Ischemic Preconditioning: Postischemic Structural Changes in the Brain

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Abstract

Ischemic brain damage can be prevented or at least significantly reduced when there is a preceding brief ischemic period that does not exceed the threshold for tissue damage—a phenomenon termed “ischemic preconditioning” (ischemic PC). Experimental PC in rodents is now considered to be a model for transient ischemic attacks in humans, and there is increasing hope for translating the knowledge of underlying mechanisms in the animal models into the clinic to enhance endogenous neuroprotective mechanisms in patients with stroke. However, although PC was originally defined as a subtoxic stimulus without any morphologic damage, there is a growing body of evidence from studies using sensitive techniques that postischemic structural alterations of brain tissue manifest not only after ischemia with prior PC but also after the PC stimulus itself. Furthermore, it has become evident over time that the primary shortcomings of many experimental studies on PC are the short observation intervals. The few studies with extended postischemic survival periods done to date provide clear evidence of considerable structural changes and even cell death, which may only be postponed by PC. Therefore, further studies are needed to elucidate structural long-term changes after PC and to validate the persistence of the neuroprotective effects.

Key Words: Brain ischemia, Ischemic preconditioning, Transient ischemic attacks.

INTRODUCTION

The term “ischemic preconditioning” (ischemic PC) describes the phenomenon of a short, by definition, harmless ischemic period that provides protection against a subsequent ordinarily injurious ischemic injury. Ischemic PC is not restricted to the brain because it has also been observed in many other organs (1). Furthermore, it has become clear that acquisition of an ischemia-tolerant state can, in principle, be achieved by any noxious stimulus that does not exceed a threshold of tissue damage (2). This phenomenon of so-called “cross tolerance” comprises a variety of PC stimuli, including seizures (3), inflammation (4), hypothermia (5) and hyperthermia (6), sleep deprivation (7), and dietary restriction (8). Even sham operation with manipulation of the middle cerebral artery has been documented to cause protection of cortical neurons after transient forebrain ischemia in rats (9). Ischemic PC must, however, be distinguished from episodes of cerebral ischemia with short recovery intervals, which have been shown to aggravate brain damage when compared with a single episode of ischemia of the same total duration (10–13). Although some kind of stress is obviously necessary to acquire an ischemia-tolerant state, energy failure per se is not a prerequisite because cortical spreading depression associated with similar ion fluxes as seen during ischemia (14), but with minimal changes in adenosine triphosphate levels (15), also induces tolerance (16). Interestingly, brief ischemia of the skeletal muscle (17) and even physical exercise render the brain tolerant against ischemic injury, a phenomenon termed “remote PC” (18).

Most authors refer to an article published by Janoff (19) in 1964 as the first to introduce the terms PC and “induction of tolerance” into the literature, when he demonstrated in rats that repeated traumas with increasing severity before a normally harmful rotational trauma rendered hepatic lysosomes stable. However, there are at least 2 earlier papers (i.e. from 1962) that had previously used the term “tolerance” to designate such phenomena (20, 21). During the next decades, PC disappeared widely from the literature until 1986, when an article by Murry et al (22) (that was frequently, but again incorrectly, cited as the first PC article) initiated a renaissance of investigations in this field. There have been an exponentially increasing number of publications on this topic since then. Using a canine model of cardiac ischemia, Murry et al demonstrated that PC with repeated short ischemic periods of 5-minute duration could reduce infarct size when followed by a sustained ischemic period of 40 minutes. However, this protection disappeared when the ischemic period was extended to 3 hours. This finding was originally interpreted as a mere delay in cell death by PC (a fact that was frequently overlooked). This would imply a longer potential time window to save the myocardium via reperfusion, for example, by thrombolytic therapy or coronary angioplasty. At that time, one major issue became evident, that is, that the acquisition of an ischemia-tolerant state is a transient phenomenon that fades over time. According to current knowledge, 2 phases of tolerance induction with different underlying molecular mechanisms can be distinguished: tolerance of the immediate and delayed type. Whereas immediate or rapid tolerance is
induced within minutes and lasts for a maximum of a few hours, delayed tolerance develops within hours and persists up to several days, fading after 1 to 2 weeks. The underlying molecular mechanisms are as different as the time windows. Delayed tolerance depends on protein synthesis, whereas rapid tolerance is based on posttranslational modifications (23). Although induction of rapid tolerance is also possible (24), it is the tolerance of the delayed type that plays a major role in the brain. Concerning the molecular basis of delayed PC, induction of heat shock proteins and immediate early genes have primarily been thought to be the first steps in the neuroprotection cascade (25, 26). Until now, many different pathways involved in mediating the neuroprotective effect have been investigated, and a considerable number of reviews have dealt with reprogramming of the genetic response by PC (2, 23, 27). From studies of genome-wide gene expression analysis after PC in experimental models of focal and global ischemia, it became apparent that numerous genes coding for structural proteins are differentially regulated by PC only (PCO) (e.g., calpactain I heavy chain [p36], fibulin 2, glial fibrillary acidic protein, β-sarcoglycan, and keratin complex 1) or after injurious ischemia with prior PC (IPC) (glial fibrillary acidic protein, matrix γ-carboxyglutamate protein, troponin T, and Homer-1C) (28, 29). One focus of this review, therefore, is to highlight the significant clue that, apart from tissue damage, other postischemic structural changes may be induced by ischemic PCO or IPC in the brain.

