Title: PENTOBARBITAL ALTERATION OF CYCLIC AMP METABOLISM

Authors: M.L. Cohn, M.D., Ph.D. and M. Cohn, C.R.N.A.

Affiliation: Department of Anesthesiology, Magee-Womens Hospital, University of Pittsburgh, School of Medicine, Pittsburgh, Pennsylvania 15213

Introduction. None of the current theories of narcosis, all of which are based on a variety of either physicochemical or biophysical properties, has been conclusively substantiated. It has never been established that anesthetics produce specific biochemical alterations in the central nervous system. Our findings that the centrally administered dibutyryl analog of adenosine 3':5'-cyclic monophosphate (cAMP) dose-relatedly shortens sleeping times induced by barbiturates and seven other structurally unrelated anesthetics (1, 2) suggested that anesthetics alter endogenous brain cAMP concentrations. However, cAMP measurements in either whole brain or specific brain structures failed to establish a relationship between cAMP brain content and state of narcosis. Therefore, using our newly devised high performance liquid chromatographic method which, unlike previously available techniques, rapidly and reproducibly permits simultaneous separation, identification and quantification of cAMP and its metabolic products (3), we performed in-vivo and in-vitro studies to determine whether anesthetics alter cAMP metabolic pathways.

Methods. Male Sprague-Dawley rats (Zivic-Miller, Pittsburgh, Pennsylvania) were anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg); untreated rats served as controls. Following sacrifice either by microwave radiation (5 sec. exposure) or decapitation, brains were immediately removed, homogenized in .4 N perchloric acid (1 ml/100 mg of wet brain), centrifuged, neutralized and filtered. Supernatant was chromatographically analyzed. After decapitation, brain cortex slices were incubated under aerobic conditions in Krebs Ringer bicarbonate buffer pH 7.4 (30°C) with or without pentobarbital (10^{-6} M). Sequentially withdrawn aliquots of bath fluid were chromatographically analyzed.

Results. Contrary to previous reports that in mammalian brain the major metabolic products of ATP and cAMP are adenosine and adenine (Fig. 1), our study of untreated rats sacrificed by microwave radiation showed smaller concentrations of the two metabolites and much higher accumulations of the deamination products inosine and hypoxanthine. Under hypoxic conditions (decapitation) we found less inosine and hypoxanthine but a significant accumulation of adenosine that was not concommitant with a cAMP rise. Under barbiturate anesthesia the shift in cAMP metabolism was similar but more pronounced. In-vitro incubation studies revealed that pentobarbital reversibly inhibits the deamination of adenosine 5'-monophosphate (5'AMP), leading to increased dephosphorylation and in turn to adenosine accumulation.

Discussion. Our in-vivo and in-vitro chromatographic evidence that barbiturates inhibit cAMP metabolism at the site where deamination occurs provides a molecular basis for the 50-year old observation that under anesthesia, brain ammonium concentrations are decreased. Our findings also suggest that narcosis is regulated by biochemical mechanism(s). Finally, that adenosine, which has been reported to regulate cerebral blood flow, accumulates under pentobarbital narcosis may explain in part why the barbiturates afford protection against ischemia and stroke.

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References.

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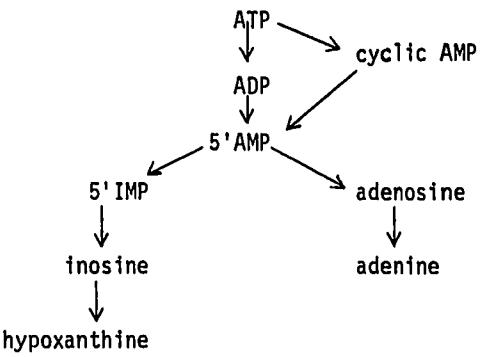

Fig. 1 Metabolic pathway of ATP and cAMP: Under the catalytic influence of adenyl cyclase, ATP is converted to cAMP. In the first and mandatory catabolic step the enzyme phosphodiesterase opens the 3':5'-phosphate link yielding 5'AMP which is then metabolized in a two-step sequence. First, the removal of a phosphate group under the catalytic influence of the nucleotidase yields adenosine. Second, the removal of the sugar ribose by nucleosidase yields adenine. Until recently, adenosine and adenine were believed the major metabolic products of ATP and cAMP. The removal of ammonium ion from 5'AMP, that is deamination, yields 5'IMP, thereby initiating what was believed to be a minor metabolic pathway. The following sequential removal of the phosphate group by the nucleotidase and the ribose group by the nucleosidase yields inosine and hypoxanthine respectively.