Therapeutic Window of CA1 Neuronal Damage Defined by an Ultrashort-Acting Barbiturate After Brain Ischemia in Gerbils

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Previous therapeutic studies on the prevention of selective vulnerability of neurons in the hippocampus have suggested that the critical period for induction of delayed neuronal injury occurs early during recirculation. To determine the onset and duration of this period, an ultrashort-acting barbiturate (methohexital) was infused into the left carotid artery of 47 gerbils after various times of recirculation following 10 minutes of bilateral forebrain ischemia. Neuronal density in the left CA1 sector was determined 7 days later by counting the number of surviving neurons per millimeter of pyramidal cell layer. In 16 saline-treated gerbils, <10% of the CA1 neurons survived the 10 minutes of ischemia. Postischemic carotid infusion of methohexital improved neuronal survival, the degree of improvement depending on the timing and duration of the methohexital infusion. When carried out during the initial 40 minutes of recirculation, methohexital infusion for 10 minutes increased the number of surviving neurons to approximately 60% of that in five sham-operated control gerbils. This increase was significant for infusions carried out between the 10th and 20th minutes (n=6, p<0.05) and between the 30th and 40th minutes (n=6, p<0.05) of recirculation. Methohexital infusion for 20 minutes increased neuronal survival to 95% and 73% of that in the controls when carried out between the 0th and 20th minutes (n=5, p<0.005) and between the 20th and 40th minutes (n=5, p<0.005) of recirculation, respectively. Protection was nonsignificant for 10- or 20-minute methohexital infusions carried out after the 40th minute of recirculation. Our results demonstrate that the pathologic processes leading to delayed neuronal injury in the CA1 sector are induced during the initial 40 minutes of recirculation and that barbiturates are able to reverse these processes only if given during this period. (Stroke 1990;21:1489-1493)
throughout the experiment and kept close to 37.5°C with a feedback-controlled heating system. The left external carotid artery was exposed through a midline cervical incision using an operating microscope. A 27-gauge needle connected to an infusion pump and a pressure transducer was inserted with a micro-manipulator (Precidor Type 5003, Infors AG, Bottmingen, Switzerland) into the left lingual artery and advanced retrogradely to place the needle tip close to the carotid bifurcation. Clotting of the needle was prevented by the continuous infusion of 2–5 μl/min saline except during ischemia.

Ischemia was induced in 77 gerbils by occluding both common carotid arteries with Biemer clips (FD 562, Aesculap Werk AG, Tutlingen, FRG) for 10 minutes. In a previous study7 bilateral common carotid artery occlusion for 10 minutes did not produce significant CA1 neuronal injury when the back pressure measured distal to the occlusion exceeded 15 mm Hg. To exclude gerbils with partial ischemia, we rejected animals in which back pressure in the carotid artery was >10 mm Hg. After release of the vascular occlusion, 0.04 mg/kg/min methohexital (30 μl/min) or 30 μl/min saline was infused into the left carotid artery for 10 minutes beginning after 0, 10, 20, 30, 40, or 60 minutes of recirculation or for 20 minutes beginning after 0, 20, or 40 minutes of recirculation. Each group consisted of five or six methohexital-treated and one or two saline-treated gerbils. After 70 minutes of recirculation the needle was removed, the skin was sutured, and halothane anesthesia was discontinued. The five remaining gerbils were used for histologic controls; they were sham-operated as described above (their common carotid arteries were not occluded) and their left carotid arteries were infused with saline.

After 7 days of recirculation the gerbils were anesthetized with 100 mg/kg pentobarbital and decapitated. The brains were removed and fixed by immersion for 1 day in 4% buffered formalin and for 4 days in 10% buffered formalin. Coronal sections 5 μm thick were prepared at a level 1.4–1.7 mm posterior to the bregma and stained with Luxol fast blue and cresyl violet. Intact neurons in the left hippocampal CA1 sector were counted, and neuronal density was only 45% of control (Figure 2, c and f), which significantly greater than for the untreated group (Figure 1); both values were approximately 200 cells/mm, or up to 64% of control (Figure 1). The 16 gerbils infused with saline at various times after 10 minutes of ischemia were lumped into one untreated group, in which neuronal density was approximately 30 cells/mm (Figure 2, a and d), or 16% of control (Figure 1).

