Ischemic Preconditioning in 18- to 20-Month-Old Gerbils
Long-Term Survival With Functional Outcome Measures

Jennifer Dowden, BSc; Dale Corbett, PhD

Background and Purpose—In young animals, ischemic preconditioning protects CA1 hippocampal neurons against global ischemia. However, cerebral ischemia occurs most frequently in individuals aged ≥65 years. This study examined the protection provided by ischemic preconditioning in a population of aged (18- to 20-month-old) gerbils.

Methods—One group of animals was exposed to two 1.5-minute episodes of global ischemia separated by 24 hours and followed 72 hours later by a 5-minute occlusion of both carotid arteries. A second group was given 2 episodes of preconditioning only. Two other groups were exposed to 5 minutes of ischemia or sham surgery. The animals survived 10, 30, or 60 days. Functional and histological assessments were used to determine the extent of protection.

Results—Ten days after ischemia there was >80% protection of CA1 neurons in ischemic preconditioned animals compared with 6% in ischemic gerbils. Nevertheless, these preconditioned animals were impaired in open-field tests of habituation. In addition, CA1 dendritic field potentials were smaller in amplitude compared with those in sham animals. While there was a complete loss of staining for CA1 microtubule-associated protein-2 in ischemic animals, staining in ischemic preconditioned animals was normal. This suggests that dendritic abnormalities per se were not responsible for the observed functional deficits. CA1 cell survival declined to ~75% of sham values (P<0.05) at 60 days after ischemia.

Conclusions—Ischemic preconditioning provided substantial neuroprotection in aged gerbils. Nonetheless, the striking dissociation between histological and functional protection provided by ischemic preconditioning in aged animals emphasizes the need to use functional end points and long-term survival when assessing neuroprotection. Although functional recovery was evident with increasing survival time, CA1 cell death continued, thereby raising the possibility that the level of neuroprotection attained was not permanent. (Stroke. 1999;30:1240-1246.)

Key Words: aging ■ cerebral ischemia ■ hippocampus ■ neuroprotection ■ gerbils

A pproximately three quarters of all stroke cases affect those aged ≥65 years.1 Despite this fact, most stroke research is performed in young animals. This may be problematic, because there are numerous age-related differences in brain biochemistry, morphology, and electrophysiology.2–6 Previous studies examining the influence of age on ischemic outcome have produced conflicting results. In experiments involving focal ischemia, infarct volume was consistently increased in aged versus young rats.7,8 Global ischemia studies, however, have yielded more variable results. In one study there was increased neuronal loss in both the hippocampal CA1 region and striatum of aged (18- to 22-month-old) versus young (5- to 6-month-old) rats.9 In contrast, another study reported an age-dependent regional vulnerability after global ischemia whereby there was less CA1 neuronal loss in old (26- to 28-month-old) than in young (2- to 3-month-old) rats but increased striatal and neocortical damage.8 Finally, Corbett and colleagues10 reported a similar degree of neuroprotection after global ischemia in old and young gerbils treated with intraischemic but not with postischemic hypothermia. These authors cautioned that the efficacy of a articular treatment should be confirmed in aged animals.

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Ischemic preconditioning is a procedure whereby brief, noninjurious episodes of ischemia are protective against a subsequent, more severe insult that would typically destroy selectively vulnerable cells. For example, in the gerbil model of global ischemia, 4 days after a 5-minute occlusion of both carotid arteries 80% to 90% of the CA1 cells are destroyed, a process termed “delayed neuronal death.”11,12 However, if the gerbil is subjected to 1 or 2 brief episodes (eg, 2 minutes) of ischemia before a more severe insult, there is approximately 60% to 90% protection of CA1 neurons.13,14 This phenomenon has received widespread interest, because it may provide insight into endogenous protective mechanisms that could be harnessed to attenuate ischemic cell death.

