THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 67:641–645, 1976

Vol. 67, No. 5, Part 2 of 2 parts
Printed in U.S.A.

CYCLIC GMP, CYCLIC AMP, AND THE YIN YANG HYPOTHESIS OF BIOLOGIC REGULATION

NELSON D. GOLDBERG, PH.D., MARI K. HADDOX, CHARLES E. ZEILIG, PH.D., SUSAN E. NICOL, PH.D., TED S. ACOTT, PH.D., AND DAVID B. GLASS, PH.D.

Departments of Pharmacology, Pathology and Laboratory Medicine, University of Minnesota, Minneapolis, Minnesota, U.S.A.

The purpose of this presentation is twofold: (1) to describe a concept of biologic regulation in which two cellular components, cyclic GMP and cyclic AMP, are envisioned to provide the facilitory and suppressive influences that control a variety of biologic events; and (2) to introduce the concept that the intracellular availability of specific cations may selectively regulate cyclic nucleotide participation in modulating cellular events.

RECIPROCAL CONTROL OF BIDIRECTIONALLY REGULATED EVENTS BY CYCLIC GMP AND CYCLIC AMP: THE YIN YANG HYPOTHESIS OF BIOLOGIC CONTROL

From the pioneering work of Sutherland and his co-workers, it is now generally understood that cyclic AMP can serve as the intracellular effector of a variety of cellular events [1]. It is now becoming apparent that another naturally occurring cyclic nucleotide, cyclic GMP, may also be of considerable importance as a regulatory effector. Experiments focused on elucidating the biologic importance of cyclic GMP have led to a series of discoveries that link cyclic GMP to the actions produced by a variety of agents that promote cellular events opposite to those believed to be promoted by cyclic AMP. From these observations a new concept of regulation has been proposed whereby the two cyclic nucleotides, cyclic AMP and cyclic GMP, are envisaged to impose opposing influences in a number of systems [2–4]. The concept has been termed the Yin Yang Hypothesis of Biological Control because in Oriental cultures this term symbolizes a dualism between opposing natural forces which may, under certain circumstances, enter into a mutual interaction that results in a synthesis. The latter situation may characterize certain biologic processes comprised of a series of regulated steps.

Before developing details of the hypothesis, it may be well to emphasize that the complexities that characterize the great variety of cellular processes susceptible to regulation are well appreciated. However, biologic regulation, in very basic terms, can be viewed as a process of imposing positive and negative controlling influences on cellular events. It would seem appropriate, therefore, to begin with a relatively simple model from which to develop a more complete understanding of the complexities that are involved.

In its simplest form, our hypothesis defines cyclic GMP and cyclic AMP as key biologic effectors involved in regulating cellular functions which could be considered to be controlled bidirectionally. A bidirectionally regulated cellular process is considered to be one which is in a functional state and susceptible to both stimulatory and inhibitory controlling influences. Such a process may involve a single pathway or opposing unidirectional pathways which are affected reciprocally. We have postulated that there are two basic types of simple bidirectionally controlled systems: A-type systems which are facilitated by cyclic AMP and suppressed by cyclic GMP, and B-type systems which are promoted by cyclic GMP and inhibited by cyclic AMP. The influence of either cyclic nucleotide may be imposed by an elevation of its cellular concentration or by a change in the composition of the cellular milieu which would allow one or the other to act effectively. This idea takes into account a change in the ratio of the two effectors as well as a possible change in a specific cell constituent such as a cation that may be required for the functional participation of the particular cyclic nucleotide. The hypothesis does not require that reciprocal changes take place in the cellular concentrations of the two cyclic nucleotides. This may occur, however, when a system is under the prior influence of an opposing signal, and lowering the level of the antagonistic cyclic nucleotide effector to a basal level (from the prestimulated level) would be required for the full expression of the newly arriving signal. An example of a simple bidirectionally controlled system of the A type may be cardiac contractility, while smooth muscle contraction would be considered to be an example of the B type.

In contrast to these relatively "simple" regulated systems there are the more complex biologic processes that may be comprised of a series of individual cellular events (i.e., the proliferative

Supported by Research Grants from U. S. Public Health Service (NS 05979), American Cancer Society (BC 166), and National Cancer Institute (Program Grant 1622801).

Reprint requests to: Dr. N. G. Goldberg, Department of Pharmacology, University of Minnesota, Minneapolis, Minnesota 55455.

