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Effect of Insulin and Growth Hormone on Rat Liver Cyclic Nucleotide Phosphodiesterase†

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ABSTRACT: Rat liver cyclic adenosine monophosphate phosphodiesterase activities, which have apparent K_m values of 6.3×10^{-7} and 7.3×10^{-7} M cyclic adenosine monophosphate, displayed negatively cooperative kinetic behavior, and appeared bound to membrane particles of differing sedimentation rates, were differentiated from cyclic guanosine monophosphate phosphodiesterase activity and lower affinity cyclic adenosine monophosphate phosphodiesterase activity by zonal centrifugation with discontinuous sucrose gradients. Thirty minutes after injection of insulin (3 units/100 g) into streptozotocinized diabetic rats, or of bovine growth hormone (1 mg/100 g) into hypophysectomized rats, the separated membrane-bound high affinity cyclic adenosine monophosphate phosphodiesterases were activated, but there was no effect on the cytosol phosphodiesterases. The activated cyclic adenosine monophosphate phosphodiesterases retained negatively cooperative kinetic behavior and showed no change in the apparent K_m values, but had increased apparent maximum velocities. Neither insulin nor growth hormone had any effect on phosphodiesterase activity *in vitro* under the conditions of these measurements. The results of these investigations support the hypothesis that membrane-bound, high affinity cyclic adenosine monophosphate phosphodiesterase may be involved in the mechanisms of insulin and growth hormone actions.

Recognition of multiple cyclic nucleotide phosphodiesterases and their associated kinetic complexities and substrate specificities (Thompson and Appleman, 1971a,b; Brooker *et al.*, 1968; Kakiuchi *et al.*, 1971; Beavo *et al.*, 1970) necessitates evaluation of hormone interaction with phosphodiesterase activity of enzyme preparations other than that of homogenate or differentially centrifuged enzyme preparations. The high affinity form of cyclic adenosine monophosphate (cyclic AMP[1]) phosphodiesterase activity in several tissues appears to have a subcellular distribution and unique regulatory properties which enable this enzyme to react to changes of substrate level (Thompson and Appleman, 1971b; Russell *et al.*, 1972b). Relatively little is known about hormonal regulation of this form of phosphodiesterase.

Recently, the multiplicity of rat liver cyclic nucleotide phosphodiesterases has been established by enzyme separation techniques and kinetic characterization (Russell *et al.*, 1972a). Previous attempts to characterize rat liver enzyme revealed that liver phosphodiesterase differs in its properties from that of other rat tissues (Beavo *et al.*, 1970; Thompson and Appleman, 1971b; Hemington *et al.*, 1971; Menahan *et al.*, 1969). However, there appears to be a low K_m, negatively cooperative, cyclic AMP phosphodiesterase in liver which may also be a membrane-bound system (Russell *et al.*, 1972a; Hemington *et al.*, 1971).

Insulin stimulation of phosphodiesterase activity has been suggested as a mechanism to account for decreased cyclic

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[1] Abbreviations used are: cyclic AMP, cyclic 3′,5′-adenosine monophosphate; cyclic GMP, cyclic 3′,5;-guanosine monophosphate; iv, intravenous; ip, intraperitoneal.

AMP levels caused by this hormone in adipose tissue and liver (Butcher *et al.*, 1966, 1968; Park *et al.*, 1972; Exton *et al.*, 1971). Experiments designed to test this hypothesis have been difficult to interpret and controversial (Menahan *et al.*, 1969; Loten and Sneyd, 1970; House *et al.*, 1972; Fain and Rosenberg, 1972; Robison *et al.*, 1971). Insulin stimulation of low K_m cyclic AMP phosphodiesterase activity in intact rat adipocytes has been reported (Loten and Sneyd, 1970). Also, insulin has been found to stimulate the cyclic AMP phosphodiesterase activity of relatively pure membrane preparations from rat liver *in vitro* (House *et al.*, 1972). In preliminary unpublished experiments we found that high doses of insulin given to streptozotocinized diabetic rats stimulate cyclic AMP phosphodiesterase activity in rat liver homogenates when measured at substrate concentrations below the low K_m of this enzyme and that bovine growth hormone administration caused stimulation of cyclic AMP phosphodiesterase activity in liver homogenates of hypophysectomized rats which provided the stimulus for the studies reported here. It has been suggested that some of the actions of growth hormone on lipolysis in adipose tissue may be related to phosphodiesterase activity or synthesis (Goodman, 1968).

