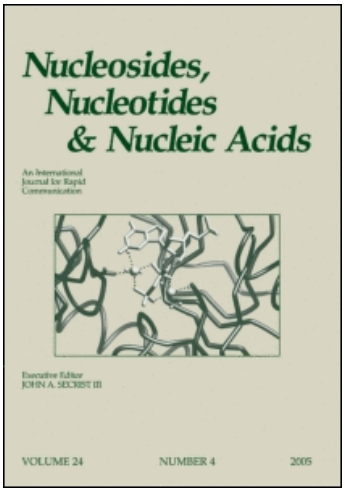

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Cytidine 3',5'-Cyclic Monophosphate: A Third Cyclic Nucleotide Secondary Messenger?

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CYTIDINE 3',5'-CYCLIC MONOPHOSPHATE: A THIRD CYCLIC NUCLEOTIDE SECONDARY MESSENGER?

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ABSTRACT: Recent evidence has now proven the natural occurrence of cyclic CMP, of specific enzymes capable of its synthesis and hydrolysis, and of cyclic CMP-binding proteins and cyclic CMP-responsive protein kinases; the effects of exogenously administered cyclic CMP are consistent with a role for cyclic CMP in the regulation of cell proliferation and/or mediation of steroid hormone actions.

Adenosine 3',5'-cyclic monophosphate (cyclic AMP) has well established functions as a biochemical second messenger, mediating the action of a wide range of mammalian hormones and neurotransmitters, while a second cyclic nucleotide, guanosine 3',5'-cyclic monophosphate (cyclic GMP) performs a similar but more restricted function. In view of the parallel functions of purine and pyrimidine nucleotides in nature, these roles of purine cyclic nucleotides as secondary messengers pose the question as to whether pyrimidine cyclic nucleotide second messengers also exist.

Initial evidence of the natural occurrence of cytidine 3',5'-cyclic monophosphate (cyclic CMP) was from liver extracts and leukaemia L1210 cultures[1,2], and several factors were later reported to stimulate increase in intracellular cyclic CMP concentrations, for example luteinizing hormone releasing hormone[3], long acting thyroid stimulator[4] and increased cell proliferation rate[5]. Cell-permeating derivatives were reported to induce various metabolic changes, and the existence of enzymes capable of cyclic CMP synthesis, cytidylyl cyclase[6], and hydrolysis, cyclic CMP phosphodiesterase[7], lent further weight to the concept of cyclic CMP as a metabolic regulator[8]. However later evidence contradicted this hypothesis in that the phosphodiesterase was found to be non-specific[9], and that the identity of both the extracted putative cyclic CMP and of the product of the cyclase reaction were contested on the basis that several different cyclic CMP-immunoreactive materials were present[10].

The natural occurrence of cyclic CMP has now been unequivocally demonstrated by means of tandem mass spectrometric analysis of sequentially purified tissue extracts[11]. The controversy regarding the identities of both the cyclic nucleotide in tissue extracts and the putative cyclic CMP product of the cytidylyl cyclase reaction was explained by the discovery of four novel cyclic CMP analogues, cytidine 3',5'-cyclic pyrophosphate, cytidine 2'-O-monophosphate-3',5'-cyclic monophosphate, cytidine 2'-O-glutamyl 3',5'-cyclic monophosphate and cytidine 2'-O-aspartyl-3',5'-cyclic monophosphate[12]. As a result of their identification chromatographic systems capable of resolving cyclic CMP from these cross-reactants have been developed and are incorporated into a specific assay for cytidylyl cyclase activity[13] and a radioimmunoassay for extracted cyclic CMP[14].

Application of these assays has shown that cytidylyl cyclase activity is of a similar level in each of nine mammalian tissues examined, but showed a dose-dependent stimulation in response to dihydrotestosterone and other steroids. Interestingly a second form of cyclic CMP phosphodiesterase[15], specific for cyclic CMP as substrate, is stimulated by both an endogenous protein and by several steroid hormones, including androsterone and dihydrotestosterone. Thus elevation of testosterone concentration would project to stimulate an increased turnover of cyclic CMP; the extremely potent effect of dibutyryl cyclic CMP in blocking testosterone-induced aggression[16] may well be related to this effect.

Application of the new, unambiguous, radioimmunoassay showed that cyclic CMP concentrations were of similar levels in a variety of tissues (Figure 1a); concentrations in regenerating liver (Figure 1b) after partial hepatonecrosis by CCl_4 were elevated, and this elevation of cyclic CMP concentrations in rapidly proliferating tissue is also evident when comparing adult heart and liver with their foetal counterparts (Figure 1c). In foetal tissue the cytidylyl cyclase activity was also elevated while the cyclic CMP phosphodiesterase activity was depressed. This elevation in cyclic CMP concentrations in rapidly proliferating cells suggests that cyclic CMP may have value as a clinical marker, as indicated by levels in leukaemic patients (Figure 1d).

