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# MOLECULAR AND PHYSIOPATHOLOGIC ASPECTS OF MAMMALIAN CYCLIC GMP–DEPENDENT PROTEIN KINASE

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## INTRODUCTION

Following the initial discovery of adenosine 3',5'-monophosphate (cyclic AMP)–dependent protein kinase (hereafter referred to as A-PK) in rabbit skeletal muscle (1), the cyclic AMP target enzyme was subsequently found to occur ubiquitously in mammalian (2) and nonmammalian (2–4) tissues. The molecular properties and biological roles of the enzyme have been extensively investigated; reviews dealing with them have appeared (5–7). Guanosine 3',5'-monophosphate (cyclic GMP)–dependent protein kinase (hereafter referred to as G-PK), activated preferentially by cyclic GMP rather than by cyclic AMP, was later found to be present in every one of many arthropod tissues examined. Its concentrations are generally comparable to those of A-PK also present in the same tissues (3, 4). One interesting tissue is the fat body of the silkmoth pupae and larvae, which was found to contain almost exclusively G-PK (4). The arthropod G-PK has many properties similar to those of A-PK of either mammalian or arthropod origins (3, 4, 8). Some differences, however, do exist between the two classes of protein kinases, such as specificity for substrate proteins (8–11) and differential effects of protein kinase modulators (12).



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Although presence of small amounts of G-PK activity had been shown earlier to occur in certain mammalian tissues (3, 13–16), substantial progress concerning mammalian G-PK had not been made until very recently (17–37). Reasons for this probably are its low tissue concentration, its relative instability, its specific assay conditions required for the maximal enzyme activity, its unique subunit structure, and the mechanism(s) by which its activity is regulated. The present chapter reviews the progress made on the mammalian G-PK during the past three years and speculates on its biological roles and future directions of research.



## MOLECULAR ASPECTS OF CYCLIC GMP–DEPENDENT PROTEIN KINASE

### *General Properties*

DISTRIBUTION Among a number of tissues and cells examined, lung (17, 18, 21), cerebellum (22, 30), femoral artery (38), and lymphocytes from lymph nodes of mice (P. R. Cheney and J. F. Kuo, unpublished) are the few sources found to contain the highest levels of G-PK activity. Its tissue concentration is comparable to or exceeds that of A-PK also present in the same tissues. Most of the tissues, such as heart, liver, cerebrum, vein, aorta, kidney, adipose, intestine, pancreatic islets, bladder, and uterus also contain significant amounts of G-PK, but the enzyme levels are generally only about 5–20% of those of A-PK (17, 18, 25, 31, 32). It seems that there is a positive correlation between the tissue concentrations of G-PK and cyclic GMP, since the lung (39, 40), and cerebellum (40–42) have been reported earlier to contain the highest concentration of cyclic GMP among many tissues examined. Skeletal muscle is probably one of the few tissues that does not contain detectable G-PK activity under the assay conditions (17).

A survey of several rat tissues for the cyclic GMP-binding proteins reveals two activity peaks upon chromatographing tissue extracts on DEAE-cellulose (26). Peak 1 is without G-PK activity, whereas peak 2 contains both the binding and G-PK activities. The size of peak 2 is the largest for the lung and cerebellum, and the smallest for the skeletal muscle (26), confirming the tissue distribution of G-PK reported earlier (17, 18, 21, 22, 30).

As to the subcellular distribution of G-PK, it was found that a great majority of the enzyme activity is in the soluble (13,000 X g supernatant) fraction from a number of tissues (17, 18, 24, 38). Only a small fraction of the total G-PK activity is associated with the particulate (13,000 X g pellets) fraction, with or without being solubilized with 0.3% Triton X-100®.