This article describes studies on rat liver cyclic nucleotide phosphodiesterases and the changes in their activity with excess or deficient insulin and growth hormone. A method for separating enzyme forms by using discontinuous sucrose gradients and a kinetic analysis made of the separated cyclic nucleotide phosphodiesterase activities, using enzyme preparations from rat liver before and after hormone treatment, are described.

Methods

Materials. 8-^{3}H-Labeled cyclic 3′,5′-adenosine monophosphate (specific activity 28.0 Ci/mmol) (Schwarz-Mann) and (G)-^{3}H-labeled cyclic 3′,5′-guanosine monophosphate (specific activity 3.96 Ci/mmol) (New England Nuclear) were purchased and purified using Sephadex A-25 ion-exchange chromatography (Thompson *et al.*, 1973). Cyclic AMP free acid and cyclic GMP sodium salt (Sigma), growth hormone (NIH-GH-B16, bovine), insulin (Lilly, semilente (Iletin) or crystalline peak IV) (Stoll *et al.*, 1969), enzyme grade sucrose (Mann), streptozotocin (U-9889, 4621-HKJ-81A, The Upjohn Co.), and snake (*Ophiophagus hannah*) venom were obtained and not further purified. Dowex 1-X8 (200–400 mesh) (Bio-Rad Laboratories) was prepared by extensive washing, as previously described (Thompson and Appleman, 1971a).

Experimental Procedure. All rats used in this study were male Wistar, weighed 85–100 g, and received Purina Lab Chow except when supplemented as noted. Food was withdrawn from all groups 2 hr before sacrifice. There were nine experimental groups: (1) normal controls, $N = 11$; (2 and 3) normal controls injected with insulin (3 units, ip), $N = 5$, or growth hormone (1 mg, ip), $N = 1$, 30 min before sacrifice; (4) hypophysectomized rats (surgery performed by Simonsen Labs) which had 5% glucose and saline in their drinking water for 4 days before sacrifice; the testes of the hypophysectomized group were atrophic, $N = 5$; (5 and 6) hypophysectomized groups treated as group 4, injected with growth hormone (1 mg, ip) 30 min before sacrifice, $N = 4$, or injected with 40 mg of glucose in the tail vein 20 min before sacrifice, $N = 1$; (7) rats made diabetic with streptozotocin (120 mg/kg, iv) and sacrificed 5–7 day later, $N = 5$; all diabetic rats were given free access to sucrose as well as the normal lab chow until 2 hr before sacrifice to minimize hypoglycemia due to insulin injection; and (8 and 9) diabetic rats injected with insulin (3 units, semilente (Iletin), ip), $N = 3$, or growth hormone (1 mg, ip), 30 min before sacrifice, $N = 1$. At sacrifice, the mean serum glucose values were 135 mg % in the normal group, 600 mg % in the untreated or growth hormone treated diabetic group, 380 mg % in the diabetic group given insulin, and 60 mg % in normals injected with insulin.

Phosphodiesterase Assay. Cyclic nucleotide phosphodiesterase activity was measured according to Thompson and Appleman (1971a) with the following modifications: the volume of the final assay mixture was 0.2 ml and contained 5 mM $MgCl_2$, 20 mM Tris-Cl (pH 8.0), 1.25×10^{-7} M to 1×10^{-4} M cyclic AMP (220,000 cpm) or 2.5×10^{-7} M to 2.0×10^{-5} M cyclic GMP (140,000 cpm), and 0.1 ml of enzyme preparation (4°). Reactions were initiated by enzyme addition and incubated at 30° for 10 min. Enzyme reactions were terminated by immersion of the reaction vessels (12 × 75 mm culture tubes) in a Dry Ice–acetone bath until frozen (10 sec), followed by immersion in a boiling H_2O bath for 45 sec. Snake venom nucleotidase action, resin precipitation of unreacted nucleotides, and measurement of labeled nucleoside products were as previously described (Thompson and Appleman, 1971a). Enzyme assays were performed using enzyme concentrations to ensure linearity with 10 min of incubation. Activities are expressed as picomoles of cyclic AMP or cyclic GMP hydrolyzed per minute per 0.2 ml.

