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Rapid communication

MK-801 protects the neonatal brain from hypoxic-ischemic damage

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Enhanced synaptic release of excitatory amino acid neurotransmitters may contribute to brain injury from hypoxia-ischemia (Meldrum, 1985; Simon et al., 1984). To examine this hypothesis in neonatal brain we tested MK-801, a novel non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, using an in vivo experimental model of hypoxic-ischemic forebrain injury (Johnston, 1983). To induce injury, seven day old rats were anesthetized with ether and the right carotid was ligated (N = 63), after which they recovered with the dam for 2 h. The pups were then placed in a warmed (37 °C) chamber and exposed to 8% oxygen balance nitrogen for 3 h, a procedure which resulted in hemispheric necrosis ipsilateral to the side of ligation.

Effect of treatment was assessed using three MK-801 treatment protocols: administration of 1 dose of MK-801 (1 mg/kg) before hypoxia (N = 10), a single dose during hypoxia (N = 18), or two doses, one before and another after 1.25 h of hypoxia (N = 9). In the second group, the timing of treatment was varied to determine if there was a critical time threshold for efficacy: a single dose was given after 1.25 h (N = 5), 1.5 h (N = 6) or 2.5 h (N = 7). Pups received one or two intraperitoneal injections of MK = 801 (N = 37) or phosphate buffered saline (N = 26); a group of untreated litter-mates served as additional controls (N = 5). Pups were sacrificed 5 days after hypoxia, and right and left hemisphere weight disparities were compared as percent reductions ($[(R - L)/L] \times 100$) by condition using Student's t-test for paired values. During this phase of rapid brain growth, reduction in hemisphere weight can be used as an indicator of injury more readily than in adults.

There was prominent neuronal loss in hippocampus, cortex and caudate-putamen of saline-treated ligates. MK-801 administration reduced hypoxic-ischemic neuronal damage both grossly and microscopically as compared the opposite side. When compared to saline treated hypoxic-ischemic controls, two MK-801 injections were most effective in reducing cerebral hemisphere damage (saline treated, -11.2 ± 2 vs. $-1.7 \pm 1.3\%$ $P < 0.001$). Figure 1 compared littermate pups treated with saline or MK-801 at the onset and 1.25 h into hypoxia, to normal controls. A single MK-801 dose at the onset (-3.7 ± 6.9 $P < 0.05$) or after 1.25 h of hypoxia ($-3.1 \pm 3.2\%$ $P < 0.01$) was similarly effective while single injections at 1.5 or 2.5 h into hypoxia were least effective. MK-801 eliminated moderate to severe injury (> 10% reduction in hemisphere weight) in protocols with two doses or a single dose at 1.25 h. The incidence of hemispheric necrosis in groups given a pre-hypoxia dose or treated at 1.5 h with MK-801 was reduced to 20 and 33%, respectively. The incidence of severe necrosis in pups that received MK-801 after 2.5 h of hypoxia (57%) was no different from saline treated controls. Thus, protocols in which MK-801 was administered before 1.25 h into hypoxia were most effective in limiting neuronal damage. The data suggest that there is a

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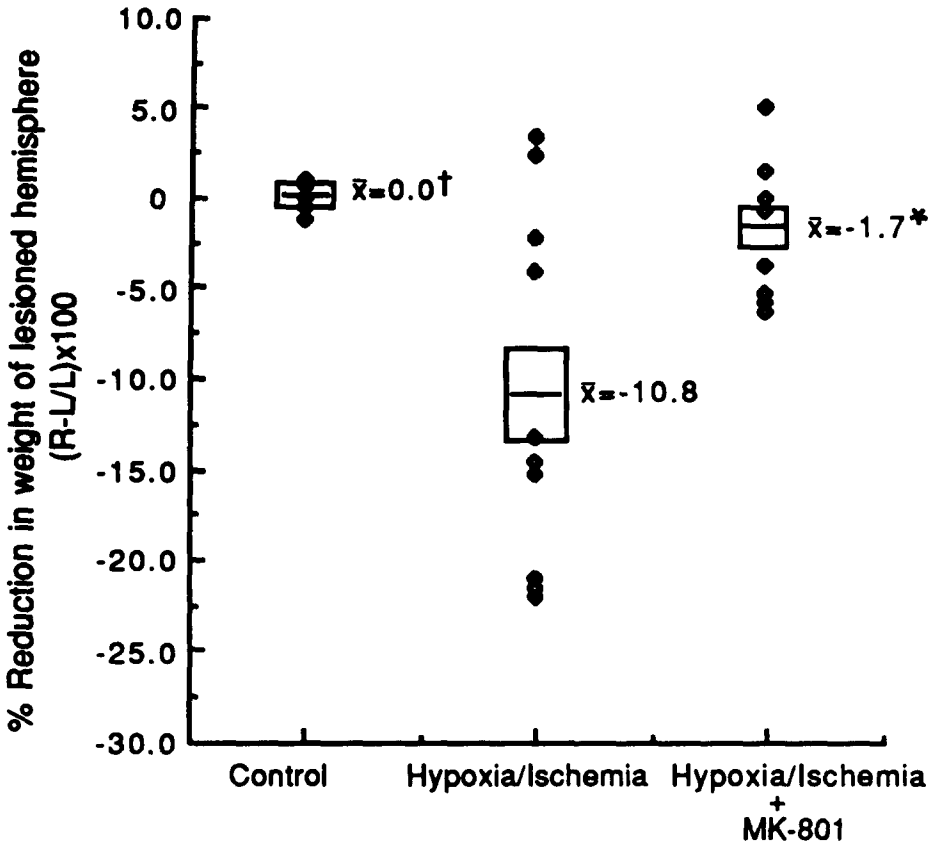

Fig. 1. Comparison of right and left hemisphere weights in animals killed on day 12. Unilateral hypoxic-ischemic forebrain injury was produced by right carotid artery ligation and subsequent exposure to 8% oxygen (balance nitrogen) for a 3 h period in 7 day old rat pups. Controls were untreated pups not exposed to hypoxia-ischemia. MK-801 and hypoxia-ischemia groups were exposed to hypoxic-ischemic conditions and were given i.p. injections of either MK-801 (1 mg/kg) or an equivalent volume of saline at the onset and 1.25 h into hypoxia. Data represent only those litters containing double MK-801 treated pups and were expressed as means ± S.E.M. Boxes represent S.E.M. * $P < 0.05$; † $P < 0.01$.

critical time threshold for drug intervention.

MK-801 may protect the immature brain from hypoxia-ischemia by binding to phencyclidine-like sites associated with the NMDA receptor operated channel complexes (Wong et al., 1986). The results are consistent with our previous observations that hypoxia-ischemia acutely disrupts glutamate binding sites in regions (e.g. striatum and hippocampus) susceptible to injury in the model (Silverstein et al., in press). In additional experiments we found that MK-801 blocks the necrosis produced by direct injection of NMDA into the 7 day old striatum. The drug may block lethal cation entry into neurons and/or diminish post-ischemia neuronal hyperexcitability. It is noteworthy that blockade of NMDA receptors alone with intracerebroventricular injection of 2-aminophosphonoheptanoic acid (APH) failed to protect from injury in the model (Silverstein and Johnston, unpublished). The data suggest that NMDA receptor channel activation plays an important role in the pathogenesis of hypoxic-ischemic brain injury. NMDA channel blockade might provide a therapeutic strategy for hypoxic disorders of the fetal and postnatal brain.

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