CELL LOSS AND TISSUE DAMAGE AFTER PCO AND IPC

Despite the acquisition of an ischemia-tolerant state, the most dramatic structural alteration both after PCO and after IPC would be cellular or tissue damage. A PC ischemic stimulus leading to tissue injury, however, would exceed the threshold defining PC and, therefore, according to the current concept, indicate only suboptimal PC. This rigorous view was nourished by most PC studies that analyzed structural integrity up to a maximum of 7 days. From the few investigations with extended surviving times in gerbil and rat models of global ischemia, however, it became evident that PC itself may trigger at least minimal cell death of vulnerable hippocampal CA1 neurons (13, 30, 31) (Table 1). This problem becomes more evident after IPC. Whereas PC before focal ischemia only reduces the infarct volume without completely preserving the tissue in the ischemic territory (32), models of global ischemia in gerbils and rats show that PC may provide near-complete protection. In fact, several reports state that there is lasting protection for up to 14 (33), 30 (34), or 56 days (35) (Table 2). However, some of these studies lack exact quantification (34) and/or use routine histology only (33), which may prevent detection of subtle damage. Other studies with long-term observation periods revealed a sometimes extremely delayed degeneration (up to more than 100 days after reperfusion) of vulnerable neurons in the hippocampal CA1 region (31, 36–38), and unpublished observations (Table 2). Because the question of whether degeneration may simply be due to suboptimal PC is a common issue in all of these studies that demonstrate cell damage, Abe and Nowak (39, 40) developed an elegant technique to address this problem. Their PC protocol is not defined by fixed occlusion times but by depolarization thresholds. Monitoring of ischemic depolarization is particularly effective in reducing the variability in the gerbil (40) and the rat model for ischemic PC (41) and is superior in predicting brain injury to the technique of only assessing failure of electroencephalographic activity (42). Despite this optimized model of ischemic PC, however, there is striking attenuation of protection after 3 months compared with that observed at 1-week survival (41) (Table 2). Therefore, future studies with observation periods longer than only a few months will need to clarify whether PC postpones neuronal death in general or only in specific neuronal subpopulations.

Another important, although frequently ignored, fact is that most PC studies in global ischemic models exclusively focus on the integrity of vulnerable hippocampal CA1 neurons. However, one of the first studies on ischemic PC in the gerbil model demonstrated that despite reduction of damage in CA1, neuronal cell loss in the hilus was not significantly reduced (49), a finding corroborated by subsequent studies (50). Furthermore, even a single short ischemic period usually used for PC has been shown to destroy hilar somatostatin neurons (51). Therefore, it must be assumed that even in an optimized PC model with at least partly prevented neuronal density in CA1, there are still other vulnerable neurons that inevitably undergo cell death. Loss of hippocampal hilar interneurons, however, is a dramatic structural alteration with consecutive rebuilding of hippocampal circuitries, the functional consequences of which have not yet been studied in detail.

