CYCLIC NUCLEOTIDES IN DISEASE

Edited by

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Preface

Volumes have been written about the biological importance of cyclic nucleotides, and much outstanding research has been carried out since the late 1950's, when Earl Sutherland and Ted Rall published their first papers on cyclic AMP. The awarding of the Nobel Prize in Physiology and Medicine to Dr. Sutherland in 1971 was not the culmination of research in this field but rather the stimulus for further research on the concepts that he and his colleagues throughout the world have promulgated.

As is natural and appropriate in a new field, most of the investigators concentrated their attention on investigating the basic properties of the cyclic nucleotides, their biological role, and the factors responsible for regulating their intracellular concentration. It was apparent from the outset, however, that these studies inevitably must lead to important and perhaps even revolutionary clinical applications. We felt the time was ripe for gathering together some of the leading researchers in the fields of cyclic nucleotide biochemistry, physiology, and pharmacology for the purpose of encouraging them to speculate on the clinical significance of their basic studies and to predict the future directions modern therapeutics might take.

This book, therefore, presents basic research viewed in the light of the possible clinical implications of cyclic nucleotides. Based largely on the editor's own bias we have divided it into four sections, namely:

Clinical Implications of Cyclic Nucleotides in Cancer and Other Diseases Affecting Tissue Growth

Clinical Implications of Cyclic Nucleotides in Diseases Affecting the Cardiovascular System

Clinical Implications of Cyclic Nucleotides in Neuroendocrine and Secretory Processes

Clinical Implications of Cyclic Nucleotides in Diseases Affecting the Central Nervous System

Our fundamental aim is to provide the background that will enable new investigators to pursue research into the biological role of cyclic nucleotides, particularly as it might relate to certain disease states, and to provide the stimulus to established investigators to evaluate their own research in terms of its possible clinical significance. Our hope, therefore, is not to close a chapter in cyclic nucleotide research but rather to continue one already begun.

Benjamin Weiss

Contents

CLINICAL IMPLICATIONS OF CYCLIC NUCLEOTIDES IN CANCER AND OTHER DISEASES AFFECTING TISSUE GROWTH

The Role of Cyclic Nucleotides in Cell Growth and Development: Regulation and Characterization of Cyclic Nucleotide Phosphodiesterases in Mammalian Cells

Samuel J. Strada and W. Jackson Pledger

Biologic responses of many hormones are associated with the formation of cyclic AMP in target tissues (Sutherland and Rall, 1960; Robison, Butcher, and Sutherland, 1971; Sutherland, 1972; Strada and Robison, 1974). It appears certain that defects not only in the formation but also in the metabolism and action of the cyclic nucleotide are involved in the etiology of a variety of human diseases. These include cancer, where recent studies appear to have linked abnormal hormone-receptor interactions, surface membrane changes, and cyclic nucleotide metabolism to the altered growth characteristics of malignant cells in culture (Abell and Monahan, 1973). Cyclic nucleotides also may play an important role during the

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immune response (see, for example, Hadden et al., 1972; Parker, 1972; Watson, Epstein, and Cohn, 1973; Bourne et al., 1974), although their exact role has yet to be clarified.

Evidence has begun to accumulate suggesting that cyclic AMP may play an important role during normal growth and development as well. Data are presently insufficient to define this role precisely, but it would appear that in some types of cells reduced levels of cyclic AMP are needed to permit rapid cell division, whereas high levels are associated with differentiation (Weiss and Strada, 1973). Changes in cyclic AMP during the cell cycle have been demonstrated (Willingham, Johnson, and Pastan, 1972; Burger et al., 1972; Sheppard and Prescott, 1972; Bombik and Burger, 1973), and a complementary role for cyclic GMP has been suggested (Hadden et al., 1972; Goldberg et al., 1973*a*). Cellular levels of cyclic AMP and/or GMP may, therefore, participate in the decision of whether or not a cell becomes malignant.

Cellular concentrations of cyclic nucleotides depend on relative rates of synthesis and hydrolysis, the former being catalyzed by an appropriate cyclase enzyme and the latter by a phosphodiesterase enzyme system. Recent studies have indicated that the hydrolysis of cyclic nucleotides involves a more complex system than once was realized; this may explain why less is known of the factors regulating phosphodiesterase activities than of adenylate cyclase systems (see Perkins, 1973). Our own studies of heterogeneous tissues such as brain have demonstrated multiple enzyme forms with divergent substrate affinities, kinetic properties, molecular sizes, and sensitivities to inhibitors and activators. We have instrumented methodology essential for characterization of the multiple enzyme forms. On the other hand, the complex nature of the cyclic nucleotide phosphodiesterase enzyme system (for a recent review, see Appleman, Thompson, and Russell, 1973) points out the need to control biologic variables and minimize the influence of heterogeneity.