SPECIFICITY FOR CYCLIC NUCLEOTIDES AND NUCLEOTIDE TRIPHOSPHATES The $K_a$ values for cyclic GMP of G-PK from several guinea pig tissues (such as lung, heart, aorta, whole brain, liver, and ileum) (17), rat epididymal fat pad and pancreatic islets (17), pig lung (21), bovine cerebellum (22), guinea pig fetal lung (23), guinea pig brain (30), fetal calf heart (31), bovine lung (25, 33, 36), and bovine aorta (32) range from 0.03 to 0.12 $\mu$M, which are generally 30 to 100 times lower than the $K_a$ values for cyclic AMP of the enzyme. This is in comparison to the

$K_a$ values of A-PK from the same tissues for cyclic GMP (2 to 150 $\mu$M), which are generally 30 to 200 times higher than those for cyclic AMP (5, 6, 17, 22, 43). The most potent activator of G-PK is 8-bromo cyclic GMP; its $K_a$ of G-PK is about 2 to 5 times lower than that for cyclic GMP (17, 23, 31, 32). Many other cyclic nucleotides and their derivatives are also capable of maximally stimulating (usually 5- to 15-fold) G-PK activity. The order of potency for activating G-PK from guinea pig fetal lung (23) and fetal calf heart (31) is as follows: 8-bromo cyclic GMP > cyclic GMP > 8-benzylamino cyclic AMP > 8-bromo cyclic AMP > 8-bromo cyclic IMP > 8-benzylamino cyclic GMP > 8-methylthio cyclic AMP > $N^2$-monobutyryl cyclic GMP > cyclic AMP > cyclic IMP > 8-benzylamino cyclic IMP > $N^6$-monobutyryl cyclic AMP > 2'-0-monobutyryl cyclic GMP > cyclic UMP > cyclic CMP. 2'-O-Monobutyryl cyclic AMP and $N^6$-2'-O-dibutyryl cyclic AMP are essentially inactive.

As previously shown for A-PK (5, 6, 44), ATP is the only phosphoryl donor for protein phosphorylation catalyzed by G-PK (3, 23). It is interesting that cyclic GMP lowers the $K_m$ for ATP from 0.63 $\mu$M in its absence to 0.21 $\mu$M in its presence in histone phosphorylation catalyzed by G-PK from the fetal lung (23).

### SPECIFICITY FOR SUBSTRATE PROTEINS AND METAL IONS, AND pH PROFILE

Histones are generally much better substrates than casein, phosvitin, protamine, and phosphorylase *b* kinase for G-PK (23, 31). All the proteins are effective substrates for A-PK (1, 5, 6, 45). Of three subfractions (Ib, IIb, and IV) of histone studied, all exhibit a higher affinity toward G-PK than A-PK. The $K_m$ values for histone Ib (lysine-rich), IIb (slightly lysine-rich), and IV (arginine-rich) of the guinea pig fetal lung G-PK are about 100, 150, and 50 $\mu$g/ml, respectively, compared to > 400, > 300, and about 100 $\mu$g/ml for A-PK from the same tissues (23). Similar results are also noted for G-PK from the fetal calf heart (31) and bovine aorta (32). The substrate protein specificity for G-PK and A-PK is also evidenced by comparing the autoradiography of an electrophoretogram of tryspin digests of radioactive mixed histone phosphorylated by the two classes of protein kinases (22). In addition, bovine lung G-PK has been shown (35) to phosphorylate pyruvate kinase, fructose 1,6-diphosphatase, glycogen synthetase, phosphorylase *b* kinase, and synthetic peptides (including "Kemptide"), even though the rate and extent of phosphorylation catalyzed by G-PK are somewhat lower than those by A-PK. Myelin basic protein is also a good substrate for G-PK from several sources (F. Chou and J. F. Kuo, unpublished).

Among many divalent ions examined, $Mg^{2+}$ and $Co^{2+}$ are most effective in stimulating G-PK from a variety of sources, with optimal concentrations of 20–40 and 0.5 mM, equivalent to $K_m$ values of about 5–10 and 0.1 mM, respectively (23, 31, 32). Some investigators reported that the optimal concentration of $Mg^{2+}$ is as high as 100 mM for G-PK from the bovine cerebellum (22). The maximal activity stimulated by $Co^{2+}$ is about 40–80% of that by $Mg^{2+}$ (23, 31, 32). It is interesting that $Co^{2+}$ stimulates lobster G-PK (3) and mammalian A-PK(46) to greater extents than does $Mg^{2+}$. Even though without effect when present alone, $Ca^{2+}$ antagonizes the stimulatory effect of $Mg^{2+}$ (21, 23, 31, 36) and $Co^{2+}$ (21, 31), with a $K_i$ of about

5–10 mM (21, 23, 31). $Mn^{2+}$, $Co^{2+}$, and $Zn^{2+}$ at 5 mM have been shown to almost completely inhibit the activity of bovine lung G-PK stimulated by 10 mM $Mg^{2+}$ (36).