Sucrose Gradient Analysis. Rats were sacrificed by a sharp blow to the head and decapitation. Livers were perfused with 6 ml of 0.32 M sucrose (4°), and 200 ± 1 mg of the liver was immediately homogenized in 5 ml of 0.32 M sucrose (4°, pH 6.8), using three strokes of a Teflon pestle at 2600 rpm in a Potter-Elvehejm glass homogenizer.
Homogenates were then diluted with 0.32 M sucrose to contain 20 mg/ml of liver (wet weight).

Discontinuous sucrose gradients were prepared in 13.4-ml cellulose nitrate tubes (iced) as three layers, using 4 ml of 1.2 M sucrose (density 1.187 g/cm^3 at 5°), 4 ml of 0.8 M sucrose (density 1.117 g/cm^3 at 5°), and 4 ml of homogenate in 0.32 M sucrose (density 1.044 g/cm^3 at 5°). Centrifugation was by a SW-36 rotor at 29,000 rpm (91,800*g* at R_{av}) for 90 min with a Beckman (Model L) ultracentrifuge. Gradient fractions were obtained at 4° after inserting a 23-gage needle just above the pellet and collecting 0.38-ml fractions dropwise through no. 50 polyethylene tubing (25 drops/fraction).

Results

Cyclic nucleotide phosphodiesterase activities of rat liver homogenates, separated by zonal centrifugation with discontinuous sucrose gradients, appeared in three zones of activity (Figure 1). Using 0.125 μM cyclic AMP as substrate, phosphodiesterase activity was present in all three regions. Using 10 μM cyclic GMP as substrate, phosphodiesterase activity was almost entirely in the 0.32 M sucrose layer. Adenylate cyclase activity was contained by gradient fractions at the 1.2–0.8 M interface, and guanylate cyclase activity occurred exclusively in the 0.32 M sucrose layer.

Other centrifugation times (3–24 hr), discontinuous gradient concentrations (six layers, 0.32–1.2 M), and homogenate protein concentrations (10–40 mg of protein) were used, but these variations showed similar enzyme distribution patterns, although migration distances varied. The method is the

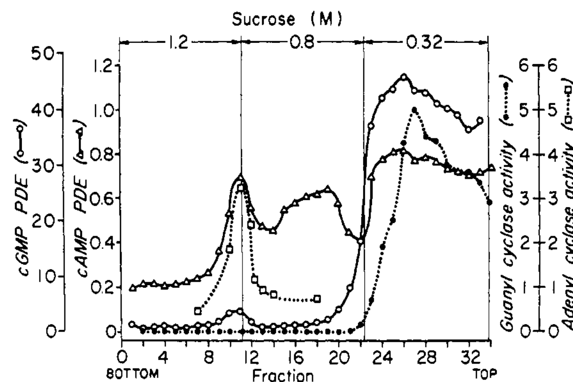

FIGURE 1: Separation of cyclic nucleotide synthetic and hydrolytic enzymes by zonal centrifugation. Discontinuous sucrose gradients were prepared, centrifuged, and fractionated as described under Methods. Adenylate and guanylate cyclase activities were determined (Thompson *et al.*, 1973) in separate experiments from cyclic AMP and cyclic GMP phosphodiesterase measurements. One-tenth milliliter of each gradient fraction (0.38 ml) was used for all enzyme assays. Adenylate cyclase and guanylate cyclase activities were determined, using 50 μM ATP and 10 μM GTP, respectively. Activities are expressed as picomoles of cyclic AMP or cyclic GMP formed after 10 min of assay. Cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase activities were determined, using 0.125 μM cyclic AMP and 10 μM cyclic GMP, respectively, and activities are expressed as picomoles of cyclic AMP or cyclic GMP hydrolyzed per minute per 0.2 ml.