Some interesting recent experiments with cultured cells suggest that cyclic AMP might regulate its own rate of metabolism through the de novo synthesis of cyclic AMP phosphodiesterase (Manganiello and Vaughan, 1972; d'Armiento, Johnson, and Pastan, 1972; Uzunov, Shein, and Weiss, 1973; Bourne, Tomkins, and Dion, 1973).

We have studied properties of cyclic nucleotide phosphodiesterases of baby hamster kidney cells (BHK–21) and BHK cells transformed by the Schmidt-Ruppin strain of Rous sarcoma virus (RSV-BHK) in relation to growth mechanisms in culture. The studies reported here indicate that cyclic nucleotide phosphodiesterase activities of cultured BHK cells may be controlled by two independent mechanisms: (a) a rapid regulatory control system, which is responsible for acute oscillatory changes in cyclic

nucleotide levels and involved in growth proliferation, and (b) a more slowly controlled system, which is responsible for long-term maintenance of cyclic nucleotide levels and related to contact-inhibited growth. Our studies further indicate that normal cells possess a mechanism for rapidly altering phosphodiesterase activity that is defective in virally transformed cells. This mechanism appears separate and distinct from the slower regulation of phosphodiesterase described here and by others.

It would appear from these studies that tissue culture offers a useful approach to the study of factors regulating enzyme activities. The potential significance of finding differences in regulatory control systems between normal and transformed cells is intriguing from the standpoint of understanding cancer in humans. Further exploration of this area could lead eventually to the molecular basis of transformation and a better understanding of mechanisms of cell growth.

ROLE OF CYCLIC NUCLEOTIDES IN CELL GROWTH AND DEVELOPMENT

Cyclic AMP has transcended its endocrinologic beginnings, since it has been shown to function in bacteria and other unicellular organisms and in almost all animal species. The ability of glucose to suppress cyclic AMP formation appears to account satisfactorily for catabolite repression in *Escherichia coli* and other gram-negative bacteria (Makman and Sutherland, 1965; Pastan and Perlman, 1972; Buettner, Spitz, and Rickenberg, 1973). Cyclic AMP also has been implicated in lysogeny (Hong, Smith, and Ames, 1971) and in bacterial transformation (Wise, Alexander, and Powers, 1973). In certain species of cellular slime molds, cyclic AMP appears to be responsible for initiating the aggregation of the slime mold amoebae, leading to the formation of a multicellular organism (Bonner, 1971; Konijn, 1972). Other than the obvious inferences from bacterial studies, a wealth of experimental evidence exists to implicate cyclic AMP in mechanisms of cell proliferation, differentiation, transformation, and tumorigenesis in higher organisms. Several theories have been proposed as a molecular basis for transformation, and one of these, now supported by independent lines of investigation, suggests an involvement of altered cyclic AMP metabolism in this process. The lines of evidence on which the theory is based will be considered briefly.

Transformed cells have certain properties distinguishing them from parent or normal cells (Todaro and Huebner, 1972). They have abnormal morphologic features; their growth is accelerated; they do not adhere to surfaces well; their ability to agglutinate upon exposure to certain plant

lecithins is increased; they grow to higher saturation densities (i.e., they lose the property of contact-inhibition of growth); their rate of production of acid mucopolysaccharides and glycolipids is either lost or reduced; and they tend to produce tumors when injected into a susceptible host. The addition of exogenous cyclic AMP or derivatives of cyclic AMP or application of agents which increase the accumulation of endogenous cyclic AMP restores to transformed cells many of the properties characteristic of untransformed cells. These would include morphologic properties (Johnson, Friedman, and Pastan, 1971; Hsie, Jones, and Puck, 1971; Hsie and Puck, 1971; Johnson and Pastan, 1971), slower rates of growth (Johnson and Pastan, 1971; Heidrick and Ryan, 1970, 1971; Yang and Vas, 1971; Schröder and Plageman, 1971; Van Wijk, Wicks, and Clay, 1972) and motility (Johnson, Morgan, and Pastan, 1972), apparent or partial restoration of contact inhibition (Heidrick and Ryan, 1971; Otten, Johnson, and Pastan, 1971, Otten, et al, 1972*a*; Sheppard, 1971; Smets, 1972; Paul, 1972), biosynthetic properties (Johnson and Pastan, 1972*b*; Goggins, Johnson, and Pastan, 1972; Puck, Waldren, and Hsie, 1972), reduced sensitivity to plant agglutinins (Sheppard, 1971), and greater adhesion to substratum (Johnson and Pastan, 1972*a*). Another line of evidence implicating cyclic AMP in cellular transformation is that concentrations of cyclic AMP are generally lower in transformed cells than in untransformed cells (Heidrick and Ryan, 1971; Otten et al., 1972*a*; Sheppard, 1972), and an inverse correlation between cyclic AMP levels and growth rate exists for a series of transformed cell lines (Otten et al., 1971). Some observations suggest that cell-to-cell contact itself may lead to increased accumulation of cyclic AMP, and that contact-inhibited cells stop growing because of the higher level of cyclic AMP. Other observations indicate that the amount of cyclic AMP per cell is always higher in untransformed than in transformed cells, regardless of the cell density. The point which does seem undisputed is that transformed cells usually contain less cyclic AMP than do untransformed cells.