Although protection provided by ischemic preconditioning appears robust, a drawback of the majority of preconditioning studies is that only histological end points have been used, invariably following short survival times (eg, 7 days). With this evaluative method it cannot be determined whether the remaining, apparently normal, cells are functioning properly.
In fact, there is evidence that this may not be the case. Hori and Carpenter\(^{15}\) revealed that after global ischemia in rats there was a reduction in the amplitude of the population excitatory postsynaptic potential (EPSP) recorded from healthy-looking CA1 neurons with normal membrane potentials and resistances. Furthermore, long-term potentiation could not be produced in these cells. On closer examination, the CA1 dendrites had an abnormal, beaded appearance, which is an early sign of neuronal injury.\(^{15-17}\) Similarly, behavioral impairments (as assessed by acquisition of operant behavior) after unilateral forebrain ischemia have been noted despite hippocampal pyramidal neuron preservation.\(^{18}\) In another study, it was found that although pentobarbital administration after global ischemia protected CA1 pyramidal cells, it was unable to preserve hippocampal cholinergic function.\(^{19}\) With respect to ischemic preconditioning, Corbett and Crooks\(^{20}\) recently reported a dissociation between histological protection provided by ischemic preconditioning and behavioral protection, as assessed by an open-field test of habituation. This is in contrast to histological and behavioral protection provided by both postischemic and intraischemic hypothermia, the latter being the “gold standard” of neuroprotection.\(^{21-23}\) Considering that behavioral outcome is of paramount importance after stroke, it is essential that functional end points be used in experimental studies.

Increased locomotor activity in an open field has been useful as an early indicator of ischemic damage. The open field tests an animal’s ability to habituate to a novel environment. The inability to do so has been linked to loss of CA1 neurons\(^{24,25}\) and not to loss of striatal or cortical neurons.\(^{26}\) However, the open-field test is not specific to hippocampal function, and even animals with extensive CA1 loss eventually recover, most likely due to compensation by other brain regions.\(^{27}\) Thus, a valuable additional functional end point is to record CA1 field potentials that have been found particularly effective when combined with behavioral and histological measures.\(^{21}\)

Traditional histological staining methods (eg, hematoxylin and eosin) are not very sensitive indicators of early neuronal injury, because cell death may not become obvious until several days after ischemia. This is even more problematic after neuroprotective treatments that may delay cell death for weeks or months after injury.\(^{23,28-30}\) It has been suggested\(^{16,17}\) that a more sensitive indicator of early neuronal injury is loss of microtubule-associated protein-2 (MAP-2), which is primarily localized to dendrites.\(^{31}\) A reduction in this structural protein could conceivably alter neuronal function in the absence of conspicuous changes in Nissl staining of CA1 somata. If so, this could account for our previous observation\(^{20,32}\) that ischemic preconditioning preserves CA1 neurons (ie, using Nissl stains) even though functionally these neurons appear compromised.

In view of the above considerations, the purpose of this study was to examine ischemic preconditioning in a population of aged gerbils (18 to 20 months) and assess its efficacy in preserving hippocampal CA1 neurons. To this end, we used behavioral, electrophysiological, immunocytochemical (MAP-2) and standard histological end points as well as long survival times (10, 30, and 60 days). The use of longer survival times ensures that treatments are providing true neuroprotection rather than merely delaying cell death.\(^{23,29}\)

### Materials and Methods

#### Subjects

A total of 69 female Mongolian gerbils weighing 55 to 114 g and aged 18 to 20 months were used for this study. All experiments met the guidelines established by the Canadian Council of Animal Care and were approved by the Memorial University of Newfoundland Animal Care Committee.

#### Temperature

Brain temperature was closely monitored in all animals before, during, and after the ischemic episode, as previously described.\(^{20,21,28}\) Under 1.5% halothane anesthesia (30% O\(_2\)/70% N\(_2\)O), all animals were implanted with a 5-mm/20-gauge guide cannula overlaying the dorsomedial striatum. Forty-eight hours later, direct measurement of brain temperature was recorded with wireless temperature probes (model XM-FX, Mini-Mitter Co, Inc) inserted into the striatal guide cannula to a depth comparable to that of CA1 neurons. Temperature was measured every 20 seconds for a period of 3 hours to establish a record of normal brain temperature.

