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SUBCELLULAR SHIFTS IN CYCLIC AMP PHOSPHODIESTERASE AND ITS CALCIUM-DEPENDENT REGULATOR IN LIVER: ROLE OF DIABETES

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SUMMARY: The distribution of the low Km cAMP phosphodiesterase shifted from the particulate to the soluble fractions of isolated liver cells of streptozotocin diabetic rats. The content of calcium-dependent regulator of phosphodiesterase was significantly depressed in cells from diabetic rats. Hence, streptozotocin diabetes is associated with both a decrease in calcium-dependent regulator and phosphodiesterase activity as well as an apparent subcellular redistribution of these components. Redistribution of these components from the sites of physiological regulation probably play a major role in the distortion of cAMP metabolism observed during diabetes.

INTRODUCTION

The mechanism by which insulin activates cAMP phosphodiesterase (PDE) is uncertain. Cheung (1) demonstrated that PDE activity was controlled by a calcium-dependent regulator (CDR). In order to understand more of the mechanisms of PDE activation by insulin and to define the role of CDR in insulin activation of PDE, the subcellular localization of PDE and its CDR were examined in isolated liver cells of normal and diabetic rats. The results show that during insulin deprivation, major shifts in subcellular distribution of PDE and its CDR occur which may relate to the reduction of PDE activity in diabetes.

MATERIAL AND METHODS

Chemicals. Cyclic AMP, snake venom (Crotalus atrox), collagenase, sucrose bovine serum albumin, DEAE cellulose, and partly purified PDE were purchased from Sigma Chemical Co. (St. Louis, MO). Anion exchange resin AG1-X2 and

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Abbreviations: PDE, cAMP phosphodiesterase (E.C. 3.1.4.17); CDR, calcium dependent regulator

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Bradford protein reagent were purchased from Bio-Rad Laboratories (Richmond, CA). ^{3}H cAMP was obtained from Schwarz/Mann, Orangeburg, N.Y. The labeled nucleotide was purified on cellulose sheets without fluorescent indicator with 2-propanol: ammonia: water (7:2:1) as the solvent.

Holtzman rats weighing 200-250 g were made diabetic by injecting 75 mg streptozotocin per kg body weight into the tail vein; the drug was a gift from the Upjohn Co., Kalamazoo, MI. Following injection the rats were maintained in metabolic cages and fed Purina Lab chow and water ad libitum. Daily weights, urine volumes, and the presence or absence of glucose and ketone bodied in the urine were recorded. At the time of sacrifice, plasma glucose measurements were made (2).

Liver cells were obtained in the following manner: after anesthesia with nembutal (50 mg per kg body weight) livers were perfused via the hepatic portal vein with Ca^{2+}-free Hanks buffer oxygenated with 95% 0_2 and 5% CO_2 to wash out blood, excised, and perfused for another 15 min. in the same buffer containing 0.05% collagenase. The livers were then incubated in a shaker for 20-30 min. in the same buffer and enzyme solution. This is a modification of the procedure described by Berry and Friend (3). Liver cells were harvested by filtering through a nylon screen (200 mesh) and collected by low speed centrifugation (1,000 xg for 30 sec.). The cells were suspended in the Ca^{2+} free buffer, filtered again to remove polymerized nucleic acids and washed two more times. Microscopic examination showed that 1-2% of the cells were broken; of the whole cells 95-99% did not stain with trypan blue. Packed liver cells were homogenized in 5.0 mM Tris-base, pH 7.4, in a dounce homogenizer. Homogenates were made up to 0.25 M sucrose by adding 50% sucrose. Subcellular fractions were collected by differential centrifugation as described by Touster et al (4). Enzyme markers were used to assess the purity of the fractions (4).

PDE activity was determined by a modification of the method of Thompson and Appleman (5). In this reaction a final volume of 0.1 ml contains 40 mM Tris-base, pH 8.0, 2 mM $MnCl_2$, nonlabeled cAMP, ^{3}H-cAMP (10-12 μci/ml), and an appropriate amount of protein (5-20 μg). The reaction was run at 30°C for 10 min. PDE activity was expressed as pmoles cAMP hydrolyzed per min. per mg protein.

CDR activity was assayed by measuring the stimulation of CDR deficient PDE; this PDE was prepared by DEAE cellulose chromatography of partly purified PDE (Sigma). After chromatography the PDE could be stimulated 4-5 fold with exogenous CDR. In the assay CDR deficient PDE was titrated with partly purified CDR (6) to determine maximal activation. Forty μg of protein from subcellular fractions were then added to CDR-deficient PDE. CDR activity was expressed as a per cent of maximal activation.