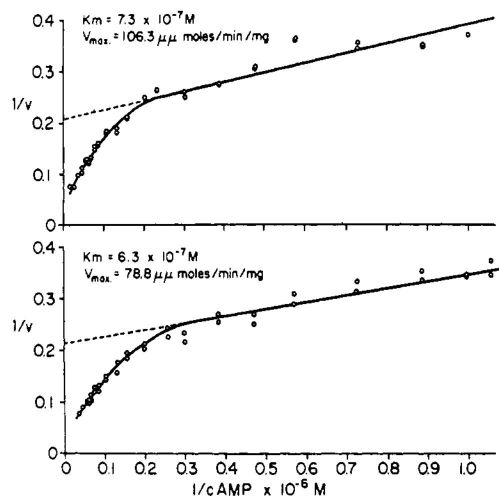

FIGURE 2: Sucrose gradient fractions 10–13 (top) and 16–20 (bottom) were pooled and assayed for cyclic AMP phosphodiesterase activity, using cyclic AMP substrate concentrations from 0.94 to 100 μM. Assays contained 60 μg (top) and 46 μg (bottom) of protein (Lowry *et al.*, 1951) and were incubated for 10 min at 30°. Data are plotted according to Lineweaver–Burk (1934), and constants were determined using slopes and intercepts from the linear portions of each curve obtained by linear least-squares analysis with correlation coefficient criteria of 0.998.

simplest, least time-consuming, and most reproducible reported for separating total cyclic nucleotide phosphodiesterase activities on the basis of substrate specificities and kinetic parameters.

The sum of the activities of all the gradient fractions, excluding the pellet, indicates a total recovery from homogenate of 38% of the cyclic AMP phosphodiesterase and 52% of the cyclic GMP phosphodiesterase. Resuspension of the pellets from these fractionations indicated 3.4% of the cyclic AMP phosphodiesterase and 1.5% of cyclic GMP phosphodiesterase activities were lost in these fractions, representing 52% of the protein in the original homogenate. Guanylate cyclase recovery was 95% and adenylate cyclase recovery was not calculated. Recovery varied by no more than 3% throughout this study but recovery calculations should be viewed with reservations because of the kinetic complexities of measuring homogenate phosphodiesterase activities (Thompson and Appleman, 1971a).

Comparison of rat liver phosphodiesterase fractionation with rat kidney and rat adipocyte phosphodiesterase fractionation by this method showed similar results, except that these two tissues lack the activity peak in the 0.8 M sucrose layer. The cyclic AMP phosphodiesterase activities of these tissues are located at the 1.2–0.8 M interface and in the 0.32 M sucrose layer. Cyclic GMP phosphodiesterase activity is almost completely contained in the 0.32 M sucrose layer.

Lineweaver–Burk (1934) analysis of cyclic AMP phosphodiesterase activity, located at the 1.2–0.8 M sucrose layer interface (Figure 2, bottom), and that of the 0.8 M sucrose layer (Figure 2, top) indicates that there is low K_m cyclic AMP phosphodiesterase activity in both regions and that both enzymes appear negatively cooperative. The apparent K_m values, derived from extrapolation of the linear portion of these Lineweaver–Burk plots, are 6.3×10^{-7} and 7.3×10^{-7} M cyclic AMP, respectively. Cyclic AMP phosphodiesterase activity of the 0.32 M sucrose layer shows biphasic Lineweaver–Burk plots (Figure 3). The extrapolated apparent K_m values are 6.7×10^{-6} and 1.3×10^{-4} M cyclic AMP. Cyclic GMP phosphodiesterase activity of the 0.32 M sucrose layer does not show linear Lineweaver–Burk plots, but appears slightly hyperbolic (data not shown). It is difficult to determine in these impure enzyme preparations if cyclic GMP is actually influencing its own hydrolysis, as previously reported for separated cyclic GMP phosphodiesterase activity, or if this kinetic behavior is a function of enzyme or effector contamination (Russell *et al.*, 1972b).