The optimal pH for the activity of G-PK is rather broad, ranging from 6.5 to 8.5 (17, 22, 25, 31, 36). For the purified G-PK, a higher activity is usually obtained in sodium acetate or Tris-Cl buffer than in potassium phosphate buffer (22, 31). A higher G-PK activity in tissue extracts, however, is seen in the phosphate buffer (14, 17).



## *Subunit Structure of G-PK and Mechanisms of Action of Cyclic GMP*

The molecular weight of G-PK from several mammalian tissues has been estimated to be between 120,000 and 165,000, with the sedimentation coefficient ranging from 7.1 to 7.8 S (22, 23, 25, 29, 31, 32, 36). It has been shown that G-PK from guinea pig fetal lung (29), fetal calf heart (31), and bovine aorta (32) can be dissociated to yield what appears to be the catalytic subunit when the holoenzyme is incubated with both cyclic GMP (50–100 $\mu$M) and histone (400 $\mu$g/ml). In order to obtain the free putative catalytic subunit it is essential that both cyclic GMP and histone are also present in the solutions used for separation in the sucrose density centrifugation or Sephadex G-200® gel filtration. Only a slight dissociation of the holoenzyme has been noted in the presence of either cyclic GMP or histone alone. Cyclic AMP, in the presence of histone, is unable to substitute for the same concentration of cyclic GMP in dissociating the holoenzyme. The isolated putative catalytic subunit, having a smaller molecular weight (about 50,000–60,000; 3.8–4.9 S) than the holoenzyme, is neither stimulated by cyclic GMP nor binds cyclic GMP. It is intriguing that the cyclic GMP-binding regulatory subunit has not been detected or recovered from the dissociated G-PK, probably as a result of its extreme instability under the experimental conditions. The phosphotransferase activity of the catalytic subunit of G-PK from bovine aorta and fetal lung is inhibited by the regulatory subunit of bovine heart A-PK, indicating a cross-interaction of the subunits from the two different classes of protein kinases (32). The resulting "hybrid" holoenzyme (6.7 S), as an A-PK, is now stimulated by cyclic AMP rather than by cyclic GMP (32). These observations described above are analogous to earlier reports that lobster muscle G-PK (7.7 S) has been dissociated to yield the catalytic subunit (3.6 S) in the presence of both cyclic GMP and histone (43, 47), and that the isolated catalytic subunit of the lobster G-PK combines with the regulatory subunit of bovine A-PK to yield a cyclic AMP–dependent "hybrid" holoenzyme (47). Interestingly, recovery of the cyclic GMP-binding regulatory subunit from the dissociated lobster G-PK has also been unsuccessful (47), even though cyclic GMP binding is readily demonstrable for the G-PK holoenzyme from the lobster (47) as well as from mammalian sources (22, 23, 25, 26, 29, 31–33, 36).

Many investigators have shown that A-PK consists of regulatory and catalytic subunits and that the mechanism of action of cyclic AMP on the A-PK holoenzyme involves binding of cyclic AMP to the regulatory subunit with subsequent formation of the fully active catalytic subunit (for review see 5, 6). The observations described above for mammalian and lobster G-PK suggest that the subunit structure of G-PK

and the mechanism of action of cyclic GMP are somewhat similar to those of the well-studied A-PK. However, the similarities cannot be firmly established unless the cyclic GMP–binding (regulatory) subunit from G-PK is isolated and its combination with the catalytic subunit of G-PK with restoration of cyclic GMP dependency in the resulting holoenzyme is demonstrated. G-PK is dissociated to yield the catalytic subunit only in the combined presence of histone and an exceedingly high concentration (50 $\mu$M or higher) of cyclic GMP; dissociation of the holoenzyme is not evident in the presence of histone and 0.5 $\mu$M cyclic GMP, a cyclic GMP concentration that maximally activates G-PK. It appears, therefore, that formation of the catalytic subunit of G-PK from mammalian tissues (29, 31, 32), as well as from the lobster muscle (43, 47), may not be a physiologic process. It is suggested that, unlike the cyclic AMP and A-PK system, G-PK may be activated by cyclic GMP without resulting in dissociation of the holoenzyme in vivo (25, 32, 33, 36).