#### Surgery

Animals were allocated to the following groups before the experiment: sham (S); preconditioned only (PO), consisting of two 1.5-minute occlusions separated by 24 hours; ischemic preconditioned (IP), two 1.5-minute occlusions separated by 24 hours and followed 3 days later by a 5-minute occlusion; and (I), a 5-minute ischemic group without prior preconditioning. All operations were performed under halothane anesthesia (1.5%, 30% O\(_2\)/70% N\(_2\)O). A ventral midline incision was made in the neck and the carotid arteries were carefully isolated. Miniature aneurysm clips were placed on the arteries, and blockage of blood flow was visually confirmed. Before and throughout occlusion, brain temperature was kept between 36.0°C and 36.5°C with the use of a heated water blanket wrapped around the head. Core temperature (37°C) was also maintained during surgery with an overhead lamp and a homeothermic heating blanket wrapped around the animal’s body (Harvard Apparatus).

At the end of the occlusion, the clips were removed and reflow was visually confirmed. After occlusions, the incisions were closed, anesthesia was discontinued, and the animals were placed in small Plexiglas cages resting on an AM receiver. Brain temperature was monitored for a period of 8 hours (following ischemic preconditioning) and for 24 hours following 5-minute occlusions, as done previously.\(^{20}\) For 30 minutes after ischemia, brain temperature was kept at ~36.5°C by use of an overhead lamp until the animals recovered and could regulate their own temperature. Sham animals underwent the same procedures without carotid artery occlusion.

#### Survival Times

Sham animals survived 30 (S-30, n = 3) and 60 days (S-60, n = 6), ischemic animals survived 10 (I-10, n = 6) and 30 (I-30, n = 7) days, preconditioned only animals survived 10 (PO-10, n = 8) and 30 (PO-30, n = 7) days, and ischemic preconditioned animals survived 10 (IP-10, n = 9), 30 (IP-30, n = 15), and 60 (IP-60, n = 8) days.

#### Behavioral Testing

Behavior in the open field was assessed on days 3, 7, 10, 30, and 60 after ischemia (depending on survival time) in a soundproof room. The position of various environmental stimuli in the room (eg, location of shelves) remained constant throughout the experiment. The floor of the open-field apparatus (72×76×57 cm) was electronically divided into 25 equal squares, and a visual tracking system (HVS Systems) recorded the number of squares entered per minute over a 10-minute test session. The total number of squares entered throughout the 10-minute test session was then used for analysis.
Electrophysiology
After the last day of behavioral testing, animals were reanesthetized with 1% to 2% halothane (30% O2/70% N2O) and cooled to a brain temperature of 30°C with use of a water blanket circulating ice cold water. The gerbils were then decapitated and their brains quickly removed and placed in ice-cold oxygenated sucrose Krebs solution containing (mmol/L) sucrose 252, KCl 2.0, NaHCO3 25, MgCl2 1.3, glucose 11.0, NaH2PO4 1.2 that was bubbled with 95% O2/5% CO2. The brains were hemisected; one hemisphere was immersed in 10% phosphate-buffered formalin (4°C) for histology, and the other hemisphere was used for electrophysiology. The hippocampus was isolated and cut in 500-μm transverse sections with a tissue chopper. Slices were again placed in the above artificial cerebrospinal fluid (ACSF) solution at room temperature for 5 minutes, as done previously.15 Slices were then transferred to an oxygenated (95% O2/5% CO2) ACSF solution containing (mmol/L) NaCl 126.0; KCl 3.5; NaHCO3 25, MgCl2 1.3; glucose 11.0; and NaH2PO4 1.2, and incubated for at least 1 hour before recordings.