The profile of rat liver cyclic AMP phosphodiesterase activity in hypophysectomized rats appears identical with that of normal untreated rats (data not shown) as was total recovery of activity. Hypophysectomized rats given bovine growth hormone displayed greater activity of both low K_m cyclic AMP phosphodiesterases after 30 min than did the untreated hypophysectomized rats (Figure 4), although total activity recovery was identical. Figure 4 is representative of one of four almost identical experiments using livers from different

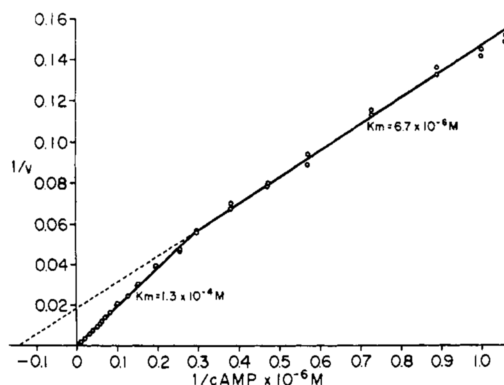

FIGURE 3: Sucrose gradient fractions 24–28 were pooled and assayed for cyclic AMP phosphodiesterase, as in Figure 2, 120 μg of protein to each assay.

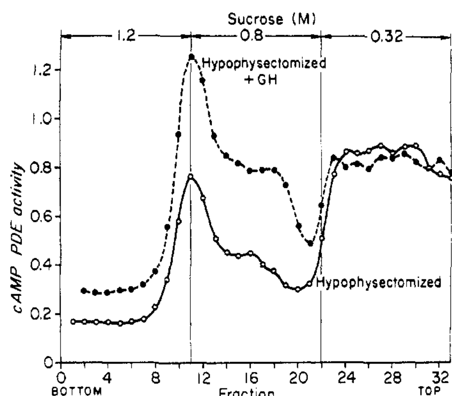

FIGURE 4: Discontinuous sucrose gradient fractionations of rat liver cyclic AMP phosphodiesterase of hypophysectomized and growth hormone injected hypophysectomized rats are compared as described. Cyclic AMP phosphodiesterase activities were measured, using 0.125 μM cyclic AMP.

rats in each case. The statistical significance using a paired observation student's distribution test for both peaks is $P < 0.02$ and for the unpaired observation student's distribution test is $P < 0.001$. Apparently, soluble cyclic AMP phosphodiesterase was unaffected and cyclic GMP phosphodiesterase activity was slightly stimulated (Figure 5).

In normal rats injected with growth hormone no effect on any phosphodiesterase fraction was seen. Growth hormone *in vitro* (10^{-12}–10^{-4} M) had no effect on fractionated or homogenate liver phosphodiesterase activity. Glucose administered to hypophysectomized rats had no effect on any of the separated enzyme activities.

Lineweaver–Burk (1934) analyses of the two particulate low K_m cyclic AMP phosphodiesterase activities stimulated by growth hormone administration indicate that the K_m values obtained by extrapolation of the linear portion of each curve for the activated enzymes was identical with those of normal rats (6.8×10^{-7} M *vs.* 6.3×10^{-7} M cyclic AMP and 7.5×10^{-7} M *vs.* 7.3×10^{-7} M cyclic AMP). The maximum velocities of the extrapolated linear portion of the curves of both stimulated activities were increased by 11 and 15% (106 and 78 to 122 and 88 pmol per min per mg of protein), respectively. Since both stimulated enzyme activities maintain their negatively cooperative appearance, computer analysis was initiated to establish the intrinsic association constants and interaction parameters necessary to interpret the kinetics of these complex enzyme species (Russell *et al.*, 1972b).

