

TITLE : EFFECTS OF PENTOBARBITAL ON cAMP IN GUINEA PIG BRAIN  
AUTHORS : G. E. DeBoer, M.D., T. W. Rall, Ph. D.  
AFFILIATION: Case Western Reserve University, School of Medicine, Departments of Anesthesiology and Pharmacology, Cleveland, Ohio 44106. Cleveland Metropolitan General Hospital, Department of Anesthesiology, Cleveland, Ohio 44109. The Cleveland Clinic Foundation, Department of Cardio-Thoracic Anesthesia, Cleveland, Ohio 44106

**Introduction:** Numerous research studies have inferred a relationship between the mechanism of anesthesia and intracerebral cyclic-AMP (cAMP). Cohn et al<sup>1</sup> showed decreased sleeping time in amobarbital-anesthetized rats when treated with intracerebral ventricular dibutyl cAMP. Kakiuchi and Rall<sup>2</sup> showed that levels of cAMP in rabbit brain following decapitation were lower in those rabbits anesthetized with pentobarbital (Pb). Dedrick et al showed increased cAMP in brain from halothane, morphine, and ketamine anesthetized rats. Rudman et al showed excellent indirect correlation between grade of coma and cAMP levels in CSF of post cerebral trauma patients. The following studies were undertaken to reveal a possible effect of barbiturate on neurotransmitter stimulated cAMP metabolism in brain slices.

**Methods:** Slices of brain from several guinea pigs (GP) were prepared, randomized, and preincubated for 50 minutes at 37°C in Krebs-Ringer-Bicarbonate-Glucose media containing 0.8 mM Ca<sup>++</sup> and with 95% O<sub>2</sub>, 5% CO<sub>2</sub> in the gas phase. Then slices were transferred to fresh media containing the substances to be tested and incubated for an additional 10 minutes. The incubation was stopped by freezing the slices in liquid N<sub>2</sub>. Extraction of frozen slices and measurements of cAMP by the protein binding assay of Gilman have previously been described. When Pb was present, it was present during both the preincubation and incubation except as indicated in Table I. When various neurotransmitters or KCl were present, they were present only during the 10 minute incubation.

**Results:** Pb had no effect on either basal cAMP levels or on the neurotransmitter (Adenosine, Histamine, and Norepinephrine) stimulated cAMP levels. However, when KCl was used as a general depolarizing agent which also elevates cAMP in GP brain, the presence of Pb diminished the KCl induced response. The variations in "Media control" cAMP levels present in the three different experiments can be expected because of animal variation and are well documented in the literature.<sup>3</sup>

**Discussion:** Richards reported that 0.6 mM Pb depressed the excitatory post synaptic potential but not the compound action potential in GP brain. Pb, 0.5 mM, should not alter nerve conduction directly, but may reduce synaptic transmitter release. Cohn has interpreted his observations to indicate that barbiturate (Bb) may decrease cAMP in brain. Our data shows that agonist enhanced

cAMP accumulation is not influenced by 0.5 mM Pb. However, Pb did reduce the KCl induced accumulation of cAMP. Zanella and Rall have shown that the KCl effect on cAMP requires extracellular Ca<sup>++</sup>. Thus it is possible that Bb exerts its effects on the release of synaptic transmitters and on cAMP via interference with Ca<sup>++</sup> availability. Furthermore, in view of Crain and Pollacks' observations that exogenous cAMP restores synaptic transmission in Ca<sup>++</sup> deprived CNS tissue cultures, it is possible that cAMP antagonizes Bb effects by augmenting the availability of Ca<sup>++</sup> for synaptic transmission and thus reduce barbiturate induced sleeping time.

| TABLE I                |          |           |             |
|------------------------|----------|-----------|-------------|
| TREATMENT              | 0.5mM Pb | pmol cAMP | mg Protein* |
| Experiment I           |          |           |             |
| Media control          | -        | 10 ± 1    | (4)         |
| Media                  | +        | 10 ± .3   | (4)         |
| .125 mM Adenosine      | -        | 419 ± 7+  | (4)         |
| .125 mM Adenosine      | +        | 446 ± 60  | (4)         |
| .1 mM l-norepinephrine | -        | 18 ± 1+   | (4)         |
| .1 mM l-norepinephrine | +++      | 17 ± .3   | (4)         |
| Experiment II          |          |           |             |
| Media control          | -        | 21 ± 1    | (4)         |
| Media                  | +        | 20 ± 1.7  | (4)         |
| .1 mM Histamine        | -        | 112 ± .6+ | (4)         |
| .1 mM Histamine        | +        | 108 ± 18  | (4)         |
| Experiment III         |          |           |             |
| Media control          | -        | 6 ± 1.9   | (3)         |
| Media                  | +        | 7 ± 1.1   | (3)         |
| 40 mM KCl              | -        | 22 ± .7   | (4)         |
| 40 mM KCl              | +        | 8 ± 1.6+  | (4)         |

\*Values expressed are means ± S.E.; the number of observations is indicated in parenthesis.

†p < .01 vs. appropriate control -Pb.

††Pentobarbital was present for only 20" of the preincubation.

#### References:

1. Cohn ML, Yamaoka H, Taylor F, Kraynack B: Action of Intracerebroventricular Dibutyl Cyclic AMP on Amobarbital Anesthesia in Rats. *Neuropharmacol* 12: 401-405, 1973.
2. Kakiuchi S, Rall TW: Studies on Adenosine 3', 5' Phosphate in Rabbit Cerebral Cortex. *Mol Pharmacol* 4: 379-388, 1968.
3. Sattin A: Cyclic AMP Accumulation in Cerebral Cortex Tissue from Inbred Strains in Mice. *L. Sci.* 16: 903-914, 1975.