Individual slices were placed in a fluid interface chamber (Fine Science Tools Inc) perfused with oxygenated ACSF at a flow rate of 2 mL/min. Temperature of the bath was maintained at 33°C. Orthodromic stimulation (0.02-millisecond constant-current pulses delivered at a rate of 0.05 Hz) of the Schaeffer collaterals was achieved using Ultrasound concentric bipolar stimulating electrodes (100 μm; Frederick Haer Company). Glass microelectrodes with a tip diameter of ~20 μm and filled with 2 mol/L NaCl were used for recording EPSPs in stratum radiatum. Responses were amplified and displayed on an oscilloscope, digitized, and stored on computer for later analysis. The viability of the slice was tested first by orthodromic stimulation of the perforant path and recording in dentate granule cells (uninjured after ischemia). In slices that failed to produce a dentate population spike, no recordings in CA1 were attempted.

Histology
After immersion fixation, the hemisphere reserved for histology was embedded in paraffin, and 2 series of 6-μm-thick coronal sections were cut. The first series was stained with hematoxylin and eosin, and the number of normally stained neurons (distinct cell membrane and nucleus, not eosinophilic) remaining in a 200-μm grid were counted in medial, middle, and lateral sectors of dorsal CA1 at 1.7 mm (level A) and 2.2 mm (level B) posterior to bregma.23 Cells were also counted from the middle sector of CA1 2.8 mm behind bregma (level C). The second series of sections were immunostained with anti-MAP-2 diluted 1:500 (Sigma Chemical Co) and developed with avidin-biotin-peroxidase complex (Vectastain ABC), followed by incubation with diaminobenzidine–nickel chloride, as previously described.32 Negative controls were run repeatedly by omitting the primary antibody. There was no staining in these sections. Semi-quantitative analysis of the stained sections at level A above was performed with NIH Image software running on a Macintosh 7600 computer to determine relative optical densities (ROD). Briefly, the ROD for the apical dendritic fields of CA1 were measured and the data normalized to the unstained corpus callosum (CC) using the following formula: (ROD CA1–ROD CC)/ROD CA1.1

Statistics
With the exception of days 3, 7, and 10 open-field data, all other data were analyzed using 1-way ANOVA. The open-field data for days 3, 7, and 10 were analyzed using repeated-measures ANOVA. Individual post hoc comparisons were performed, when necessary, using the Newman-Keuls test to compare group means. Temperature profiles, behavior in open field, and cell counts were all obtained from the same animals, and electrophysiological and MAP-2 immunocytochemical data were obtained from subsets of the animals in each group.

Results
There were no group differences in weight at the start of the experiment. Eight animals died throughout the course of the experiment. Six died as a result of respiratory failure, 1 from a severed artery, and 1 animal was killed because of weight loss resulting from a brain infection. One animal in the PO group was excluded from the study. In this animal, there was complete loss of CA1 cells on histology, most likely due to impaired in ischemic preconditioned animals, although there is a trend for recovery in the latter group with repeated exposure and longer survival time. *P<0.01, †P<0.05 with respect to group S; ‡P<0.05 comparing group IP with group I.

Temperature
The mean baseline temperature for the groups ranged from 36.8°C to 36.9°C. Similarly, there were no significant differences in the intraischemic (5 minutes) brain temperatures between groups (F1,51 = 2.991, P = 0.0591). The mean postischemic brain temperatures recorded over 24 hours were 36.66±0.23, 36.96±0.34, and 37.14±0.29°C for the S, IP, and I groups, respectively. These postischemic temperatures of the IP and I groups were significantly different from those of group S (P<0.05), reflecting the brief period of hyperthermia that is common after global ischemia in this model.28