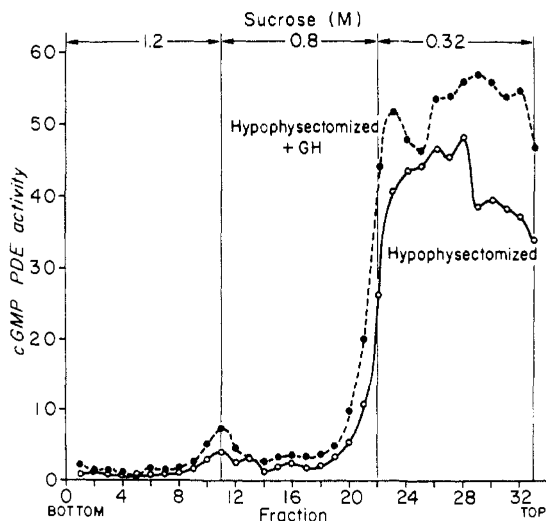

FIGURE 5: Comparison of discontinuous sucrose gradient fractionation of rat liver cyclic GMP phosphodiesterase of hypophysectomized and growth hormone injected hypophysectomized rats. Procedures are as in Figure 4; cyclic GMP phosphodiesterase activity is measured using 10 μM cyclic GMP.

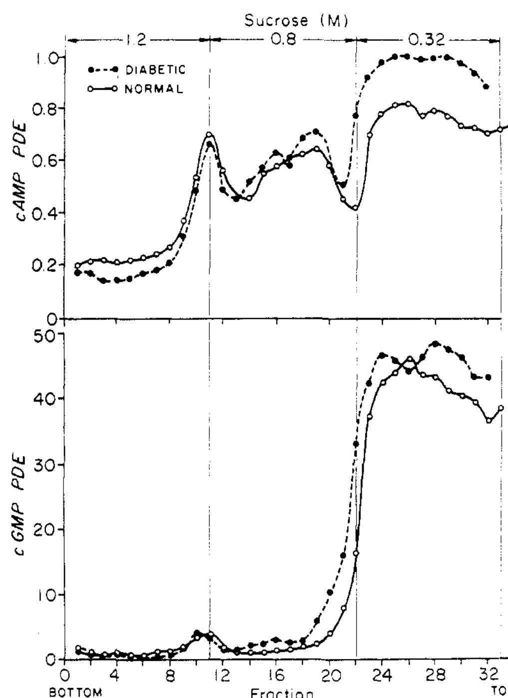

FIGURE 6: Discontinuous sucrose gradient fractionation of rat liver cyclic nucleotide phosphodiesterase activities of normal and streptozotocinized diabetic rats compared as in Figure 4 (top) and Figure 5 (bottom).

In contrast to comparison of liver phosphodiesterase activities of the hypophysectomized rats and control rats, the sucrose gradient fractionated enzyme activities of streptozotocinized diabetic rats showed some differences from those of normal rats (Figure 6). Cyclic AMP phosphodiesterase activity of the 0.32 M sucrose layer was slightly elevated ($P < 0.001$), while that of the particulate fractions was unchanged (Figure 6, top). The cyclic GMP phosphodiesterase activity of the 0.32 M sucrose layer was, however, not appreciably different from the normal (Figure 6, bottom).

The results of treating streptozotocinized diabetic rats with insulin showed stimulation of both the particulate low K_m cyclic AMP phosphodiesterase activities after 30 min ($P < 0.02$), while the activity of the 0.32 M sucrose layer is unaffected (Figure 7, top). Cyclic GMP phosphodiesterase activity was not influenced by identical insulin treatment (Figure 7, bottom). Growth hormone after administration to diabetic rats had no effect on any phosphodiesterase activities. Recovery of activity in all cases was as given for normal rats.

Insulin administration to normal rats generally showed stimulation after 30 min of the particulate cyclic AMP phosphodiesterases as in diabetic rats, but these effects were less pronounced and more variable than was the consistent stimulation seen in diabetic rats and no statistical significance of these differences could be established. Insulin administration had no effect on cyclic GMP phosphodiesterase activity. Insulin (85 ng/ml; crystalline peak IV) (Stoll *et al.*, 1969),

like growth hormone, had no effect *in vitro* on any of the phosphodiesterase activity fractions.

Discussion

Evaluation of the role of cyclic nucleotide phosphodiesterases in hormone action is complicated by multiple substrate specificities and multiple separable forms of this enzyme and associated cooperative kinetic behavior. Interpretations of hormonal effects on cyclic nucleotide phosphodiesterase are hampered by the absence of techniques to separate enzyme forms for comparative purposes, and the lack of enzyme activating mechanisms *in vitro*.

