Chronic Ischemia: Memory Impairment
And Neural Pathology in the Rat

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Recent findings, such as the presence of diseased cortical microvasculature in Alzheimer’s dementia (DAT) and the reduced cerebral blood flow in those at risk for DAT, suggest that brain hypoperfusion may be pathogenic in the etiology of this disease. To assess this possibility in an animal model of cerebral hypoperfusion, we permanently ligated both common carotid arteries in the early middle-aged (~10 months old) Sprague Dawley rats. In Wistar rats, this procedure (2-VO) chronically (and perhaps permanently) reduces cortical and hippocampal blood flow by approximately 30%. Using this model, we do not observe the behavioral and neuropathologic sequelae that are characteristic after transient ischemia in the rat. Surgery is performed with the rats under barbiturate plus ketamine anesthesia. Ketamine may prevent the immediate neurodegenerative consequences of the relatively severe reduction in blood flow resulting from the carotid ligation procedure in the early postligation period.

2-VO progressively impairs the ability of the rats to perform the Morris water maze. This task requires that the rat escape to a submerged platform in a circular pool of water. The rats use extra-maze cues to guide their navigation to the platform-containing area. As shown in Figure 1 (upper panel), the Morris maze impairment appears as early as 7 days post surgery and persists over time despite repeated training. It is unlikely that this early-appearing deficit reflects impaired learning or memory mechanisms. A more plausible explanation is that the spatial ability of the rats is compromised by 2-VO and that this deficit is exacerbated by the moderately stressful water maze task. In support of this hypothesis, we have shown that 2-VO rats demonstrate a greater plasma corticosterone elevation to water immersion than do sham-operated animals. As well, presurgical training attenuates the deficit, indicating that if the animals are allowed to adapt to the stress of water immersion prior to 2-VO surgery, they are capable of normal performance. It should be noted that spatial disorientation and “getting lost” are characteristics of DAT and are also anxiogenic and thus self-fueling.

In rats that have been pre-trained to perform the radial arm maze task, 2-VO causes a strikingly late-emerging increase in errors. Rats will effectively forage for food located on the arms of this maze, shunning arms that are never baited with food (unbaited-arm entries, a measure of long-term, habit memory) and avoiding

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FIGURE 1. Upper panel: the average session latency to locate the hidden platform in the Morris water maze at various postoperative intervals for 2-VO and sham control rats. Lower panel: unbaited-arm entries in the radial-arm maze. Asterisks denote differences between the 2-VO and control groups. The data are from Pappas et al.\(^3\)

re-entry of arms from which they have already obtained food (baited-arm re-entries, a measure of short-term memory). As shown in the lower panel of FIGURE 1, 2-VO rats show a significant elevation of unbaited-arm entries at 63, 105 and 168 days, but not at 7 and 21 days post surgery.

The neuropathological consequences of 2-VO are unusually late to emerge. Hippocampal CA1 pyramidal cells appear unaffected 14 days after 2-VO, but are reduced at 180 days. Cell loss is, apparently, an active process exhibiting biochemical (terminal deoxynucleotidyl dUTP nick end-labeling), morphologic (cytoplasmic shrinkage, chromatin condensation, nuclear blebbing), and protein expression (enhanced clusterin immunoreactivity), all indices of an apoptotic process. Similarly, immunohistochemical assessment of glial fibrillary acidic protein (GFAP) indicates that reactive gliosis is evident throughout the CA1 cell field at 180 but not at 14 days, suggesting enhanced phagocytic activity in and around degenerating tissue. CA1 GFAP is significantly correlated with the incidence of unbaited-arm entries on the radial-arm maze. Furthermore, if animals are followed for increasingly longer periods after 2-VO surgery, a striking correlation emerges between regions demon-
demonstrating characteristics of cellular apoptosis at 180 days and evidence of β-amyloid-containing neurodegenerative lesions at 280 days after surgery. Within the CA fields of the hippocampal formation, CA1 and CA3c exhibit the highest percentage of active cell loss 180 days after carotid occlusion. By 280 days, these areas fail to exhibit continued cell death but demonstrate a marked accumulation of aberrant, extracellular, amyloid precursor protein (APP) immunoreactivity (FIG. 2).

APP is membrane-bound and neuronally localized in sham-operated animals (FIG. 2, upper panel). In 2-VO animals, APP appears as discrete extracellular deposits localized in brain parenchyma particularly around microvasculature (FIG. 2, lower panel). Significantly, these deposits are also the site of de novo oxidative stress, a common characteristic of human neurodegenerative lesions. Oxidative stress was assessed by immunolocalization of heme oxygenase 1 (HO-1) protein (FIG. 2). Heme oxygenase (HO) is the rate-limiting enzyme in the breakdown of heme-containing proteins. The enzyme oxidatively cleaves heme to produce both biliverdin, a potent anti-oxidant, and carbon monoxide. Two HO isoforms, constitutively active HO-2 and inducible HO-1, have been identified. In brain, HO-1 expression is elicited by oxidative stress and heat shock, and changes in HO-1 mRNA and protein have been used to identify cerebral tissue undergoing oxidative stress after experimental induction of transient cerebral ischemia.5 Our data indicate that HO-1 expression is elicited only at later time points after 2-VO surgery in regions demonstrating both coincident extracellular deposition of APP and previous increases in cell loss with apoptotic characteristics.

**FIGURE 2.** Representative photomicrographs from adjacent sections. Upper panel: sham-operated animal (40 weeks after surgery) immunoreacted for APP and hemeoxygenase-1 (HO-1) (inset). Note the cell membrane association of APP and lack of HO-1 immunoreactivity. Lower panel: 2-VO animal immunoreacted for APP and HO-1 (inset). APP immunoreactivity is found in extracellular deposits or is blood vessel–associated. Areas that exhibit this aberrant localization of APP demonstrate high de novo levels of HO-1 immunoreactivity suggestive of oxidative stress. Photomicrographs depict CA3c of the hippocampal formation. Scale bar, 10 μm.
In summary, with the exception of an early-emerging impairment on the Morris water maze, the behavioral and brain pathologies caused by 2-VO are unusually late-appearing. We have yet to accurately characterize their temporal course. Certainly they do not appear before 14 days, and pathological neural events are still occurring in the brain after 280 days. This timeframe greatly exceeds the latency period for behavioral and neural pathologies after more severe global or focal brain ischemia that typically fully manifest themselves within a week.

It may be coincidental that impaired spatial ability, reactive gliosis, active neuronal death, enhanced clusterin immunoreactivity, extracellular deposition of amyloid precursor protein, and heme oxygenase-1 expression in the temporal lobe are also observed in DAT. On the other hand, 2-VO-induced neuropathology might model the premonitory events of DAT. Furthermore, it is hypothesized that these histopathologic markers, which are only modestly elevated in the 2-VO rat, could progressively accelerate if other conditions prevail. For example, it would be instructive to map the progression of these changes as the rat becomes aged or in the context of basal forebrain cholinergic lesions, a hallmark of the DAT brain. The possibility that chronic low-grade metabolic dysfunction attributable to microvascular abnormalities can mediate neurodegenerative disease(s) is currently of great interest. Our observations using the 2-VO rat support this hypothesis.

REFERENCES