G-PK has recently been purified about 6000-fold from bovine lung extracts to apparent homogeneity by means of either 8-$NH_2$-$(CH_2)_2$NH-cyclic AMP-Sepharose (25, 36) or 8-$NH_2(CH_2)_2$S-cyclic GMP-Sepharose (33) affinity chromatography. G-PK activity bound to the affinity gel is eluted by solutions containing very high concentrations (0.1 or 20mM) of cyclic GMP. The enzyme thus obtained still remains as the holoenzyme form, namely, retaining the original molecular size (6.9–7.8 S; mol wt, 150,000–165,000) and the abilities both to bind cyclic GMP and catalyze histone phosphorylation (25, 33, 36). This is in contrast to earlier observations that A-PK is separated into respective subunits upon chromatographing on the cyclic AMP affinity medium (47-49); the catalytic subunit is unabsorbed while the cyclic AMP–binding (regulatory) subunit is absorbed and can be recovered by eluting with appropriate ligands. The purified bovine lung G-PK displays a single polypeptide of a molecular weight of 74,000 (25, 36) or 81,000 (33) in sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, suggesting that the holenzyme is a dimer composed of an identical subunit (25, 33, 36). Dissociation of G-PK into its monomers by SDS absolutely requires the presence of a reducing agent (dithiothreitol or 2-mercaptoethanol), indicating an involvement of interchain disulfide bridge in the association of the two subunits (monomers) to yield the holoenzyme (36). The bovine lung G-PK binds 2 mol of cyclic GMP per mol of enzyme at equilibrium (33, 36), corresponding to 1 mol of cyclic GMP for each subunit.

A limited proteolysis of bovine lung G-PK also yields a subunit (mol wt 75,000) possessing both the cyclic GMP–binding and catalytic activities (33), which is slightly smaller than the subunit with a molecular weight of 81,000 produced by the SDS treatment (33). This is to compare with an earlier report that limited proteolysis of silkworm G-PK (mol wt 140,000) yields the catalytic fragment (mol wt 34,000) with a full enzymatic activity and the binding fragment (mol wt 36,000) with cyclic GMP–binding activity (50). It is possible, therefore, that dissociation of mammalian G-PK to yield the catalytic subunit by cyclic GMP observed for G-PK from guinea pig fetal lung (23), fetal calf heart (31), and bovine aorta (32) can occur after proteolytic cleavage or modification of the holoenzyme or its monomeric subunit. This seems to enhance the possibility of the impurity of the G-PK preparations from the above three mammalian tissues (23, 31, 32) and the lobster muscle (43, 47), from

which the putative catalytic subunit is derived by incubating the holoenzymes with cyclic GMP and histone. It is suggested (32) that a contaminating protease in these G-PK preparations may be activated by the conditions employed for dissociation, thus yielding the putative catalytic subunit with a molecular weight of about 50,000-60,000 (29, 31, 32).

Earlier evidence indicates that bovine heart A-PK can incorporate phosphate from ATP into its own cyclic AMP–binding (regulatory) subunit (51, 52), and this autophosphorylation results in an enhanced dissociation of the holoenzyme by cyclic AMP (53). A self-phosphorylation by ATP has also been shown recently for bovine lung G-PK; phosphorylation of the enzyme, however, appears to be without effects on its dissociation (34).

Currently available evidence has led some investigators (35, 37) to propose that A-PK and G-PK are homologous proteins evolved from a common ancestral protein. This is based upon their similarities in physical characteristics (molecular size and shape), cyclic nucleotide binding (2 mol per mol of holoenzyme), affinity for ATP and their respective cyclic nucleotides, amino acid composition (70–90% homology), and substrate protein specificity. It is further suggested (37) that G-PK is a dimer composed of two identical protomers in isologous association with the chains arranged in antiparallel fashion, which prevents its dissociation into subunits upon activation of the holoenzyme by cyclic GMP. In contrast, A-PK has a similar structure with a dyad axis of symmetry with a discontinuity in each chain, which permits dissociation of the holoenzyme into subunits upon activation by cyclic AMP.