NEUROGENESIS AFTER PCO AND IPC

During the past decade, it has been demonstrated that neurogenesis also takes place in the adult mammalian brain in 2 regions: the dentate gyrus (DG) and the subventricular zone (52). Apart from its cell-damaging effect, ischemia has also been shown to enhance neurogenesis in both the DG and the subventricular zone (52). The influence of a short PC ischemia or a severe ischemia subsequent to PC on neurogenesis, however, has been investigated in very few studies. Using the gerbil model of global ischemia, Liu et al (43) demonstrated that PCO does not trigger neurogenesis in the granule cell layer of the DG, whereas both ischemia with and without prior PC led to a more than 10-fold increase in neurogenesis within this region, peaking at 11 days after reperfusion. These results indicate that ischemic PC does not prevent the increased neurogenesis usually seen after a severe ischemic challenge (53). More importantly, the loss of CA1 neurons is not a prerequisite for increased cell proliferation in the DG. In a rat model of focal PC, it was demonstrated that PCO, which did not cause infarcts, led to a 2.6-fold enhanced proliferation of progenitor cells in the ipsilateral DG, with 21% thereof maturing into neurons (45). However, combining PC with ischemia 3 days later had no additional effect on the ischemia-induced increase in 5-bromo-2-deoxyuridine–positive cells in the DG. Recent work from another laboratory indicates that focal ischemic PC enhances neurogenesis...
in the subventricular zone even without subsequent severe ischemia (46). The functional consequences of PC-induced neurogenesis, however, remain unknown. Nevertheless, it is reasonable to assume that neurogenesis in combination with induction of growth factor expression (45) might positively influence plastic processes with or without subsequent severe ischemia.

**DENDRITES: TARGETS OF SUBTLE OR EVEN REVERSIBLE CHANGES AFTER PCO/IPC**

Dendritic structural changes induced by PCO or IPC may easily escape detection because they are partly transient in nature. It has been known for a long time that only a few minutes of cerebral ischemia can cause focal dendritic swelling and the disappearance of dendritic spines (54–56). Similar dendritic alterations can be observed in vitro in slice preparations or cell culture models after hypoxic injury (57, 58). A short period of anoxia-hypoglycemia causes only minimal damage to the tissue but leads to extensive remodeling of hippocampal networks in slice preparations (59). With respect to dendritic spines of neurons exposed to brief sublethal hypoxia or excitotoxicity (i.e. a situation similar to PC), time-lapse microscopy revealed that despite widespread loss, the spines recovered within 2 hours after termination of hypoxia/excitotoxicity; moreover, they remained stable for at least 24 hours (60). Interestingly, in a global model for ischemic PC, PCO triggered an increase in spine density of hippocampal CA1 neurons 3 days after reperfusion (48). Unfortunately, longer reperfusion time points were not investigated in this study, and, thus, according to the authors’ statements, it cannot be excluded that this increase is only a rebound effect and transient in nature. Our data support these results because we found altered dendritic integrity in CA1 neurons after a 6-week reperfusion period subsequent to PCO in a gerbil model of global ischemia despite the preservation of neuronal cell density in CA1 (30). In the above-mentioned study by Corbett et al (48), IPC caused an increase in CA1 dendritic spines after 10 and 30 days accompanied by normalization of