In addition to the sensitivity of cytidylyl cyclase and cyclic CMP phosphodiesterase to hormonal agonists, for cyclic CMP to have a second messenger function a cyclic CMP-binding protein or cyclic CMP-responsive protein kinase must exist. We have demonstrated several such binding proteins, two of which possess protein kinase activity selectively

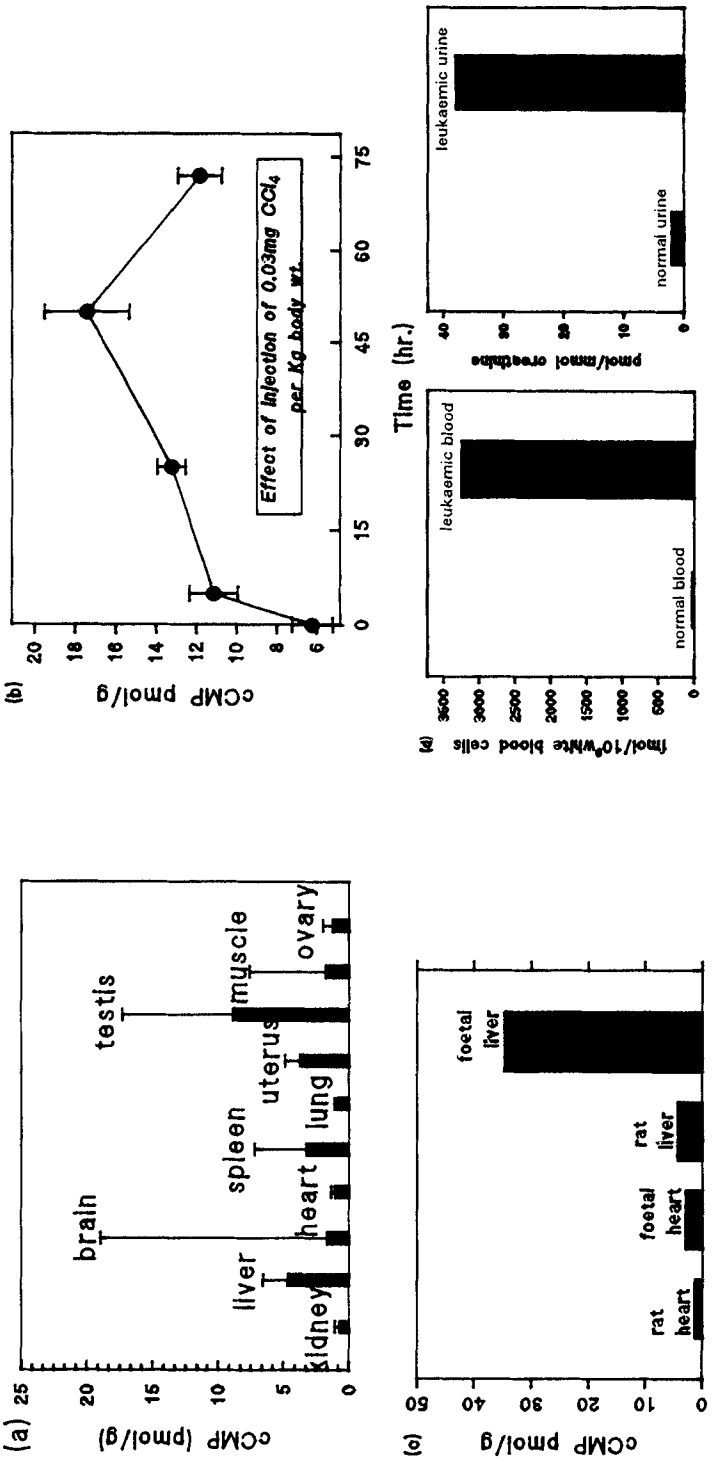

FIGURE 1. Cyclic CMP concentrations, determined by radioimmunoassay, in (a) mouse tissues, (b) regenerating liver, (c) foetal tissues and (d) leukaemic patient samples.

sensitive to cyclic CMP[17] ; studies with [γ-^{32}P]-ATP incorporation have indicated at least 17 endogenous protein substrates of this kinase, but they have yet to be characterized.

Synthesis of a cell-permeating derivative of cyclic CMP, N^4,2'-O-dibutyryl-cytidine-3',5'-cyclic monophosphate[18], and administration to animals evoked a variety of responses dependent upon dose, frequency of administration and tissue examined, but in general an increase in DNA synthesis, decreases in RNA synthesis, increase in free amino acid concentration, decrease in total protein and increase in total lipid concentrations were observed.

Collectively the available data are consistent with a role for cyclic CMP in mediating the action of steroid hormones, and/or in the regulation of cell proliferation. However deductions of the function of cyclic CMP as a third cyclic nucleotide metabolic regulator will only be credible after the cyclic CMP binding proteins and the substrates of cyclic CMP-sensitive protein kinases have been characterized and demonstrated to be integral components of systems responsive to exogenously applied cyclic CMP, and to be shown sensitive to factors modifying cytidylyl cyclase and cyclic CMP phosphodiesterase activities.

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