## *Stimulatory Modulator of G-PK*

A heat-stable protein factor which inhibits A-PK was originally isolated from skeletal muscle (54). The protein inhibitor, shown to exert its effect by interacting with the catalytic subunit of A-PK (55, 56), was subsequently found to occur in many other tissues. During the course of investigating the effects of the crude preparations of the factor (obtained by precipitating the factor with 5% trichloroacetic acid from the heated tissue extracts) from various sources on the then newly isolated G-PK from the lobster tail muscle, it was observed, unexpectedly, that they stimulate G-PK (12). Because of the dual effects (i.e. inhibiting A-PK and stimulating G-PK), it has been proposed that the factor(s) be called *protein kinase modulator* (*s*). The crude modulator preparations obtained from many mammalian tissues, with a possible exception of the skeletal muscle, stimulate also G-PK from mammalian origin; in fact, this phenomenon has helped establish the ubiquitous occurrence of G-PK in mammalian tissues (17, 18) and led to its isolation from some representative tissues (23, 30–32). The modulator from the lobster tail muscle has been purified to homogeneity and found to possess both the inhibitory and stimulatory activities as a single entity (12). There are two questions remaining to be answered: (*a*) whether the purified protein inhibitor of A-PK (54–56) has any effects on G-PK and (*b*) whether the two distinct activities of protein kinase modulator(s) seen in its crude preparations from many tissues reside in the same protein or different proteins.

Recent experiments (27, 28) indicate that crude protein kinase modulator obtained from several mammalian tissues (aorta, heart, lung, skeletal muscle, small intestine, and testis) can be separated into their stimulatory and inhibitory modulator components by means of Sephadex G-100 gel filtration. In contrast, the two modulator activities are eluted as a single peak when the crude modulator from the lobster muscle is chromatographed under the same conditions (27). The isolated stimulatory modulator from the mammalian tissues, eluted in the void volume, augments the activity of G-PK from either mammalian and arthropod origins; it has no effect, however, on the A-PK activity from all sources. The isolated inhibitory modulator, eluted after the void volume and presumably the same as the protein inhibitor reported earlier (54), on the other hand, depresses the activity of A-PK without affecting the G-PK activity.

The above-mentioned studies (27, 28) provide evidence to support the following contentions: 1. The stimulatory and inhibitory modulators are separate proteins, which are present as a mixture in the crude modulator preparations obtained from mammalian tissues. This is in contrast to the lobster muscle protein kinase modulator, which possesses dual modulator activities as a single entity. 2. Stimulatory modulator, which specifically augments the G-PK activity, is different from inhibitory modulator (protein inhibitor) which specifically inhibits the A-PK activity. 3. The stimulatory and inhibitory modulators can occur in any proportion in mammalian tissues, because it has been found that the skeletal muscle contains almost exclusively the inhibitory modulator and that the small intestine contains almost exclusively the stimulatory modulator, whereas most other tissues contain both modulator activities at comparable levels.

Stimulatory modulator, or a crude protein kinase modulator preparation containing the stimulatory component, has been shown to be absolutely required for the G-PK activity purified from any source, including guinea pig fetal lung (23), guinea pig brain (30), fetal calf heart (31), and bovine aorta (32) for histone phosphorylation. The enzyme is not stimulated by any concentration of cyclic GMP or cyclic AMP in the absence of added stimulatory modulator. The requirement of the factor is further demonstrated by the observation that it stimulates the isolated putative catalytic subunit of G-PK (29, 31, 32), as in the case of G-PK in the presence of cyclic GMP. Inhibitory modulator, on the other hand, has no effects. There appears to be no tissue- or species-specificity for the effects of the modulator on the G-PK activity: Stimulatory modulator from the heart stimulates G-PK from other tissues as it does the enzyme from the heart (23, 31, 32). Lack of tissue- or species-specificity has been shown earlier for the action of inhibitory modulator on A-PK (54–56).