Zonal centrifugation using discontinuous sucrose gradients allows comparative experiments in which changes in the activities of the various forms, relatively free of the kinetic complexities associated with homogenate or differentially centrifuged enzyme preparations, can be observed. Zonal centrifugation, even on the small scale shown here, has quantitative analytical advantages over electrophoretic separation (Monn and Christiansen, 1971; Uzunov and Weiss, 1972) and has both preparative and comparative advantages over gel filtration (Thompson and Appleman, 1971a,b; Kakiuchi *et al.*, 1971).

This technique indicates that liver has two fractions of low K_m, membrane-associated cyclic AMP phosphodiesterase, a cyclic AMP phosphodiesterase fraction of multiple binding constants, and cyclic GMP phosphodiesterase activity, the latter two apparently being associated with the lightest density fractions, probably cell cytoplasm. Since the kinetic parameters of both the higher density fractions and their stimulation by insulin and growth hormone administration are similar, both low K_m phosphodiesterase activities may have been originally the same system. Procedural alterations such as homogenizing medium variants and physical manipulations are needed to test these interpretations. Fractionation of rat kidney and of rat fat cell homogenates by identical procedures indicates similar phosphodiesterase distributions, except that the second low K_m cyclic AMP phosphodiesterase peak seen in liver homogenates is undetectable. It is possible that this peak is peculiar to rat liver or a function of multiple hepatic cell types, as liver phosphodiesterase differs characteristically from that of other tissues (Beavo *et al.*, 1970; Thompson and Appleman, 1971b; Russell *et al.*, 1972a; Hemington *et al.*, 1971; Menahan *et al.*, 1969). Although quite unlikely, nonspecific adsorption of originally "soluble" cyclic AMP phosphodiesterase to membrane surfaces cannot be completely negated by these studies.

Adenylate cyclase activity is found in the same fractions that contain low K_m cyclic AMP phosphodiesterase activity. A similar subcellular distribution of these fractions is inferred and may be important in the regulation of the subcellular levels of cyclic AMP, particularly in initiation of hormone action. Guanylate cyclase, on the other hand, appears to be entirely in the light-density fractions along with high K_m cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase activity, indicating that these enzymes may be cytoplasmic in origin, as previously thought (Beavo *et al.*, 1970; Thompson and Appleman, 1971b,c; Menahan *et al.*, 1969).

Since high K_m cyclic AMP phosphodiesterase activity is detectable using cyclic AMP substrate concentrations below the apparent affinity of the low K_m enzyme, interpretive problems arise with data obtained from unfractionated enzyme preparations, particularly with liver tissue, where the ratio of high K_m enzyme to low K_m enzyme is high. Enzyme separations are therefore necessary to confirm hypotheses based on homogenate activities using substrate concentrations below the low K_m enzyme. We found that insulin and growth hormone effects can be demonstrated in rat liver homogenate preparations, but they are small and many determinations needed to be made to provide data which establish even the statistical significance (unpublished observations).

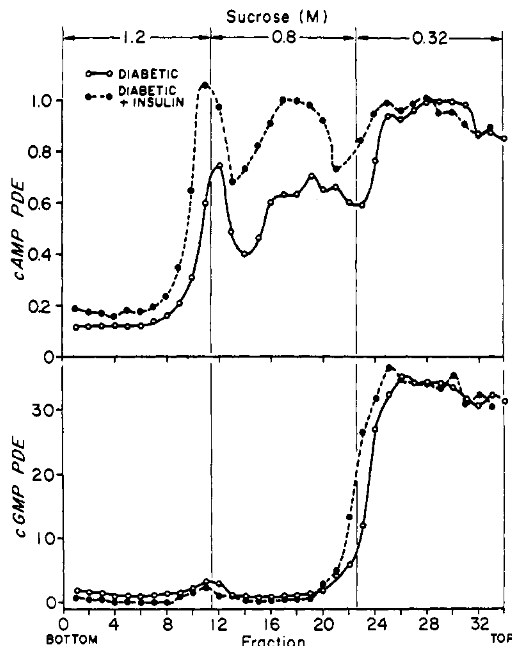

FIGURE 7: Rat liver cyclic nucleotide phosphodiesterase activities of streptozotocinized diabetic rats are compared with those of insulin-injected streptozotocinized diabetic rats. Procedures are as in Figure 6.