Correlations between cyclic AMP levels and effectors of growth proliferation have been made in a number of studies. Transformation of fibroblasts in vitro by both wild-type and temperature-sensitive viruses (Otten et al., 1972*b*; Rein et al., 1973; Raska, 1973; Carchman et al., 1974) produces a fall in cyclic AMP; the reduction in cyclic nucleotide level precedes the induced morphologic transformation. Agents such as trypsin, insulin, or serum which stimulate proliferation of cells in culture produce transient falls in levels of cyclic AMP (Otten et al., 1972*a*; Sheppard, 1971; Froehlich and Rachmeler, 1972; Burger et al., 1972; Rosengurt and Pardee, 1972; Willingham, Johnson, and Pastan, 1972). Addition of exogenous cyclic AMP (or agents that increase endogenous

cyclic AMP during this period) prevents the proliferation that would otherwise occur (Millis, Forrest, and Pious, 1972; Willingham et al., 1972). Low levels of cyclic AMP are found during mitosis, and fluctuations in cyclic nucleotide levels are found during the cell cycle of cultured fibroblasts (Burger et al., 1972; Sheppard and Prescott, 1972) and lymphoid cells (Millis et al., 1972, 1974).

Of related interest from the standpoint of regulatory influences of cyclic AMP on cell growth are studies showing that reduced levels of cyclic AMP in epidermal cells correlate with the excessive cell proliferation rates of psoriatic lesions (Voorhees and Duell, 1971; Voorhees et al., 1972*a*), and that increasing the level of cyclic AMP inhibits epidermal mitosis (Voorhees et al., 1972*b*; Bronstad, Elgjo, and Øye, 1972; Marks and Grimms, 1972).

Supporting the role of cyclic AMP in growth and development, in a converse way, are studies showing that the replication of thymic lymphocytes (Whitfield et al., 1973), hematopoietic stem cells (Byron, 1972), salivary acinar cells (Guidotti, Weiss, and Costa, 1972), and adrenocortical cells (Nussdorfer and Mazzocchi, 1972) are stimulated rather than inhibited by high levels of cyclic AMP. Similarly, cultured neuroblastoma cells differentiate (as evidenced by neurite formation) in response to dibutyryl-cyclic AMP or agents that enhance cyclic AMP formation in these cells (Prasad and Hsie, 1972; Roisen, Murphy, and Braden, 1972).

Although the rapid growth and relatively undifferentiated character of certain tumor cells could be understood in principle in terms of reduced levels of (or sensitivity to) cyclic AMP, it is not clear that the ability to metastasize could be understood in similar terms. Defects in the formation, regulation, or action of cyclic AMP could be a prerequisite for malignancy, however. Along these lines, hepatoma cells (Granner, 1972), an adrenocortical carcinoma (Ney et al., 1969), and mutants of lymphoma cells (Daniel, Litwack, and Tomkins, 1973*b*) have been shown to be insensitive to the growth inhibitory effects of cyclic AMP. The actual level of cyclic AMP in these cells might be quite irrelevant, therefore, since reductions in sensitivity to the action of cyclic AMP could lead to effects essentially similar to those produced by reduced levels of the nucleotide.

Studies of the effects of cyclic GMP on the growth and development of cells are not as extensive as they are for cyclic AMP. Goldberg and his colleagues (Hadden et al., 1972) have reported large increases in lymphocyte cyclic GMP in response to a preparation of phytohemagglutinin possessing mitogenic activity but free of agglutinating activity. This preparation of phytohemagglutinin did not affect the level of cyclic AMP. These investigators have suggested that cyclic AMP and cyclic GMP may act "dualistically" as antagonists of each other. Opposing influences of the