It is speculated that the two modulator activities may be artifacts because of the drastic procedures (i.e. boiling and acid precipitation) employed for their initial isolation. This possibility seems to be excluded, however, since the modulators with the same activities can be prepared using mild methods conventionally employed for the preparation of labile enzymes (27, 28). Several recent observations that the modulator activities alter under certain physiologic and pathologic conditions also tend to support the suggestion that the modulators are not only the natural constituents of the cells but also play certain biological roles. It has been reported that inhibitory modulator in the heart decreases during starvation and alloxan treatment,

while its level is restored upon feeding and insulin treatment (57); that the level of inhibitory modulator in the brown fat is the highest perinatally and that it declines 10 days after birth (58); that the levels of both the inhibitory and stimulatory modulator decrease in the fat pad while they increase in pancreas of the alloxan-induced diabetic rats (19); and that the level of stimulatory modulator, relative to that of inhibitory modulator, is higher in hepatoma 3924A than the liver (59).

Some investigators have reported that stimulatory modulator is not required for the G-PK activity (21, 22, 25, 33–37). The discrepancy appears to be due to differences in assay conditions employed rather than differences in the enzyme species studied. Exceedingly high concentrations of $Mg^{2+}$ (50 or 100 mM) and rather high concentrations of ATP (1 to 10 mM) were employed in studies in which the effect of stimulatory modulator on G-PK was not demonstrated. It has been shown (23) that the effects of the modulator are independent of the ATP concentration, since the modulator is essential for the cyclic GMP–stimulated activity of G-PK over a wide range of ATP concentrations. It has been shown most recently that the optimal $Mg^{2+}$ concentration for guinea pig fetal lung G-PK in the absence of stimulatory modulator is about 100 mM, compared to about 20 mM in its presence, with the maximal activity seen for the former condition being about only one fifth of that for the latter. Moreover, at 100 mM $Mg^{2+}$ the G-PK activity is stimulated only about twofold by cyclic GMP and this stimulation is independent of stimulatory modulator. At 10 mM $Mg^{2+}$, on the other hand, the enzyme is stimulated over tenfold by cyclic GMP and this stimulation depends on the presence of the modulator (60). The above experimental data indicate that stimulatory modulator is indeed required for not only the maximal activity of G-PK but also for the maximal stimulation of the enzyme by cyclic GMP when it is assayed under a more physiologic condition of 10 mM $Mg^{2+}$. Histones have been used as substrate proteins for all of the above-mentioned studies. Whether stimulatory modulator is also required for phosphorylation of other substrate proteins by G-PK remains unclear.

Stimulatory modulator was recently purified to homogeneity from the heart (M. Shoji, N. L. Brackett, J. Tse, R. Shapira, and J. F. Kuo, to be published). The preparation appears as a single protein band in the SDS-polyacrylamide gel electrophoresis, with a molecular weight of 34,000. Analytical polyacrylamide gel electrophoresis of the native modulator reveals several bands. A similar electrophoretic pattern is seen for the reassociated modulator obtained by dialyzing the SDS-treated modulator. The apparent molecular weight of the native and the reassociated modulator, the latter being as active as the former, is estimated by gel filtration to be about 180,000. It appears that stimulatory modulator can exist in multiple forms of molecular aggregates consisting of a common subunit, and the dissociation-association of the modulator is a reversible process. The amino acid composition of stimulatory modulator from bovine heart is strikingly similar (about 90% homology) to that of the lobster muscle protein kinase modulator, a factor shown to have dual activities (12), suggesting, as A-PK and G-PK, that they may be homologous proteins evolved from a common ancestral protein.

The mechanism of action of stimulatory modulator in augmenting the G-PK activity is not yet clearly understood. It appears that, unlike inhibitory modulator

that interacts with the catalytic subunit of A-PK, stimulatory modulator does not interact directly with G-PK. Preliminary results indicate that stimulatory modulator interacts with histones or other substrate proteins, thus making them better substrates for G-PK (M. Shoji and J. F. Kuo, unpublished).

In addition to inhibitory modulator of A-PK and stimulatory modulator of G-PK mentioned above, the occurrence of yet another protein factor inhibiting both A-PK and G-PK has been recently reported (61). Since A-PK is the major, and G-PK is the minor, class of protein kinases in most mammalian tissues, a possible regulatory significance of the new modulator in depressing both the cyclic AMP and cyclic GMP target enzymes is beyond comprehension at the present.