<table>
<thead>
<tr>
<th>Reference</th>
<th>Species</th>
<th>Design</th>
<th>Survival</th>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>(43)</td>
<td>Gerbil</td>
<td>2 min I</td>
<td>Up to 8 days</td>
<td>Progenitor cell proliferation (BrdU) in the DG</td>
<td>No increased progenitor cell proliferation</td>
</tr>
<tr>
<td>(44)</td>
<td>Rat</td>
<td>15 min I</td>
<td>Up to 14 days</td>
<td>TTC, HE, TUNEL, PANT HSP27, 32 ferritin, microglia</td>
<td>No morphologic change, no apoptosis</td>
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<tr>
<td></td>
<td></td>
<td>focal (tMCAO)</td>
<td></td>
<td></td>
<td>Uregulation of HSPs, ferritin, microglia activation</td>
</tr>
<tr>
<td>(33)</td>
<td>Rat</td>
<td>3 min I</td>
<td>Up to 14 days</td>
<td>Hippocampal histology, prostaglandin receptor IHC</td>
<td>No hippocampal damage</td>
</tr>
<tr>
<td>(13)</td>
<td>Rat</td>
<td>5 min I</td>
<td>Up to 21 days</td>
<td>Hippocampal morphology</td>
<td>No or minimal CA1 damage</td>
</tr>
<tr>
<td>(45)</td>
<td>Rat</td>
<td>10 min I</td>
<td>Up to 21 days</td>
<td>Morphology/infarct volume Progenitor cell proliferation (BrdU) in the DG</td>
<td>Increased progenitor cell proliferation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>focal (tMCAO)</td>
<td></td>
<td></td>
<td>No infarcts</td>
</tr>
<tr>
<td>(46)</td>
<td>Rat</td>
<td>10 min I</td>
<td>Up to 28 days</td>
<td>Morphology/infarct volume Progenitor cell proliferation/ neurogenesis in the SVZ</td>
<td>No infarct</td>
</tr>
<tr>
<td></td>
<td></td>
<td>focal (tMCAO)</td>
<td></td>
<td></td>
<td>Induction of progenitor cell proliferation and of neurogenesis</td>
</tr>
<tr>
<td>(31)</td>
<td>Gerbil</td>
<td>1.5 min I</td>
<td>Up to 30 days</td>
<td>Hippocampal neuronal density</td>
<td>No significant reduction of CA1 neurons</td>
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<tr>
<td></td>
<td></td>
<td>+ 24 h +</td>
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<td></td>
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<td>1.5 min I</td>
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<tr>
<td></td>
<td></td>
<td>global (tCCAO)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>(37)</td>
<td>Gerbil</td>
<td>1.5 min I</td>
<td>Up to 30 days</td>
<td>Hippocampal neuronal density</td>
<td>No reduction of CA1 cell count</td>
</tr>
<tr>
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<td></td>
<td>+ 24 h +</td>
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<td>1.5 min I</td>
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<td></td>
<td></td>
<td>global (tCCAO)</td>
<td></td>
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<tr>
<td>(32)</td>
<td>Rat</td>
<td>10 min I</td>
<td>Up to 4 weeks</td>
<td>Morphology, TUNEL</td>
<td>No injury, no increase of TUNEL-positive cells</td>
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<tr>
<td></td>
<td></td>
<td>focal (tMCAO)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(47)</td>
<td>Rat</td>
<td>10 min I</td>
<td>Up to 4 weeks</td>
<td>Astroglia proliferation</td>
<td>Prolonged astrogliosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>focal (tMCAO)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>(30)</td>
<td>Gerbil</td>
<td>2.5 min I</td>
<td>Up to 6 weeks</td>
<td>Hippocampal histology, dendritic markers (MAP2, MAP1B, synaptopodin)</td>
<td>Some reduction of CA1 neuronal density In case of preserved CA1 neuronal density, loss of dendritic integrity (synaptopodin)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>global (tCCAO)</td>
<td></td>
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</tbody>
</table>

BrdU, 5-bromo-2-deoxyuridine; CCAO, common carotid artery occlusion; DG, dentate gyrus; HE, hematoxylin and eosin; HSP, heat shock protein; I, ischemia; IHC, immunohistochemistry; MAP, microtubule-associated protein; MCAO, middle cerebral artery occlusion; PANT, DNA polymerase I-mediated biotin-dATP nick-translation; p, permanent; PCO, ischemic preconditioning only; t, transient; SVZ, subventricular zone; TTC, 2,3,5-triphenyltetrazolium; TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine 5-triphosphate-biotin end labeling; VAO, vertebral artery occlusion.
behavior investigated by open-field habituation. A feasible explanation for this might be that this increase takes place in compensation for functional loss due to death of other CA1 neurons. Immunohistochemical analysis of dendritic integrity in hippocampal CA1 after IPC in the gerbil model with an antibody against synaptopodin, however, revealed reduced immunolabeling suggestive of disturbed dendritic structure (unpublished observations).