Thirty minutes after administration, insulin and growth hormone consistently caused activation of low K_m, membrane-bound cyclic AMP phosphodiesterase activity of rat liver in diabetic and hypophysectomized rats, respectively. Cyclic GMP phosphodiesterase activity was unaffected. Since growth hormone has no effect in the diabetic rat, and an iv glucose pulse causes no stimulation in hypophysectomized rats, neither effect appears to be a function of the release of the other hormone. The experimental design and the use of large doses of each hormone, however, preclude the conclusion that enzyme activations are a function of direct hormone action.

Insulin activation differs from that of growth hormone, since insulin affects low K_m phosphodiesterase activity of normal rats but growth hormone has no effect in normal rats. Insulin stimulation in normal rats is, however, not consistent and complicated by associated hypoglycemia. We, unlike House (House *et al.*, 1972), were unable to demonstrate any effect of either hormone on any liver phosphodiesterase activity *in vitro* under the conditions of these measurements.

Lineweaver–Burk analysis of growth hormone-caused stimulation of the higher density enzyme fractions indicated no change in the apparent K_m values for cyclic AMP, but an increase in the apparent maximum velocity of the linear portion of each curve. These data confirm the observations of Loten and Sneyd (1970), who found that insulin treatment of intact fat cells activated apparent low K_m cyclic AMP phosphodiesterase activity. We concur with these authors that increased enzyme synthesis seems unlikely.

We emphasize that the kinetic constants obtained from this study are derived from computer-analyzed, graphically expressed data and are extrapolated values. Intrinsic association constants and interaction coefficients derived from extensive computer analysis of the entire curves based on mathematical models of negative cooperative enzymes (Russell *et al.*, 1972b) indicate that the growth hormone activated phosphodiesterase has a threefold increased maximum velocity of the higher K_m catalytic site.

Separated cyclic nucleotide phosphodiesterase activity of liver from normal and hypophysectomized rats appears identical but activities of diabetic rats differ from the normal by having slightly elevated cyclic AMP phosphodiesterase activity in the low-density gradient fractions. No change in the cyclic GMP phosphodiesterase activity is found. Insulin treatment did not affect the activity of either of these apparently soluble enzymes, although the low K_m membrane-bound cyclic AMP phosphodiesterase activities were clearly increased. These findings may indicate that not only are the soluble and membrane-associated phosphodiesterases under separate regulatory mechanisms, but that the apparently cytoplasmic cyclic AMP and cyclic GMP phosphodiesterase activities of hepatic tissues may also be independently regulated. This is consistent with the finding that liver cyclic GMP phosphodiesterase can be isolated from an enzyme form possessing both cyclic AMP and cyclic GMP hydrolyzing capacities (Russell *et al.*, 1972a). These data cause speculation that if insulin controls hepatic cyclic AMP levels through regulation of phosphodiesterase activity, the mechanisms whereby cyclic AMP levels are kept elevated in the diabetic state may be dissociated from the mechanisms by which insulin either reduces or assists in the maintenance of normal levels of cyclic AMP.

Low K_m, membrane-bound cyclic AMP phosphodiesterase activity, appears to have at least two types of regulation: (1) proposed negative cooperativity regulation as a function of enzyme structure, location, and catalytic site interactions, whereby it responds to rapid changes in low substrate concentrations; and (2) the regulation of activity in direct response to changes in circulating hormone concentrations and/or other effectors. Our results suggest that insulin and growth hormone administration causes increases in membrane-bound low K_m liver cyclic AMP phosphodiesterase activity, but that these hormones have no immediate effect on the activities of the soluble phosphodiesterases. The activations require intact cell systems that are metabolically adjusted to the deficiency of each hormone before their mechanisms are readily apparent. It also seems that the subcellular location and kinetic characteristics of cyclic AMP phosphodiesterase may effect the manifestation of the proposed binding mechanisms of insulin and growth hormone at the level of the liver plasma membrane.

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