## PHYSIOPATHOLOGIC ASPECTS OF G-PK

### *General Consideration*

Current evidence indicates that A-PK and G-PK mediate respectively the actions of cyclic AMP (1–7) and cyclic GMP (3, 4, 17), whose intracellular concentrations are in turn regulated by appropriate physiologic and pharmacologic agents. The effects of cyclic AMP and cylic GMP have been shown to be mutually opposing (Yin-Yang) in many instances where the cellular functions are regulated bidirectionally (62). The evidence for the roles of A-PK is quite compelling (5–7). The functional roles of G-PK based upon comparisons with the A-PK system, however, are largely speculative. Intracellular concentrations of cyclic AMP and cylic GMP, by virtue of their nature as second messengers, could fluctuate widely. This would post some difficulties in obtaining their "true" values that reflect some specific physiologic or pathologic conditions under studies. It is conceivable, therefore, that A-PK and G-PK, the more stable cellular constituents and the receptor enzymes for the more transient cyclic nucleotides, could serve as additional or alternate parameters for characterizing certain physiopathologic conditions. Modifications in their absolute and relative tissue levels would be most likely to occur if these conditions are chronic in nature. Recent experimental evidence shown below indeed indicate that this is the case. Cellular sites of action of G-PK are still largely unknown. An earlier demonstration (63) of the cyclic GMP-stimulated phosphorylation of membrane proteins of the mammalian smooth muscle suggests that endogenous substrate proteins for G-PK are not limited to the cytoplasm.

### *G-PK in Some Physiologic and Pathologic Conditions*

ONTOGENY Changes in levels of G-PK, compared to A-PK, from some tissues of developing guinea pigs have been studied (18, 64). The G-PK level in the fetal lung, the tissue shown to contain the highest G-PK level in all mammalian tissues examined, declines progressively during development. The lung A-PK level, on the other hand, is the lowest in the fetus, the highest in the adult, with an intermediate value seen in the neonate. Thus the G-PK to A-PK ratio in the lung, which is the highest in the fetus, declines as the animals reach maturity. A qualitatively similar change in the G-PK to A-PK ratio is also seen for the heart of developing guinea pigs. In contrast, an increase in the ratio is noted for the developing brain. Unlike

in the lung, heart, and brain, no change in the levels and ratios of protein kinases have been noted in the liver during development. These results provide evidence for the first time to suggest that a balance between the effects of G-PK and A-PK, hence the actions of cyclic GMP and cyclic AMP, may be crucial in normal development of certain tissues.

It should be pointed out that the lung is a heterogeneous tissue consisting of many cell types. Increases in the type and number of cells occur during early stages of development. The changes in the levels of G-PK and A-PK and their ratio observed, therefore, may not necessarily reflect the development-related changes in a given cell type.



COMPARISON OF ARTERY AND VEIN AND EFFECTS OF ARTERIOVENOUS FISTULA AND ARTERIAL OCCLUSION Earlier studies suggest that cyclic AMP and cyclic GMP may mediate the actions of vasodilators and vasoconstrictors, respectively (65). In view of distinct differences in the hemodynamic properties of the artery and vein, possible involvements of protein kinases in vascular functions have been explored (38). Both G-PK and A-PK, localized in the smooth muscle–rich inner layer of the vascular tissues, have been shown to be invariably higher in the arteries than in the veins of the dog. The peripheral (adrenal, femoral, renal, saphenous, carotid, hepatic, and radial) arteries are disproportionately more abundant in G-PK (as indicated by higher G-PK to A-PK ratios) than the corresponding veins, with an exception of the pulmonary artery, an atypical arterial tissue exposed to low blood pressure. Interestingly, the protein kinase ratio for the aorta, an elastic type of arterial tissue without a significant role in blood pressure regulation, is not any higher than the vena cava and any other peripheral veins.

