth of that available from aerobic oxidation, anaerobic glucose utilization must be expected to increase and to become rate-limited by glucose availability. Mies and Paschen\(^{27}\) showed that after a wave of CSD in the normally perfused rat brain in vivo, the tissue glucose pool remained depressed for \( \approx 160 \) seconds. There is also evidence to link diminished tissue glucose availability with destabilization of glutamate (and by implication cation) homeostasis in the extracellular space, which is a possible cause of PID initiation. Swanson et al\(^{28}\) showed that microdialysis of glucose into globally ischemic deep gray matter could reduce ischemic glutamate release to 20% of the value seen with glucose-free dialysate. De Courten-Myers and colleagues\(^{29}\) found that MCAO infarct size in cats was increased both by hyperglycemia and by hypoglycemia, and given the dependence of infarct size on PID number\(^8\) and the likely dependence of glutamate homeostasis on tissue glucose, it is possible that their observations in hypoglycemia could be explained on the basis of increased numbers of PIDs.

**A Species Hierarchy for PID Frequency, and Its Biological Basis?**

What is the basis for the suggestion that PIDS might be less frequent in humans than in the MCAO models? There is experimental evidence that species-related hierarchies may exist for more than 1 relevant variable. First, regarding PID frequency, Nedergaard and Astrup\(^4\) recorded 5.1±2.3 PIDs in their observation period of 80 minutes \( (3.8 \text{ PIDs per hour}) \) in rats (MCAO, pentobarbital anesthesia). In only 1 cat experiment have we observed a frequency in the range of 3.5 to 5 transients per hour; this was in an experiment (number 7, Table) in which plasma glucose was low, and the mean value for the present study group was 1.3 PIDs per hour. Our earlier, unpublished review of 32 MCAO experiments in cats (detection of PIDs with potassium-sensitive electrodes) also yielded a value of 1.3/h. The present experiments represent, so far as we are aware, the first specific attempt to establish a value for PID frequency in primate MCAO experiments. That we have been unable to demonstrate a difference in PID frequency between cats and squirrel monkeys may be due to interexperiment variability but possibly also to the fact that the sizes of the squirrel monkey and cat brains are similar. In consequence, their glial:neuronal ratios and PID frequencies may be similar\(^{30}\) (please see below).

A second variable for which a hierarchy may exist is the flow threshold for homogenous ischemic cell change or massive, sustained potassium release after MCAO. In rats, a flow value of \( 24 \text{ mL} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1} \) is required for homogenous infarction.\(^{31}\) In cats, the flow threshold for sustained, major potassium ion release is \( \approx 16 \text{ mL} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1} \), whereas in the baboon, the corresponding value lies in the range of 8 to 11 \( 100 \text{ g}^{-1} \cdot \text{min}^{-1} \).

Third, in view of the role of the glia in homeostasis of extracellular concentrations of both potassium and glutamate, the ratio of glia to neurons in the cortex is a further potentially relevant variable for which a species hierarchy has been proposed, and it is possible that interspecies variation in this ratio accounts for the differences between cats and rats discussed above. Tower and Young\(^{30}\) described a hierarchy for glial:neuronal ratio in a broadly based group of mammalian species and demonstrated a striking, linear relationship of glial:neuronal ratio with brain size, the hierarchy being: mouse, rat, guinea pig, rabbit, cat, dog, monkey (macaque),...
ox, horse, human, elephant, fin whale. Thus, it may be increasing brain size rather than membership in the primate order that reduces PID frequency.

How might an increased glial:neuronal ratio confer such potential benefits as lower PID frequency and infarction flow thresholds? The glia are essentially the only location of glycogen in the cortex,32,33 are fully capable of anaerobic glycolysis in vitro34 and generation of pyruvate or lactate, and contribute substantially to homeostasis of potassium35 and glutamate36 in the extracellular space. These functions must necessarily become critical (and an increased glial:neuronal ratio an advantage) at the particular stage of progressive focal ischemia when anaerobic metabolism has become the sole source of ATP and glycolytic rate, now enhanced, outpaces glucose availability.

It must be recognized that the 3 sets of findings on which the discussion above is based lack the strength of results from a single, specifically designed study, but it seems that any consideration of species differences in PID frequency must take account of the issue of brain size in relation to glial:neuronal ratio.