Behavior
Data from the open field are presented in Figure 1. Repeated-measures ANOVA indicated a significant treatment effect
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...vided robust protection of CA1 neurons (P < 0.01) as well as a significant treatment by day interaction (F = 5.504, P < 0.01). On the first test day, sham animals exhibited normal levels of exploration and showed habituation both within the test session (data not shown) and on subsequent test days. The PO group followed the same behavior pattern as the S group, and although levels of recorded activity were slightly higher on all test days, they were not significantly different from sham levels. In contrast, the I group displayed heightened levels of activity during all test sessions that were significantly different from those of the S group on days 3, 7, 10, and 30 (P < 0.01). In addition, these ischemic animals showed very little within-session habituation (data not shown). Interestingly, similar levels of increased activity were seen in the IP group on all test days, and although overall activity levels did decline with repeated exposure, the behavior of these gerbils in open field remained significantly different from the S group on days 3, 7, and 10 (P < 0.01) as well as on days 30 and 60 (P < 0.05). A comparison of the IP and I animals revealed significant differences on days 7 (P < 0.05) and 10 (P < 0.01) in open-field activity, indicating a more rapid recovery in the IP group.

Electrophysiology

CA1 field potentials were recorded from approximately 100 slices taken from 35 animals (S-60, n = 5; PO-10, n = 5; I-10, n = 6; IP-10, n = 6; IP-30, n = 6; and IP-60, n = 7). Three recordings of maximal fEPSP amplitude were averaged for each rostral, middle, and caudal section from every animal. These means were then averaged to yield an overall maximum amplitude representative of the whole hippocampus. Following stimulation of the Schaeffer collaterals, the maximum amplitude of the field EPSPs (fEPSPs) recorded from stratum radiatum of gerbils in the S and PO groups were not significantly different (–4.04 mV versus –3.514 mV). In keeping with the deficits in behavior in open field, fEPSPs measured in IP group at 10-day survival were significantly lower than those of the S and PO groups (P < 0.05). However, with longer survival times (Figure 2), there was recovery of the evoked responses from –2.487 mV at 10 days to –2.975 mV and –3.173 mV at 30 and 60 days, respectively, which was not significantly different from that in the sham animals. In comparison, the mean amplitude of the fEPSP of the I group was –1.07 mV, which was significantly different from all groups (P < 0.01). Previous studies from this laboratory have shown that the field potentials recorded from I group animals do not recover over time, because recordings taken 3 to 4 weeks after ischemia are not different from those at 10 days.

Histology

The results of the histological assessment are presented in Figure 3. Five minutes of ischemia resulted in 93.8%, 88.5%, and 70.4% loss of CA1 pyramidal cells at levels A, B, and C, respectively (P < 0.01 versus S, PO, and IP groups). PO animals were not significantly different from S animals. Ischemic preconditioning with 10- and 30-day survival provided robust protection of CA1 neurons (>80%) at all levels measured. However, the percent savings at levels A and B did decline with continued survival, such that by 60 days (IP-60) 75% of CA1 neurons were remaining at level A (P < 0.05 versus S group).

Figure 2. Maximum amplitude of CA1 fEPSPs (mean ± SD) recorded in stratum radiatum from group S (n = 5), PO (n = 5), I (n = 6), IP-10 (n = 6), IP-30 (n = 6), and IP-60 (n = 8) animals. Note the recovery of CA1 fEPSPs in IP animals with increasing survival time. Ischemic preconditioning provided significant protection at all survival times (P < 0.01, versus group I). Recordings from S, PO, and I animals were performed after 60-, 10- and 10-day survival, respectively, because survival time (ie, 10 versus 30 versus 60 days) would not affect fEPSP amplitude in these groups (see “Results”). *P < 0.01, †P < 0.05 with respect to group S.

There was a significant difference in MAP-2 immunostaining between the S and I groups (P < 0.01). There was essentially a near-absence of MAP-2 staining in the apical dendritic region of CA1 from ischemic animals, whereas the area under CA3 remained normal (Figure 4). IP gerbils, irrespective of survival time, showed no significant changes in MAP-2 compared with S animals. However, the mean ROD of the IP group was slightly lower than that of the S group (0.66 versus 0.71, respectively).

Discussion

Numerous studies have confirmed the neuroprotective efficacy of ischemic preconditioning in animal models. However, these studies have typically used young subjects, and it was therefore unknown whether similar levels of protection would be obtained in older animals.