Abbreviations:
Con-A: concanavalin A
EGTA: ethylene glycol tetraacetic acid
IP: induced protein
PHA: phytohemagglutinin
PMN: polymorphonuclear

process; hormone biosynthesis, storage, and secretion; etc.). Each of the individual steps comprising these overall processes may be susceptible to regulatory influences imposed through the two cyclic nucleotides. For certain of the processes, the individual sequential steps may be dependent on the progression of the preceeding step, and influences by one or the other effector in sequential fashion may be required to promote the overall process. A suppressive influence by one cyclic nucleotide effector on an individual step in the sequence may prevent the process from proceeding to completion. Although several steps in the sequence may be affected reciprocally by the two cyclic nucleotides, there may also be a specific step that is stimulated by both effectors (i.e., a "monodirectionally" regulated step [4]). An example of a system in which such a step could occur is a synthesis–secretion system where the biosynthesis of product is needed to meet two different requirements: to satisfy the current demand of the stimulated state or to replenish depleted stores of secretory product. The foregoing is still a matter of conjecture but is meant to underscore the importance of thoroughly defining the biologic system under investigation in terms of the individual steps that may be involved, the various sites at which regulation may be imposed, and the different circumstances that may arise and call for a particular combination of controlling influences. Although it is proposed that this general model involving the two cyclic nucleotides may apply to most forms of hormonal regulation and to other biologic signals, it does not exclude the existence of alternative mechanisms for affecting cellular function.

The evidence in support of the concept we have proposed derives from two lines of investigation. The first has shown that a number of biologically active agents that cause the accumulation of cellular cyclic GMP also promote cellular events opposite to those elicited by agents that stimulate cellular AMP accumulation. The second has shown that exposure of cells to cyclic GMP (or appropriate derivatives) can cause cellular alterations that are the reciprocal of those that occur upon exposure to cyclic AMP (or appropriate derivatives).

The first line of investigation had its beginning when it was observed that acetylcholine-induced suppression of cardiac contractility occurred in association with a rapid increase in the tissue concentration of cyclic GMP [5]. Considering that epinephrine-induced stimulation of heart function occurs in association with a rapid accumulation of cellular cyclic AMP, it was apparent that the two cyclic nucleotides might be involved in promoting opposing regulatory influences in this tissue and that some aspect of acetylcholine action in cardiac muscle might be expressed through some intracellular influence of cyclic GMP. Isoproterenol-induced stimulation of contraction was also shown to diminish the levels of cyclic GMP by about 50% [5]. Acetylcholine can now be shown in a variety of different tissues to promote cellular cyclic GMP accumulation [6] and in each of the tissues or cells in which the influence of acetylcholine on cell function is definable (and of the muscarinic type) the effect produced by this neurohormone is opposite to that produced by agents (i.e., β-adrenergics) that elevate cellular cyclic AMP concentration. In addition to acetylcholine, a number of other agents and conditions that promote so-called "cyclic AMP antagonistic" events in highly differentiated cells have been shown to do so in association with an elevation of cellular cyclic GMP concentration [6]. This list now includes hormonal and neurohormonal agents such as prostaglandin $F_{2\alpha}$, serotonin, oxytocin, α-adrenergics, insulin, cholescystokinin, somatostatin, and nonhormonal agents such as phorbol myristate acetate, serum-coated zymosan particles (which promote lysosomal enzyme release), and the ionophore-A23187 which appears to promote a variety of calcium-dependent processes. The list of agents now shown to promote cyclic GMP accumulation is as diverse as the list of cellular processes that are affected. However, the commonality exists in the nature of the cellular responses, all of which are opposite to those that occur in response to agents that stimulate the cellular accumulation of cyclic AMP.

Although the unequivocal proof establishing cyclic GMP or cyclic AMP as the mediators of opposing influences in various systems is still forthcoming, the continuing effort to test the hypothesis has produced new information that helps to extend our understanding of how cyclic nucleotides and other cell constituents may be interrelated. An illustration of the latter is the recent observation that cyclic GMP metabolism can be markedly affected by ascorbic acid.