In the groups with 10 minutes of methohexitol infusion beginning during the initial 40 minutes of recirculation, neuronal density increased to >100 cells/mm, or up to 64% of control (Figure 1). This increase was significant (p<0.05) for the 10–20 and 30–40 minutes of recirculation groups. Neuronal density decreased to <30% of control for the 40–50 and 60–70 minutes of recirculation groups.

In the groups with 20 minutes of methohexitol infusion, neuronal density was 95% of control for the 0–20 minutes of recirculation group (Figure 2, b and e), and 73% of control for the 20–40 minutes of recirculation group (Figure 1); both values were significantly greater than for the untreated group (p<0.005). Neuronal density for the 0–20 minutes of recirculation group was also significantly greater than that for the 0–10 and the 10–20 minutes of recirculation groups. Methohexitol infusion for 20 minutes beginning after 40 minutes of recirculation did not consistently result in neuroprotection; neuronal density was only 45% of control (Figure 2, c and f), which did not differ significantly from that for the untreated group (Figure 1).

**Discussion**

Pharmacologic studies of the selective vulnerability of neurons in the hippocampus after brief periods of global forebrain ischemia in gerbils suffer from a
Inhibiting metabolic activity may be of benefit under two circumstances: when barbiturates are applied either before ischemia to delay breakdown of energy metabolism or during postischemic hypoperfusion to improve the mismatch between the oxygen requirement and the oxygen consumption of the brain. In our present experiment, the barbiturate effect must depend on another mechanism because the drug was given after ischemia, before hypoperfusion developed. For similar reasons, suppression of hyperexcitability and inhibition of edema formation are probably of lesser importance because neuronal hyperactivity does not appear until a few hours after ischemia and edema does not appear until 2 days after recirculation, that is, long after barbiturates lose their protective effect.

It is, therefore, more likely that methohexital ameliorated some kind of early reperfusion injury. One mechanism of such amelioration may be interference with postischemic free radical formation. Yoshida et al. showed that the increase in the concentration of arachidonic acid that results from peroxidation of lipid membranes during and after ischemia is reversed within 30 minutes of recirculation. These authors also observed that malondialdehyde, which is a by-product of lipid peroxidation, transiently accumulates during the initial 30 minutes of recirculation,
indicating that reactive oxygen radical species are generated during this interval. In view of the fact that barbiturates are free radical scavengers and that the therapeutic effect was most pronounced during this interval, such a mechanism could be involved. However, interference with other reperfusion-associated changes (such as increased calcium fluxes and the release of neurotransmitters) cannot be excluded and should also be considered.

A puzzling question that remains to be solved is the long interval between the early appearance of barbiturate-sensitive pathophysiologic processes and the late manifestation of CA1 neuronal injury.

During the maturation of the injury, certain metabolic disturbances (such as dysregulations of protein and polyamine metabolism) have been described that apparently are of pathogenetic importance for the development of delayed neuronal death. Our demonstration of an amazingly narrow postischemic window during which short-acting barbiturates are protective suggests that the onset of these disturbances must be linked to pathophysiologic events that are established during this short interval. The elucidation of this relation will be of fundamental importance for the understanding of selective vulnerability and therefore warrants further investigation.

**FIGURE 1.** Bar graph of mean±SD neuronal density (surviving neurons per millimeter of CA1 sector of dorsal hippocampus) in gerbils. Cross-hatched bars: control, halothane-anesthetized sham-operated group; untreated, 10 minutes of ischemia without postischemic methohexital infusion. Time of methohexital infusion during recirculation: solid bars, 20 minutes of methohexital infusion; shaded bars, 10 minutes of methohexital infusion. Methohexital infusion during initial 40 minutes of recirculation significantly increased neuronal density (prevented CA1 injury). **p<0.05, 0.005, respectively, different from untreated by analysis of variance.

**FIGURE 2.** Light micrographs of dorsal hippocampus in gerbils. a, d: 10 minutes of ischemia without postischemic treatment with methohexital. b, e: 10 minutes of ischemia with 20 minutes of postischemic methohexital infusion beginning at 0th minute of recirculation. Note nearly complete preservation of CA1 neurons. c, f: 10 minutes of ischemia with 20 minutes of postischemic methohexital infusion beginning at 40th minute of recirculation. CA1 neurons were not protected by this treatment. Luxol fast blue and cresyl violet stain, calibration bars=500 μm for a, b, and c; 50 μm for d, e, and f.
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