In the present study there was significant histological protection of CA1 neurons across 3 anterior-posterior levels of the hippocampus (88.2%, 83.5%, and 89.4% of sham controls) at 10 days in preconditioned animals. The rostral CA1 region is known to be highly vulnerable to cerebral ischemia. In this study, the degree of neuronal protection found at this level was similar to that seen in similar studies from our laboratory using young (3-month-old) animals. Interestingly, there was not as rapid nor as great a decline in CA1 cell numbers in the aged animals with longer survival times. Thirty days after ischemic preconditioning there were ≈53% and ≈66.5% savings of rostral CA1 in the young animals. This is in contrast to the 75% savings found 60 days after a 5-minute ischemic episode in aged ischemic preconditioned gerbils. This suggests that ischemic preconditioning...
is at least as, if not more, effective in aged versus young animals. Nonetheless, as in other long-term survival studies using both hypothermia and neuroprotective compounds, neuronal loss continued with increasing survival time (88.2% at day 10 compared with 75% at day 60, level A).

As previously mentioned, several studies have reported a dissociation between histological and functional protection after both ischemia and ischemic preconditioning. In this study, animals subjected to ischemic preconditioning displayed heightened levels of locomotor activity in the open field similar to those of ischemic animals without any preconditioning. This was despite the fact that cell counts at this time in preconditioned gerbils were near normal (e.g., 88% of group S at Day 10). Cell counts from S, PO, and I groups have been pooled since they did not differ with survival time (see "Results").

Increased open-field activity has consistently been a reliable indicator of ischemic damage; however, it is not a specific measure of CA1 or even hippocampal function. Therefore, measurement of fEPSPs provides a more direct assessment of hippocampal CA1 function. Recordings from gerbils that had received ischemic preconditioning were characterized initially by a decreased amplitude in CA1 evoked potentials. This coincides with the deficits seen in the open field and occurs at a time when cell counts are virtually normal. In conjunction with the improvement in behavior, there was recovery of the fEPSP amplitudes in IP animals with increasing survival time, such that by day 60 they were not significantly different from sham values. Although there was a trend for increased cell death with longer survival times, it is not known whether cell death would continue past day 60, and if it did, how this would ultimately affect field potentials. It is possible that through alteration of postsynaptic receptor density or increased transmitter release from Schaeffer collaterals the fEPSPs would remain essentially normal. Alternatively, if CA1 cell death continues, these compensatory mechanisms may not be able to sustain normal fEPSP amplitudes.

MAP-2 has been reported to be an early indicator of ischemic injury. In this study, animals subjected to ischemic preconditioning displayed heightened levels of locomotor activity in the open field similar to those of ischemic animals without any preconditioning. This was despite the fact that cell counts at this time in preconditioned gerbils were near normal (e.g., 88% of group S at Day 10). With repeated exposure to the open field, the animals in all groups showed habituation; however, testing on day 60 still revealed significant differences between S and IP animals (P<0.05). These results are consistent with those of previous studies in that ischemic animals tend to recover with repeated exposure to a novel environment, especially when tests are conducted at short intervals. However, persistent increases in locomotor activity are observed when test sessions are spaced out over weeks or months. Moreover, when apparently recovered animals are exposed to a semi-novel environment, they again show elevated levels of activity.

Figure 4. Representative photographs of MAP-2 immunocytochemistry from groups S (top left), I (top right), and IP after 10- and 60-day survival (bottom left and right, respectively). Note the complete absence of MAP-2 staining in the apical dendritic region of CA1 in group I compared with group S. Ischemic preconditioning did not significantly alter the pattern or intensity of MAP-2 immunocytochemistry assessed at 10, 30, and 60 days after ischemia. Scale bar=0.5 mm.
tioning. In addition, differences in the activation of microglia (cytotoxic) and astroglia (neurotrophic) may play a role in ischemic preconditioning.