ASCORBIC ACID

During the course of experiments in which the role of cyclic GMP was under investigation as a modulator of platelet function, it was found that ascorbic acid, which was employed as an antioxidant in solutions of epinephrine used to induce platelet aggregation, promoted 180-fold increases in platelet cyclic GMP concentration within 5 sec after exposure [6]. This effect of ascorbic acid is selective with regard to cyclic GMP since there was no significant change in cyclic AMP levels under these conditions. The increase in platelet cyclic GMP concentration which appears to be attributable to ascorbic acid is not induced by equimolar concentration of several reducing agents which have been tested, including dithiothreitol, glutathione, sodium metabisulfite, and mercaptoethanol, or by a number of other weak acids which have been examined. The ability of ascorbic acid to promote an accumulation of cyclic GMP certainly provokes a new interest in effects that this vitamin may have on cyclic nucleotide metabolism in this and other systems.

Since the ascorbic acid-induced increase in platelet cyclic GMP does not by itself lead to aggregation, the role played in this process by cyclic GMP remains to be established. Since it is now known that various aggregating agents (i.e., prostaglandin endoperoxides, collagen) elevate cyclic GMP levels shortly (1 min) after initiation of platelet aggregation (D. Glass, J. Gerrard, D. Towsend, J. White, and N. Goldberg, unpublished data), cyclic GMP may not be involved in the first stage of reversible aggregation but it may be involved in the processes leading to irreversible aggregation.

CELL PROLIFERATION

In addition to the effects that cyclic nucleotides may have on the function of so-called "differentiated" cells, there is evidence that cyclic AMP and cyclic GMP may play major regulatory roles in the proliferative and differentiative processes. It has been shown in a number of laboratories [7,8] that elevated levels of cellular cyclic AMP inhibit proliferation and promote differentiation. Although it has been proposed that a lowering of cellular cyclic AMP concentration may represent a state that permits proliferation to occur, it has now been shown in a number of cases that conditions which stimulate cell proliferation do so in association with elevations of cellular cyclic GMP concentration [4]. The possibility that cyclic GMP might represent an intracellular mediator of the proliferative process was first suggested on the basis of the observation that mitogenic concentrations of phytohemagglutinin (PHA) or cancanavalin A (Con-A) produced greater than 10-fold increases in the cyclic GMP levels of human peripheral blood lymphocytes within 10 min with little or no change in the levels of cyclic AMP [9]. Since then it has been shown that several agents which promote proliferation in different cell types produce similarly striking increases in cyclic GMP concentrations [6]. In fibroblasts, for example, insulin, fresh serum, fibroblast growth factor, and phorbol myristate acetate have been shown to produce marked elevations of cyclic GMP within just seconds or minutes. In mouse epithelium, phorbol myristate acetate and histamine, which stimulate these cells to divide, elevate the levels of cyclic GMP. The meristematic (proliferative) regions of pea and bean seedling roots and stems contain much higher concentrations of cyclic GMP (by as much as 70-fold) than the nonproliferating, elongating cells. From the time course of the changes of cyclic GMP following addition of various mitogens such as serum, insulin, or fibroblast growth factor, it appears that the increase in cyclic GMP occurs only during a specific period of time in the early G_1 phase of the cell cycle and that this may represent an important signal for initiating the progression of events involved in cell proliferation.

Even in some malignant cells which generally exhibit low basal levels of cyclic GMP in asynchronous cultures, synchronization reveals the existence of transient increases in cyclic GMP during G_1 [6].

ESTROGEN

After the relationship between cell proliferation and cyclic GMP was established with mitogenic agents which were thought to act at the level of the plasma membrane, one of the questions that arose was whether mitogenic agents such as steroids which are thought to act intracellularly also promoted cellular cyclic GMP accumulation. Estrogen, which promotes cell proliferation of uterine endometrium, was examined in this regard [10]. It was found that estradiol-17β or diethylstilbestrol [10] promoted striking increases of 7- to 10-fold in uterine cyclic GMP while at the same time the concentration of cyclic AMP was lowered (approximately 50%) in this tissue.

There is a time-dependent lag of 60 to 90 min before the rise of cyclic GMP occurs and the rise is blocked by puromycin, cycloheximide, and high concentrations of actinomycin D. These experiments suggest that a protein is induced which might function to enhance cyclic GMP generation, retard its degradation by inhibiting phosphodiesterase, or prevent its degradation by complexing with it. The possibility that a protein inducible by estrogen is involved in bringing about the accumulation of tissue cyclic GMP (and/or lowering of cyclic AMP levels) is of considerable interest since it could represent a component similar or identical to the formerly described estrogen "induced protein" (IP) [11].