### TABLE 2. IPC: Structural Changes After More Than 7 Days of Reperfusion

<table>
<thead>
<tr>
<th>Reference</th>
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<th>Design</th>
<th>Survival</th>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>(43)</td>
<td>Gerbil</td>
<td>2 min I (PC) + 3 d + 5 min I global (tCCAO)</td>
<td>8 days</td>
<td>Progenitor cell proliferation (BrdU) in the DG</td>
<td>50% of animals with CA1 damage Increased progenitor cell proliferation in intact CA1 similar to a 5-min I</td>
</tr>
<tr>
<td>(33)</td>
<td>Rat</td>
<td>3 min I (PC) + 3 d + 10 min I global (pVAO + tCCAO)</td>
<td>Up to 14 days</td>
<td>Hippocampal histology, prostaglandin receptor IHC</td>
<td>No hippocampal damage</td>
</tr>
<tr>
<td>(13)</td>
<td>Rat</td>
<td>5 min I (PC) + 2 d + 10 min I global (pVAO + tCCAO)</td>
<td>Up to 21 days</td>
<td>Hippocampal morphology</td>
<td>No or minimal CA1 damage</td>
</tr>
<tr>
<td>(45)</td>
<td>Rat</td>
<td>10 min I (PC) + 3 d + 60 min I focal (tMCAO)</td>
<td>Up to 21 days</td>
<td>Morphology/infarct volume</td>
<td>Reduction of infarct volume compared with 60 min I without PC Increased progenitor cell proliferation</td>
</tr>
<tr>
<td>(46)</td>
<td>Rat</td>
<td>10 min I (PC) + 2 d + 120 min I focal (tMCAO)</td>
<td>Up to 28 days</td>
<td>Progenitor cell proliferation (BrdU) in the DG</td>
<td>Reduction of infarct volume Induction of progenitor proliferation and neurogenesis</td>
</tr>
<tr>
<td>(34)</td>
<td>Gerbil</td>
<td>2 min I (PC) + 24 h + 2 min I (PC) + 48 h + 5 min I global (tCCO)</td>
<td>Up to 30 days</td>
<td>Cell count in CA1 and CA3</td>
<td>Almost all neurons survived</td>
</tr>
<tr>
<td>(31)</td>
<td>Gerbil</td>
<td>1.5 min I (PC) + 24 h + 1.5 min I (PC) + 3 d + 5 min I global (tCCO)</td>
<td>Up to 30 days</td>
<td>Hippocampal neuronal density</td>
<td>Reduction of CA1 cell loss, but increase over time</td>
</tr>
<tr>
<td>(48)</td>
<td>Gerbil</td>
<td>1.5 min I (PC) + 24 h + 1.5 min I (PC) + 3 d + 5 min I global (tCCO)</td>
<td>Up to 30 days</td>
<td>CA1 dendritic spine morphology (Golgi Cox)</td>
<td>Increase in spine density after 10 and 30 d</td>
</tr>
<tr>
<td>(32)</td>
<td>Rat</td>
<td>10 min I (PC) (tMCAO) + 6/12 h /1/2/7/14/21 d + pMCAO (focal)</td>
<td>Up to 4 weeks</td>
<td>Morphology, infarct volume, TUNEL</td>
<td>Significant infarct volume reduction with 1/-2/-7-d interval</td>
</tr>
<tr>
<td>(35)</td>
<td>Rat</td>
<td>3 min I (PC) + 2 d + 9 min I global (tCCAO) + hypotension</td>
<td>Up to 8 weeks</td>
<td>Hippocampal, striatal and neocortical histology, neuronal density</td>
<td>No histologic damage</td>
</tr>
<tr>
<td>(37)</td>
<td>Gerbil</td>
<td>1.5 min I (PC) + 24 h + 1.5 min I (PC) + 3 d + 5 min I global (tCCO)</td>
<td>Up to 60 days</td>
<td>Hippocampal neuronal density</td>
<td>Slight reduction of CA1 cell count, which increased at 60 d</td>
</tr>
<tr>
<td>(36)</td>
<td>Gerbil</td>
<td>1.5 min I (PC) + 24 h + 1.5 min I (PC) + 3 d + 5 min I global (tCCO)</td>
<td>Up to 90 days</td>
<td>Hippocampal neuronal density</td>
<td>Reduction of CA1 cell loss, but increase over time</td>
</tr>
<tr>
<td>(38)</td>
<td>Gerbil</td>
<td>1.5 min I (PC) + 24 h + 1.5 min I (PC) + 3 d + 5 min I global (tCCO) ± enrichment</td>
<td>Up to 110 days</td>
<td>Hippocampal neuronal density</td>
<td>Not enriched: reduction of CA1 loss enriched: lower reduction of CA1 loss</td>
</tr>
<tr>
<td>(39)</td>
<td>Gerbil</td>
<td>tCCAO 2.5 to 3.5 min depolarization (PC) + 2 d + 6.5 to 8.5 min depolarization</td>
<td>Up to 2 months</td>
<td>Cell count CA1</td>
<td>Slight but significant reduction of CA1 cell count</td>
</tr>
<tr>
<td>(41)</td>
<td>Rat</td>
<td>4VO 2 to 3 min depolarization (PC) + 48 h + 7 to 9 min depolarization</td>
<td>Up to 12 weeks</td>
<td>Cell count CA1</td>
<td>Significant reduction of CA1 cell count</td>
</tr>
</tbody>
</table>

