NEUROSCIENCES AND ANESTHETIC ACTION V

TITLE: THE EFFECT OF MIDAZOLAM AND GABAERGIC INHIBITION ON ANOXIC DAMAGE IN THE RAT HIPPOCAMPAL SLICE

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Introduction. Brain ischemia and hypoxia are common clinical problems in the practice of anesthesiology. Much effort has been devoted to examining the potential cerebral protective effect of anesthetic agents. It has been suggested that the inhibition of neuronal activity and the related profound decrease in cerebral metabolic rate for oxygen (CMRO₂) caused by large doses of barbiturates are responsible for their protective action in focal brain ischemia. Midazolam (MDZ), a benzodiazepine, decreases CMRO₂ and EEG frequency in a dose related fashion. Benzodiazepines bind to the GABA receptor complex and enhance the inhibitory action of GABA. Nugent et al have found that MDZ increases survival time in a hypoxic mouse model. We studied the effect of MDZ and GABA on electrophysiological recovery from anoxia in an in vitro rat hippocampal slice preparation.

Methods. Hippocampal slices were prepared from adult male rats and superfused with oxygenated artificial cerebrospinal fluid (aCSF) at 37°C. An evoked population spike was recorded from the CA1 pyramidal cells. Anoxia was generated by superfusing the slices for 5 minutes with aCSF preequilibrated with 95% nitrogen - 5% carbon dioxide. MDZ, GABA or 0.5% DMSO was added to the aCSF 10 minutes before and washed out 10 minutes after the anoxic insult. The percent recovery was calculated as the amplitude of the population spike 60 minutes after anoxia, divided by its preanoxic, predrug amplitude. At the end of the anoxic period in the biochemical experiments, slices were frozen in liquid nitrogen and prepared for measurement of ATP levels in the CA1 region. Significance was calculated using Student's t-test.

Results. Slices were subjected to 5 minutes of anoxia, with either no drug present or with 0.5% DMSO in aCSF. There was virtually no recovery of the CA1 postsynaptic spike amplitude in untreated (2 ± 1%; mean ± se) and DMSO treated slices (2 ± 1%). There was no improvement of recovery after the slices were incubated with 1 mM MDZ dissolved in either water (2 ± 2%) or 0.5% DMSO (0 ± 0%). Similarly, 100 mM MDZ dissolved in water offered no significant protection against anoxia (5 ± 2%). The water solubility of MDZ at physiologic pH is limited. We therefore repeated our experiments with 100 mM MDZ using 0.5% DMSO as the solvent. These slices recovered to 27 ± 7% of their preanoxic amplitude. This is a statistically significant protective effect as compared to both the untreated and 0.5% DMSO treated slices (p < 0.001).

The amplitude of the evoked response was increased when 100 mM MDZ was added to the aCSF. It increased over its predrug level by 40 ± 8% after 10 minutes of exposure to MDZ. GABA (10 mM) abolished the evoked population spike after it was added to the aCSF.

This inhibition of neuronal excitability was reversed in normoxic slices when GABA was washed from the system. There was no recovery of the postanoxic evoked response when the slices were exposed to 10 mM GABA (0 ± 0%).

The ATP concentration in the CA1 region of normoxic slices preincubated with 10 mM GABA was not different from the concentration in control tissue (4.64 ± 0.3 vs 4.83 ± 0.24 nM ATP/mg dry weight respectively). In slices subjected to 5 minutes of anoxia, GABA (10 mM) caused a significant decrease of the ATP concentration compared to tissue not pretreated with the drug (1.29 ± 0.09 vs 2.03 ± 0.18 nM ATP/mg dry weight p < 0.001).

Discussion. Our results demonstrate that high doses of MDZ significantly increase the amplitude of the evoked population spike recorded from the CA1 pyramidal cells of the rat hippocampus in vitro. Despite the increase neuronal activity, these cells are protected against damage after a 5 minute anoxic insult. It seems unlikely that MDZ protects merely by potentiating the inhibitory effects of GABA since we were unable to demonstrate any protection from GABA at concentrations that completely abolish neuronal transmission. Moreover, 10 mM GABA caused a more profound ATP depletion during anoxia than found in untreated hippocampal slices.

In conclusion, our findings suggest that high dose MDZ offers protection against anoxia in this in vitro model, but this is not caused by a depression of neuronal activity. We also conclude that despite its inhibition of neuronal transmission, high dose GABA depletes ATP stores during anoxia which may worsen the impact of the insult. This is an unexpected finding since the ability of a drug to decrease neuronal activity is currently considered to be an important mechanism of its cerebral protective properties. Our results lead us to believe that other cellular effects of anesthetics might be more important in providing protection against oxygen deprivation.

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