OPPOSING EFFECTS OF EXOGENOUS CYCLIC GMP AND CYCLIC AMP

One of the problems that is still not completely resolved and continues to impede progress in establishing the precise biologic role of cyclic GMP is the fact that exposure of intact cells to this cyclic nucleotide often produces no effect or the same effect as exogenously added cyclic AMP. It has been suggested [3] that excessively high concentrations of exogenous cyclic GMP (i.e., millimolar range) interact nonspecifically with components more selective for cyclic AMP, resulting in an effect similar to that obtained with cyclic AMP. However, addition of cyclic GMP (or the 8-bromo or dibutryl derivatives) in the micromolar or submicromolar concentration range often induces effects on cell functions that are opposite to those promoted by the addition of any concentration of cyclic AMP. An example of an intact cell system in which opposing effects of the two cyclic nucleotides can be demonstrated is the chemotactic responsiveness of polymorphonuclear leukocytes (PMN) [12,13]. With the PMN it can be shown that agents, such as β-adrenergic catecholamines or PGE_1 (which elevate cyclic AMP levels in the cells) or exogenous cyclic AMP, inhibit motility induced by bacterial chemotactic factor. On the other

hand, α-adrenergic agents, acetylcholine, low concentrations (10^{-8} and 10^{-7} M) of $PGF_{2\alpha}$ (which can be shown to elevate cyclic GMP levels in the PMN), or exogenous cyclic GMP in the concentration range of 10^{-9} to 10^{-7} M enhance chemotaxis 2- to 3-fold. The peak effect is achieved at 10^{-8} M cyclic GMP. Concentrations of cyclic GMP between 10^{-6} to 10^{-5} M are without effect, and at 10^{-4} M an inhibitory effect like that produced by cyclic AMP is obtained.

Opposing effects of the two cyclic nucleotides (or appropriate derivatives) have also now been demonstrated on short-circuit current generation in frog skin (unpublished observations), histamine release from mast cells [14], lysosomal enzyme release from leukocytes [15,16], sheep red blood cell binding to mouse lymphocytes [17], transport of several substances by fibroblasts [18], the polarization of postsynaptic membranes in superior cervical ganglia [19], the rate of neuronal firing in somatosensory cells of the cortex [20], the cytotoxicity of lymphocytes [21], and the rate of cardiac muscle cell contraction [22]. With the greater appreciation for what appears to be a more subtle concentration dependence of effects elicited by cyclic GMP (bell-shaped rather than sigmoidal concentration-response curves) and a more directed intuition as to the effect that it may produce with respect to cyclic AMP, it can be predicted that a number of other systems will be shown to respond reciprocally upon exposure to the two cyclic nucleotides.

CATION-INDUCED MODULATION OF CYCLIC NUCLEOTIDE INTERACTIONS WITH CELLULAR COMPONENTS

Skeletal Muscle Cyclic AMP-Dependent Protein Kinase

A variety of hormonal agents can be demonstrated to promote increases in the cellular concentration of cyclic AMP (or cyclic GMP) and this fact has been extremely useful in implicating cyclic nucleotides as cellular mediators of hormonal actions. This relationship between hormone and cyclic nucleotide concentration served as an important criterion for the development of Sutherland's "second messenger hypothesis." However, there is evidence accumulating which indicates that alterations occur in the levels of certain cell constituents that may modulate the interaction of a cyclic nucleotide with a specific cellular component. In this case a cyclic nucleotide could be envisaged to influence a cellular event without any immediate change occurring in its cellular concentration.

Consistent with this concept is the observation that ATP (Mg^{2+}) can inhibit cyclic AMP binding to a major species of skeletal muscle protein kinase [4]. The inhibitory effect requires both the triphosphate and Mg^{2+}; neither alone is effective. The inhibitory effect of ATP (Mg^{2+}) results in a 10-fold [23] to 50-fold [24] increase in the requirement for cyclic AMP in both the protein kinase complexing reaction and the activation of phosphotransferase activity [25]. The inhibition of cyclic AMP reactivity was found to be reciprocally related to the binding of [^{3}H]ATP to the protein kinase and the apparent K_d value ATP binding was approximately 10^{-7} M. The binding of ATP (Mg^{2+}) to the protein kinase and the inhibitory influence imposed as a result did not lead to any detectable transformation of the ATP [23]. The high affinity site of STP (Mg^{2+}) in addition to the catalytic subunit was required for the release of bound cyclic AMP from the regulatory subunit [25].