Regardless of the mechanism(s), the results of this study clearly show that ischemic preconditioning is quite effective in aged gerbils, possibly more so than in young animals. Although evidence in the literature generally supports the notion that there is increased damage following ischemia in aged animals, one study found less damage in CA1 in aged rats after global ischemia. Undoubtedly some of these differences are due to variations in the model used (global versus focal), the severity of the insult, the survival times used, and the adequacy of temperature control during and after ischemia. Increased efficacy of ischemic preconditioning in older animals may reflect differences in the vulnerability of the aged population to ischemia and/or ability to tolerate treatment procedures compared with that in young subjects. There is evidence for decreased density of N-methyl-D-aspartate (NMDA) receptor complexes as well as decreased responsivity to glutamate in the aged rodent brain. Prolonged activation of NMDA receptors by glutamate and entry of calcium into the cells has been thought to be a key component of the excitotoxic theory of ischemic cell death. A decrease in the number and sensitivity of NMDA receptors in aged animals could potentially reduce this excitotoxic response and decrease neuronal death.

The results of the current study raise several important issues concerning neuroprotection. First, there may be subtle differences in the ability of aged versus young animals to tolerate ischemia. If so, it is important that future experiments be performed to confirm these findings and investigate the factors that can account for the influence of age. Second, a functional approach is necessary for determining the extent of neuroprotection. In studies using intraischemic and postischemic hypothermia, deficits in behavior were directly related to degree of neuronal loss. This is clearly not the case with ischemic preconditioning and may not be the case with other (eg, drug) protective treatments. In addition, several studies have shown a similar dissociation between histological end points and other outcome measures after ischemia. Considering that functional outcome is of paramount clinical importance, the aim of any potential treatment should be to provide long-term physiological and behavioral protection and not just histological preservation. Therefore, extended survival times and multiple end points are essential for determining true neuroprotection.

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References

Ischemic preconditioning in Old Gerbils


**Editorial Comment**

Ischemic preconditioning, in which prior exposure to brief episodes of ischemia renders the brain resistant to more sustained ischemic attacks, offers a new and potentially beneficial strategy for cerebroprotection. In the accompanying article, Corbett and colleagues examine the protection provided by such preconditioning in a population of aged gerbils and compare the results with those obtained from a comparable study in young gerbils.1 In addition to assessing the ability of preconditioning to preserve hippocampal CA1 neurons, the investigators used electrophysiological techniques to monitor CA1 dendritic field potentials as an indicator of synaptic function, together with open-field locomotor activity measurements of ability to habituate to a novel environment. A significant aspect of this study is the prolonged survival time (60 days) over which measurements were recorded.

The results demonstrate that ischemic preconditioning by two 1.5-minute episodes of global forebrain ischemia (bilateral carotid artery occlusion) separated by 24 hours, followed 72 hours later by a 5-minute ischemia, confers substantial protection of CA1 neurons, together with preservation of CA1 dendritic field potentials, in comparison with non-preconditioned ischemic controls. Preconditioning appeared to be at least as protective, if not more so, in aged versus young gerbils. Even so, there appears to have been a slow loss of CA1 neurons over the 60-day period. A dissociation between the histological preservation and functional protection became apparent when preconditioned animals were evaluated for ability to habituate to a novel surrounding in open-field trials. Increased locomotor activity in such trials is regarded as a reliable indicator of ischemic damage, possible due to a failure of cognitive functions,2,3 with a reduction in ability to form spatial maps of the surroundings. In the present study, preconditioned animals displayed elevated levels of locomotor activity during open-field trials at 30 days comparable with those displayed by the ischemic controls, even though CA1 neuronal loss was more severe in the latter group.

Although the mechanisms underlying ischemic preconditioning are unresolved at this time, the results clearly demonstrate its efficacy in the aged gerbil. The study is noteworthy in that a prolonged (60-day) period of observation followed the exposure to ischemia. During this interval a degree of functional recovery was evident in the open-field trials, even though some further CA1 neuronal loss occurred. The authors stress that extended survival times, with measurements of multiple end points, are essential for a meaningful assessment of cerebroprotective activity following an ischemic episode.

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