Dibutyryl Cyclic Adenosine Monophosphate Effects in the Ischemic-Hypoxic Cat

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SUMMARY The effects of dibutyryl cyclic adenosine monophosphate (dB-cAMP) were studied in fifty cats, twenty anesthetized with pentobarbital and thirty with halothane. Nasopharyngeal temperature and Paco₂ were maintained at normal values. Somatosensory evoked response was monitored and used as an indicator of cerebral cortical function. Ischemic hypoxic injury was produced by an orthopedic tourniquet snugly applied around the animal's neck and inflated for a period of fifteen minutes. This method produces a reliable and reproducible injury. Times for recovery of the evoked response to 10% of control value, as well as immediate and long-term animal survival, were noted. The dB-cAMP was administered at the end of the hypoxic insult. Treated animals recovered the evoked response earlier than the untreated controls and had better immediate and long-term survival rates.

CYCLIC ADENOSINE 3',5' monophosphate (c-AMP) has been proved to be the intracellular messenger of a wide variety of endogenous hormones. Among these are epinephrine, norepinephrine, dopamine and pituitary hormones. These hormones, by interacting with a cell membrane receptor, stimulate the activity of intracellular adenyl cyclase causing the formation of c-AMP from adenosine triphosphate stores. c-AMP is degraded by phosphodiesterase which limits the intracellular effect of c-AMP. This phosphodiesterase is itself inhibited by aminophylline, theophylline, caffeine and dibutyryl N₆O₂ adenosine 3',5' cyclic monophosphoric acid (dB-cAMP). The dibutyryl derivative also has an inhibitory effect on phosphodiesterase.

c-AMP is rapidly metabolized, and being ionized, does not penetrate cell membrane. Its dibutyryl analogue, dB-cAMP, is un-ionized and therefore passes through both cell membrane and blood-brain barrier and mimics the intracellular actions of c-AMP.

Cohn et al. reported that dB-cAMP administered into the lateral cerebral ventricles of rats reversed, in a dose related fashion, the duration of action of structurally unrelated anesthetics. Later this same group reported an antagonism between dB-cAMP and amobarbital. No other cyclic nucleotides were demonstrated to possess this effect. Welch et al. observed elevated c-AMP levels in cerebral ischemic-hypoxic injury. The time required for the somatosensory evoked response wave complex. The somatosensory evoked response was amplified and displayed on a storage oscilloscope for observation and photography. Continuous arterial pressure and unipolar EEG were recorded on a Beckman Dynograph. After the surgical preparation was complete, the end tidal halothane was brought to 0.1% over a one-hour equilibration period.

An orthopedic tourniquet was placed around the neck and inflated to 1200 mm Hg for 15 minutes to produce the standard ischemic injury. This method has been shown to be a reliable and reproducible method of producing cerebral hypoxic injury. The time required for the somatosensory evoked response and EEG to disappear with cuff application and to reappear with cuff deflation were noted. A response which was 10% of the control amplitude was taken as the reappearance point of the evoked response. Neurologic status was evaluated for up to thirty days post-injury.

Evoked response recovery times were compared to dB-cAMP dosage and initial anesthetic technique. dB-cAMP was dissolved in sterile saline and administered intravenously. The drug was given at an original set dose at the time of cuff release and repeated at 30 minutes. One-half the original dose was administered at 60 and 90 minutes after cuff release. The original doses were 1, 2, 4 and 6 mg/kg. Treated groups were compared to untreated animals receiving the same type of anesthetic. The student t-test was used to evaluate differences in evoked response recovery times between groups. A "t" < 0.05 was accepted as indicating significance.

Results

There was no significant difference in the time for disappearance of either the evoked response or the EEG in any of the groups. The time for reappearance of EEG activity

Methods

Fifty adult cats were anesthetized with intraperitoneal pentobarbital (20 cats) or halothane 0.9% (30 cats), paralyzed with pancuronium, intubated and ventilated (halothane in room air) with a Harvard small animal respirator to maintain a Paco₂ of 37 ± 4 torr. Under these circumstances Paco₂ was equal to or greater than 80 torr. Nasopharyngeal temperatures were maintained at 37 ± 0.25°C. The femoral artery and vein were cannulated for pressure measurement and fluid and drug administration respectively. Through a small burr hole, a silver-silver chloride recording electrode was placed transdurally over the sensory cortex and the contralateral forepaw stimulated (0.4 Hz, 1.0 msec) by a Grass S4 stimulator and isolation unit. The voltage selected produced the largest somatosensory evoked response wave complex. The somatosensory evoked response was amplified and displayed on a storage oscilloscope for observation and photography. Continuous arterial pressure and unipolar EEG were recorded on a Beckman Dynograph. After the surgical preparation was complete, the end tidal halothane was brought to 0.1% over a one-hour equilibration period.