The physiologic significance of these observations is not yet understood, but since the ATP requirement for this effect (10^{-7} M range) is several logs lower than the levels of ATP (10^{-3} M range), it is conceivable that the cation might serve as the allosteric determinant. This possibility was examined by testing cations other than magnesium for their ability to support [^{3}H]ATP binding to protein kinase and to suppress reactivity of the kinase with cyclic AMP. Mn^{2+} and Ca^{2+} were both found to be as effective as Mg^{2+} in promoting ATP binding to the kinase but unlike ATP (Mg^{2+}) or ATP (Mn^{2+}), ATP (Ca^{2+}), when in complex with the enzyme, did not inhibit cyclic AMP binding. Variations in the availability of free intracellular calcium might allow for an exchange of calcium for magesium complexed with the protein kinase (by active or passive mechanisms). Such an exchange would result in a decrease (1/10 to 1/50) in the requirement of the protein kinase for cyclic AMP. The tissue concentration of cyclic AMP which may have been inadequate to stimulate the kinase when it was complexed with magnesium could now become effective. If such a mechanism is operative, it would represent an alternative means for promoting an event through an influence of a cyclic nucleotide without the concentration of this component necessarily increasing in the cell.

Cation-Dependent Cyclic GMP Binding Protein

One feature of many cellular events, promoted by agents which have been shown to stimulate cyclic GMP accumulation, is an apparent requirement for divalent cation, namely calcium [26,3]. When approaching the problems of identifying the cellular component(s) with which cyclic GMP may interact to influence certain cellular events, it would seem reasonable to expect that interaction of this cyclic nucleotide with specific cellular components would exhibit a dependence upon divalent cation. It was with this view of the problem that investigations were conducted to uncover a cellular component with which cyclic GMP might specifically interact.

Preliminary experiments along these lines were conducted by determining what influence Ca^{2+} or Mg^{2+} might have on the formation of protein complexes between cyclic GMP and soluble cellu

lar components contained in a 40,000 x *g* supernatant fraction of uterine homogenates that contained EDTA (5 mM) and the phosphodiesterase inhibitor MIX (1-methyl-3-isobutylxanthine) (2 mM). It was found that each of the cations promoted a significant enhancement (greater than 100%) of [^{3}H]cyclic GMP binding as determined by the retention of complexes on Millipore filters. Under similar conditions [^{3}H]cyclic AMP binding was affected only slightly but in an opposite manner (i.e., diminished 16 to 25%).

In subsequent experiments it was possible to demonstrate the occurrence of cellular constituents in uterine tissue separable by sucrose density gradients that bind cyclic GMP rather selectively with an apparent dependence on Ca^{2+} for the interaction. However, only when the homogenate was exposed to [^{3}H]cyclic GMP and Ca^{2+} before centrifugation could appreciable binding to a low-molecular-weight component be domonstrated. Furthermore, the addition of ethylene glycol tetraacetic acid (EGTA) (5 mM) to the density gradient-separated cyclic GMP binding fraction obtained after centrifugation in the presence of Ca^{2+} and [^{3}H]cyclic GMP did not promote release of the labeled cyclic nucleotide. This suggests that calcium probably does not bind to the same subunit that complexes with the cyclic nucleotide or that, once bound together with cyclic GMP, the calcium is not removed by EGTA. Experiments conducted to determine the distribution of bound $^{45}Ca^{2+}$ under similar conditions, tentatively eliminated the latter possibility. The binding of cyclic GMP to this component was specific in that 100-fold excess concentrations of cyclic AMP and 5'-GMP did not interfere with cyclic GMP binding.

When examining the cation specificity for [^{3}H]cyclic GMP binding it was found that Mn^{2+} enhanced cyclic GMP binding to two components, one of which was very different from the major peak that emerges in the presence of Ca^{2+} and another that migrates in close proximity to the [^{3}H]cyclic GMP binding peak obtained in the presence of Ca^{2+}. Experiments conducted to identify the "calcium dependent" cyclic GMP binding peak indicate that it has a molecular weight of approximately 23,000 and that it exhibits no phosphotransferase or phosphoprotein phosphatase activity. The possibility that it may modify activities of either of the latter enzymes has not been entirely eliminated.