4VO, 4-vessel occlusion; BrdU, 5-bromo-2-deoxyuridine; I, ischemia; t, transient; p, permanent; CCAO, common carotid artery occlusion; DG, dentate gyrus; MCAO, middle cerebral artery occlusion; PC, ischemic conditioning; IHC, immunohistochemistry; SVZ, subventricular zone; TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine 5-triphosphate-biotin end labeling; VAO, vertebral artery occlusion.
ALTERED ABUNDANCE OF NEUROTRANSMITTER RECEPTOR DENSITY AFTER PCO AND IPC

Numerous studies have demonstrated involvement of glutamate, γ-aminobutyric acid (GABA), or adenosine receptors in the acquisition of an ischemia-tolerant state (2). Only a few studies, however, have thoroughly analyzed postischemic levels of various neurotransmitter systems as a means of assessing potential structural alterations. An early autoradiographic study by Kato et al could not detect any changes in binding densities of MK-801, muscimol, or PN200-110 labeling N-methyl D-aspartate (NMDA), GABA_A, and adenosine A1 receptors, respectively, after a single 2-minute PC ischemia in the gerbil hippocampus 7 days after reperfusion (61). However, quinuclidinyl benzilate binding at muscarinic acetylcholine receptors decreased by approximately 30% after 1 hour and 1 day and did not reattain control levels at 7 days after reperfusion in hippocampal CA1 neurons. Analyzing ligand-binding densities of NMDA α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and GABA_A receptors in a gerbil model for PC and IPC, we were able to demonstrate transient upregulation of [3H]muscimol labeling GABA_A receptors in various hippocampal subfields with observation periods of up to 4 days after reperfusion (62, 63). Similarly, semiquantitative analysis of protein expression of AMPA receptor subunits GluR 1 and GluR2 in the same model showed only slight transient reductions of GluR2 in the hippocampal CA1 sector up to 4 days after reperfusion (64). The same holds true for different metabotropic glutamate receptor (mGluR) subtypes. Although Group I mGluR1b and mGluR5 were transiently reduced only after IPC in an observation window of up to 4 days, Group II mGluR2/3 remained completely unaltered (65). In contrast, a recent study from our group demonstrated significantly reduced ligand binding to central cannabinoid receptors in hippocampal CA1 and CA3 subfields after PCO beginning after 24 hours and continuing up to 4 days (66). Interestingly, 3 weeks after reperfusion, receptor autoradiography revealed reduced binding values for NMDA and AMPA receptors after PC, but not IPC (unpublished observations). This, again, indicates that valid statements on postischemic effects must include observation periods longer than those used in many previous studies.

GLIAL RESPONSE AFTER PC/IPC

The role of glial cells in PC has been investigated over many years (67), but detailed studies on structural changes of glial cells (particularly astrocytes) after PC are comparatively rare. Using the 4-vessel occlusion model of global ischemia in rats, ischemic PC with a 3-minute period of ischemia was shown to cause prolongation of astrocytic processes that was even more pronounced after IPC and was maintained up to 7 days (68). The clamping of vulnerable hippocampal CA1 neurons by these astrocytic processes with additional upregulation of glial glutamate transporter 1 has been suggested to contribute significantly to tolerance induction. In contrast, microglia proliferation seems to be restricted to lethal ischemia, whereas PCO does not significantly enhance the microglial response (69, 70). Similarly, PC by a short focal ischemic period and lacking any infarct resulted in a massive and prolonged astrogliosis in the ipsilateral hemisphere (47).

TRANSIENT ISCHEMIC ATTACKS: CLINICAL MODEL FOR PC WITHOUT MORPHOLOGICAL CORRELATE?