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Results

There was no significant difference in the time for disappearance of either the evoked response or the EEG in any of the groups. The time for reappearance of EEG activity
varied considerably and showed no correlation to the presence or absence of a treatment modality. Significant differences were found for evoked response recovery times between control and treated groups.

In the barbiturate anesthetized groups, nine untreated animals recovered their evoked responses in 23.3 ± 1.5 minutes (fig. 1a). Eight of these animals died within 48 hours (fig. 2a). One animal survived for 30 days and manifested a spastic quadriparesis. Eleven barbiturate-anesthetized animals, who were treated with 1 mg/kg dB-cAMP immediately after cuff release, recovered their evoked responses in 11.0 ± 0.7 minutes (p < 0.01) (fig. 1a). Seven survived 30 days and were neurologically normal at the end of the observation period (fig. 2a). Two animals died of infected surgical wounds at 5 and 7 days. The remaining two animals died within 24 hours of aspiration pneumonitis.

In the halothane anesthetized groups, five untreated animals recovered their evoked responses in 20.3 ± 1.7 minutes. Six animals treated with 1 mg/kg dB-cAMP recovered their evoked responses in 11.7 ± 1.3 minutes (p < 0.02). Six animals who received 2 mg/kg had an evoked response recovery time of 11.9 ± 1.2 minutes (p < 0.02), while those eight animals receiving 4 mg/kg had combined recovery times of 8.6 ± 0.2 minutes. Five animals receiving 6 mg/kg recovered in 9.5 ± 0.8 minutes (fig. 1b). In both of these groups, p was less than 0.01 when compared with the control recovery time.

All the halothane anesthetized treated groups had significantly shortened evoked response recovery times compared to the halothane anesthetized control groups. The 4 and 6 mg/kg groups had statistically significant improvement in recovery over the 2 mg/kg group (p < 0.05 and p < 0.01 respectively).

In the halothane anesthetized control group, four animals died within 24 hours while one survived for 30 days and manifested severe spasticity and ataxia. Four of six halothane anesthetized animals treated with 1 mg/kg dB-cAMP survived for 30 days and were neurologically normal. Of the remaining two animals, one died of pulmonary edema and one had a massive subarachnoid hemorrhage. Five of six animals receiving 2 mg/kg dB-cAMP survived. One animal died of pulmonary edema. Of those animals receiving 4 mg/kg, five survived intact while three died, two of infection and one from unknown cause. Four of five animals receiving 6 mg/kg dB-cAMP survived without defect while one died of spinal cord transection, probably related to cervical trauma from the orthopedic tourniquet (fig. 1b).

Discussion

Results demonstrate that the addition of dB-cAMP as a treatment modality markedly decreased the time to recovery of the evoked response after a standard ischemic injury. The use of the drug also markedly increased the percentage of survival of the injured animals. With the relatively small numbers of animals in each of the seven groups of this study, there was no significant difference seen between the two anesthetic techniques utilized.

The use of the tourniquet technique for producing a stan-
dand ischemic anoxic injury has been discussed elsewhere. The use of the somatosensory evoked response as an index of the integrity of a multineuronal system has been demonstrated by Hossmann and Kleihues. In previous studies we have demonstrated that the reappearance times for the evoked response is a sensitive indicator of the duration of a preceding hypoxic insult. With the use of signal averaging procedures, both a rapid and slow component of the evoked response can be seen. In our procedure, only the fast component is seen. The fast component utilizes fewer neurons than the slow component. If the interruption of transmission caused by hypoxia occurs at synaptic junctions, then the fast component may be less sensitive than the slow to depression. This has been seen with barbiturates. The benefit of using the fast component is that it can be seen with single stimuli rather than the 256–512 stimuli needed for an averaged response.

With the currently available information about the many actions of dB-cAMP, it is impossible to pinpoint a specific mechanism of action for our results. In fact, the cerebral metabolic effects of intravenously administered dB-cAMP have not been investigated. Speculation as to a mechanism of action is therefore fruitless.

This study has demonstrated that the intravenous administration of dB-cAMP shortens the time for the return of the evoked somatosensory evoked response after an ischemic anoxic insult. The number of animals surviving the ischemic anoxic insult is increased by dB-cAMP.

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