**PIDs in Humans: Implications for Treatment of Acute Brain Injury**

Were extracellular ion/neurotransmitter homeostatic capacity indeed related to brain size and glial:neuronal ratio and the proposed hierarchy a reality, a rather lower PID frequency might be predicted for humans than for the experimental species. Evidence for the occurrence of PIDs in humans is extremely limited, but this is possibly due to lack of appropriate methods for detection. There is one recent report12 of transient changes in NADH, extracellular potassium, and laser Doppler flow in the frontal cortex of 1 patient of 14 with severe head injury. We and others agree the transients reported are suggestive of a CSD-like phenomenon, but the significance remains unclear; the finding appears to have been a rarity, and the changes observed may have been preterminal. However, in the patient concerned, the PID detection system was sited over the right frontal convexity, whereas the traumatic lesion was left parietal, and the data may therefore underestimate the frequency of peri-infarct PIDs.

The principal conclusion from this study is that cortical fluorescence transients believed to indicate peri-infarct depolarizations, occur in the nonhuman primate brain during focal cerebral ischemia. Furthermore, we found considerable interspecies variation in PID frequency within the 2 species we studied, so that the possibility of a modest difference in PID frequency between cats and nonhuman primates is not excluded by our results.

Our data suggest that the risk of a substantial increase in frequency of PIDs associated with focal cerebral ischemia rises when plasma glucose falls toward 4 mmol/L. This suggestion is consistent with other studies and may offer at least a partial explanation for other published data relating low plasma glucose with increased infarct size. If confirmed by deliberate plasma glucose reduction in specifically designed experiments, the finding would influence the application of the therapeutic concept of control of plasma glucose in patients with acute brain injury. It also follows that any rigorous interspecies comparison of “natural” PID frequency must be controlled for plasma glucose as well as for anesthesia, temperature, and other factors affecting excitatory neurotransmission, such as pH.

Clear information on the occurrence, frequency, and properties of PIDs in human disease states would be an important addition to our understanding of the pathophysiology of ischemic (and traumatic) brain injury in humans and would provide important guidance on the value of continued efforts to develop neuroprotection strategies based on the use of EAAAs. The scientific case for their use in acute brain lesions in humans remains to be either established or dismissed.

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**Editorial Comment**

In the preceding article, Strong and colleagues have definitively demonstrated that peri-infarct depolarizations (PIDs) occur in the squirrel monkey. The frequency of the fluorescence transients averages 0.7 per hour during an observation period of 4 hours after middle cerebral artery occlusion. This is an important finding, this phenomenon has rarely been observed in human brain injury, and has been generally thought to be restricted to lower species. It appears likely that the species difference is not so great and that transient depolarizations have not been found more commonly in human brain injury because of the difficulty in doing these types of studies in the clinical setting.

Although spreading depression is not associated with injury in the normal brain, it may be reasonable to hypothesize that the additional metabolic stress caused by PIDs may be deleterious in the ischemic brain. As the authors discuss in their article, some evidence has begun to accumulate that the occurrence of PIDs may actually increase the severity of ischemic damage. Infarct size is related to the number of PIDs observed in focal ischemia models, and treatments that reduce
the number of PIDs observed have protective effects during focal ischemia. However, these studies do not establish a cause-and-effect relationship for PIDs and increase injury. The presence of PIDs may simply be a marker of a more severe ischemic injury. The study of Busch et al., lends more support to this idea by demonstrating that repeated episodes of cortical spreading depression induced by potassium are associated with a larger volume of tissue injury. However, because these studies involved induced spreading depression, the role that depolarizations which occur spontaneously after focal ischemia play in worsening injury is still not entirely clear.

In attempting to identify factors that might explain the variability of PID frequency among individual animals, the authors also observed an interesting association between plasma glucose concentration, and the frequency of PIDs in the cat studies. The animals with low plasma glucose had a significantly higher frequency of PIDs after middle cerebral artery occlusion. Because the numbers of animals in the study are small, and the studies were not really designed to examine this relationship, the association must be considered speculative. Although additional studies are needed to definitively answer this question, this issue of optimal plasma glucose concentration is very timely. Hyperglycemia has long been associated with a higher mortality rate and poorer neurological recovery after stroke. In a recently published analysis of 1259 patients involved in the trial of ORG 10172, a higher plasma glucose concentration on admission was significantly associated with a poorer neurological recovery at 3 months. This relationship was especially strong for the subgroup of patients with nonlacunar stroke. A recent pilot trial has demonstrated the feasibility of reducing plasma glucose concentrations after acute stroke with glucose potassium insulin infusion. Because trials are conducted to study the efficacy of this treatment strategy for stroke, it will be important to consider that there may also be adverse consequences for a plasma glucose concentration that is too low for the ischemic brain.

Claudia Robertson, MD, Guest Editor
Department of Neurosurgery
Baylor College of Medicine
Houston, Texas

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