The concept and definition of transient ischemic attacks (TIAs) have changed considerably over the years. The classic definition of TIA is that of a sudden, focal neurologic deficit characterized by duration of less than 24 hours, association with complete resolution of brain ischemia, and absence of morphologically detectable permanent brain injury. In this context, the fascinating hypothesis arose that TIAs may function as a PC stimulus in humans, thereby representing the clinical correlate of ischemic tolerance in the various animal models. Enthusiasm for this concept was nourished by retrospective studies on patients with a history of TIAs who had more favorable clinical outcomes after stroke compared with patients without preceding TIAs (71), a finding also corroborated by recent studies (72–74). Furthermore, the new definition of TIAs as “brief episodes of neurologic dysfunction caused by focal brain … ischemia … without evidence of acute infarction” better meets the demands originating from a more complex reality (75). This definition explicitly permits structural alteration except for acute necrosis. In fact, due to the use of more sensitive magnetic resonance imaging (MRI) techniques, it has become evident that a considerable number of patients with TIAs suffer from permanent ischemic brain damage (76). Thus, for instance, resolution of diffusion-weighted imaging lesions does not necessarily indicate tissue salvage from ischemia because selective neuronal necrosis can be detected in these regions (77). This observation can explain the phenomenon of persisting neurologic deficits in patients presenting with normal diffusion-weighted imaging and perfusion-weighted imaging results after cerebral ischemia. A recent study in rats by Sicard et al (78), using multimodal MRI after transient focal cerebral ischemia of 20-minute duration, demonstrated that, although lesions on diffusion-weighted imaging and perfusion-weighted imaging fully recovered within 30 minutes after reperfusion, fMRI responses did not fully normalize for up to 24 hours. Therefore, normal findings on diffusion, perfusion, and T2 imaging shortly after transient ischemia may not indicate normal tissue status. This may be of further help in explaining the persistence of neurologic deficits in patients with normal conventional MRI after cerebral ischemia (78).

In view of these findings, it is reasonable to assume that both the more sensitive imaging and more sensitive histologic techniques may detect irreversible or long-lasting structural alterations originating from short PC ischemia. Similarly (according to its original definition), the ischemic penumbra was thought to represent ischemia less than the threshold for irreversible damage but more than the threshold for neuronal dysfunction (79). However, it was verified in a rat model of focal ischemia (middle cerebral artery occlusion) that very short ischemic periods of 5-minute duration, comparable to the penumbral range, cause scattered apoptotic cell death.
that, if not specifically analyzed, would have been overlooked (80). The conclusion drawn from this animal study is that in patients who have experienced a TIA apparently without clinical or radiologic sequelae, a favorable outcome does not necessarily exclude apoptotic cell death or subtle structural changes. The situation is further complicated by studies exhibiting functional improvement after PC focal ischemia in rats without histologically detectable damage by use of TTC and terminal deoxynucleotidyl transferase–mediated deoxyuridine 5-triphosphate–biont end labeling staining (44). Although the limitation of this study is reflected in the fact that no dendritic or other cytoskeletal “structural” markers were investigated, the behavioral deficits observed might have been caused by changes in cellular morphology of morphologically intact but functionally impaired cells.

CONCLUSIONS

It is widely accepted that, by definition, ischemic PC does not cause any kind of damage or structural alterations applied at a subtoxic level. However, there is a growing body of evidence that this ideal form of PC does not correspond to reality for several reasons. First, detection of potential lesions may frequently be missed because the observation periods are too short or the techniques too insensitive to render visible structural changes. Second, the question arises whether ischemic PC necessarily involves some form of brain damage, leading to functional impairment with behavioral deficits, although without any lesion. Because some form of structural brain injury is hypothesized to be a conditio sine qua non for plastic processes, the same may hold true for ischemic PC (44). Third, hibernation is frequently considered to represent the ideal PC model. Although there is great similarity in the genomic response both after ischemic PC and hibernation, the crucial difference between these 2 situations is probably their initiation. When considering clinical applications, learning more about the gentle induction of a hypoxic-ischemic–tolerant state may be at least as helpful as elucidating the detailed pathways providing protection. In any case, future studies on PCO and IPC should be designed to detect even the most subtle posts ischemic structural changes and investigate whether protection after PC is really maintained in the long term.

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