Although the identity of the cyclic GMP binding proteins demonstrable under the conditions described remains to be determined, one interesting characteristic they seem to exhibit is a dependence upon specific cations for interaction with cyclic GMP. These observations are consistent with the concept that cyclic nucleotide participation in the process of biologic regulation may be modulated by cytoplasmic cations and they raise the possibility that a change in the intracellular level of either cyclic nucleotide or of appropriate cation may serve to promote the participation of both as regulatory effectors.

REFERENCES

1. Robison G, Butcher R, Sutherland E: Cyclic AMP. New York, Academic, 1971, p 17
2. Goldberg N, Haddox M, Hartle D, Hadden J: *In* Pharmacology and the Future of Man, Proceedings of the Fifth International Congress on Pharmacology, San Francisco, vol 5. Basal, Karger, 1972, p 146
3. Goldberg N, O'Dea R, Haddox M: *In* Advances in Cyclic Nucleotide Research. Edited by P Greengard, A Robison. New York, Raven, 1973, p 155
4. Goldberg N, Haddox M, Dunham E, Lopez C, Hadden J: *In* The Cold Spring Harbor Symposium on the Regulation of Proliferation in Animal Cells. Edited by B Clarkson, R Baserga. Cold Spring Harbor, NY, Cold Spring Harbor Laboratories, 1973, p 609
5. George W, Polson J, O'Toole A, Goldberg N: Proc Natl Acad Sci USA 66:398, 1970
6. Goldberg N, Haddox M, Nicol S, Acott T, Zeilig C, Glass D: *In* ICN, UCLA Symposia on Molecular and Cellular Biology, vol 2. Edited by CF Fox, D McMahon. Menlo Park, Calif, WA Benjamin Publishing, 1975, p 440
7. Sheppard J, Bannai S: *In* The Cold Spring Harbor Symposium on the Regulation of Proliferation in Animal Cells. Edited by B Clarkson, R Baserga. Cold Spring Harbor, NY, Cold Spring Harbor Laboratories, 1974, p 571
8. Pastan I, Anderson W, Carchman R, Willingham M, Russell R, Johnson G: *In* The Cold Spring Harbor Symposium on the Regulation of Proliferation in Animal Cells. Edited by B Clarkson, R Baserga. Cold Spring Harbor, NY, Cold Spring Harbor Laboratories, 1974, p 563
9. Hadden J, Hadden E, Haddox M, Goldberg N: Proc Natl Acad Sci USA 69:3024, 1972
10. Kuehl F Jr, Ham E, Zanetti M, Sanford C, Nicol S, Goldberg N: Proc Natl Acad Sci USA 71:1866, 1974
11. Notides A, Gorski J: Proc Natl Acad Sci USA 56:230–246, 1966
12. Estensen RD, Hill HR, Quie PG, Hogan N, Goldberg ND: Nature (Lond) 245:458, 1973
13. Hill HR, Estensen RD, Quie PG, Hogan NA, Goldberg ND: Metabolism 24:447, 1975
14. Kaliner M, Orange RP, Austen KF: J Exp Med 136:556, 1972
15. Ignarro LJ: Nature [New Biol] 245:151, 1973
16. Zurier RB, Hoffstein S, Weissman G: J Cell Biol 58:27, 1973
17. Luckasen JR, Sabod A, Hauge N, Goldberg ND, Kersey JH: Fed Proc 33:594, 1974
18. Kram R, Tomkins GM: Proc Natl Acad Sci USA 70:1659, 1973
19. McAfee DA, Greengard P: Science 178:310, 1972
20. Stone TW, Taylor DA, Bloom FE: Science 187:845, 1975
21. Strom TB, Carpenter CB, Garovoy MR, Austen KF, Merrill JP, Kaliner M: J Exp Med 138:381, 1973
22. Krause EG, Halle W, Wollenberger A: Advances in Cyclic Nucleotide 1:301, 1972
23. Haddox MK, Newton NE, Hartle DK, Goldberg ND: Biochem Biophys Res Commun 47:653, 1972
24. Beavo JA, Bechtel PJ, Krebs EG: Proc Natl Acad Sci USA 71:3580, 1974
25. Brostrom CO, Corbin JD, King CA, Krebs EG: Proc Natl Acad Sci USA 68:2444, 1972
26. Schultz G, Hardman JG, Schultz K, Baird EE, Sutherland EW: Proc Natl Acad Sci USA 70:3889, 1973