Creations of side-by-side femoral arteriovenous fistulae lead to a preferential reduction in the G-PK level in the artery both proximal and distal to the anastomotic site, probably secondary to a minute reduction in blood pressure as a result of part of its blood being shunted away to the adjacent vein. The G-PK level increases greatly in the stressed (arteriolarized) vein distal to the anastomotic site, but its level proximal to the site remains unchanged. The G-PK level is also preferentially reduced in the saphenous artery distal to ligation. Changes in G-PK levels appear to precede gross atrophy or hypertrophy and hyperplasia of the vascular tissues. In all cases, changes in A-PK level are relatively small compared to those in the G-PK level. It appears, therefore, that vascular G-PK, perhaps not A-PK, may be closely related to peripheral resistance and its regulation.

CARDIAC HYPERTROPHY It has been shown that the cardiac level of G-PK in spontaneously hypertensive (SH/N) rats is lower than that in normotensive (WKY/N) rats, whereas that of A-PK is the same in both groups (24). The levels of G-PK and A-PK in the lung, which is used as a control tissue, are the same in both groups of rats, indicating that depression of cardiac G-PK is specifically associated with cardiac hypertrophy secondary to hypertension.

Several lines of evidence suggest that cyclic AMP and cyclic GMP may mediate the cardiac stimulatory and inhibitory effects of catecholamines and acetylcholine,

respectively [for example, see ref. (62)]. In a state of sustained elevated blood pressure, the heart adapts to work more efficiently. Physiologically, this manifests itself as a compensatory hypertrophy. Biochemically, a decreased ratio of the effects mediated by cyclic GMP to those mediated by cyclic AMP may be important. This can be accomplished, in part, by either depressing the G-PK levels, or increasing the A-PK levels, or both. The finding of a specific depression of G-PK in cardiac hypertrophy seems to be of some physiological significance, since A-PK is present at much higher levels than G-PK in the heart, and consequently it would require a great deal of the newly synthesized A-PK to lower the enzyme ratio significantly.

HEPATOMA When compared with the normal or the host liver, modifications in the cyclic nucleotide systems favoring the expression of cyclic GMP effects have been reported to occur in the transplanted fast-growing Morris hepatoma 3924A (59). These include disproportionately elevated activities of G-PK and its stimulatory modulator, relative to the activities of A-PK and its inhibitory modulator. This is in line with an earlier finding showing that the tissue concentration of cyclic GMP in the hepatoma is exceedingly high (66). Taken collectively, hepatoma 3924A, therefore, may be characterized as a neoplastic tissue in which cyclic GMP effects become predominant.

## CONCLUSION AND FUTURE DIRECTIONS OF RESEARCH

The occurrence of G-PK in mammalian tissues has now been firmly established, owing to progress made in the last three years. Even though G-PK and A-PK appear to be homologous proteins having many characteristics in common, a few differences do exist between them, the most notable ones being the mechanisms of activation of the enzymes by the cyclic nucleotides, and the effects of stimulatory and inhibitory modulators on their activity.

In the presence of histone, a high concentration (50 $\mu$M) of cyclic GMP causes dissociation of the G-PK holoenzyme to yield the putative catalytic subunit; its activity is augmented by stimulatory modulator and is independent of cyclic GMP, and it combines with the regulatory subunit of A-PK to form a cyclic AMP–dependent "hybrid" holoenzyme. The G-PK holoenzyme also yields either monomeric subunits by SDS, or fragments (possessing both cyclic GMP–binding and catalytic activities) by limited proteolysis. It seems, however, that the in vivo action of cyclic GMP in activating G-PK may not be involved in dissociation of the holoenzyme. As to the discrepancy concerning the requirement of stimulatory modulator for G-PK, it appears to arise largely from the differences in the assay conditions employed by the investigators. If G-PK is assayed in a more physiologic concentration of 10 mM $Mg^{2+}$ (rather than 100 mM), the factor is indeed essential for a maximal enzyme activity as well as a maximal stimulation of the enzyme by cyclic GMP.

Future directions of research would be to further refine the molecular structure of G-PK and the exact mechanism by which the enzyme is activated by cyclic GMP. Elucidations of physiopathologic roles for G-PK would represent a new frontier of

scientific endeavor. This would probably be best carried out by seeking and identifying the modifications in the concentrations, localizations, and properties of G-PK and its endogenous substrate proteins under the various conditions studied. Similar information should also be obtained for the A-PK system, so that the regulatory roles of the cyclic nucleotides can be suggested at the new level of protein kinases.

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