Protein concentration was determined by the method of Bradford (7). Bovine serum albumin was used as the standard. All assays were done in duplicate. All experiments were done in pairs. Statistical analysis was performed by the paired student "t" test.

RESULTS

Three days after intravenous injection of streptozotocin, the animals had lost an average of 5 grams per day and were excreting about 50 ml urine per day. The urine contained glucose and ketones. At the time of sacrifice the plasma glucose was in excess of 300 mg %. All of these parameters are in con-

TABLE I

SUBCELLULAR DISTRIBUTION OF PHOSPHODIESTERASE IN LIVER CELLS OF NORMAL AND DIABETIC RATS

Fraction	Phosphodiesterase Activity pmoles/min/mg protein (% of total activity)		
	Normal	Diabetic	
Nuclear	19.7 ± 1.7*(19.0 ± 3.0)	12.1 ± 1.3(9.0 ± 1.0)	$p < 0.02$**
Mitochondria	12.6 ± 1.3 (6.1 ± 0.7)	8.5 ± 0.6(5.7 ± 0.7)	NS
Membranes	30.0 ± 2.2 (16.5 ± 1.4)	23.7 ± 3.6(12.4 ± 0.7)	$p < 0.005$
Soluble	42.0 ± 1.9 (48.7 ± 4.0)	68.3 ± 7.4(71.2 ± 3.1)	$p < 0.01$

*Numbers represent mean ± S.E.M. of eight separate experiments.
**p values are for specific activities.

trast to normal animals which gain weight each day, excrete about 10 ml of urine per day, do not spill sugar or acetone into urine, and maintain blood glucose under 150 mg % (2).

PDE activity in liver cell homogenates decreased from 34 pmoles cAMP hydrolyzed per min. per mg protein in control animals to 26 pmoles cAMP hydrolyzed per min. per mg protein in diabetic animals ($p < .005$). Table I shows specific activities of PDE in different subcellular fractions. Specific activities of PDE were significantly lowered in nuclear and membrane fractions of diabetics by comparison to their non-diabetic controls. By contrast PDE in the soluble fraction from diabetic cells was significantly higher (about 63%) than from nondiabetic controls. Table I also shows the data expressed as per cent distribution in various subcellular fractions. In nondiabetic controls PDE activity was almost equally distributed between the particulate (41.6%) and the soluble fraction (48.7%); however in the diabetic the particulate fractions contained roughly one quarter (27.1%) of the PDE activity while the soluble component contained almost three fourths (71.2%) of the total PDE activity in the cells.

CDR content in whole homogenates and subcellular fractions of isolated liver cells from normal and diabetic animals is shown in Figure 1. The content of CDR in homogenates of isolated liver cells is significantly

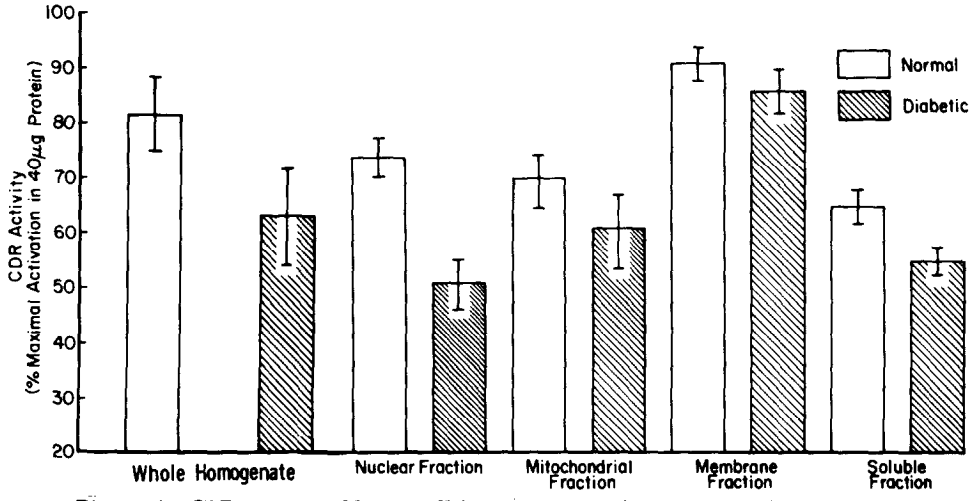

Figure 1 CDR content of liver cell homogenate and subcellular fractions from normal and diabetic rats. Values represent mean ± S.E.M. of eight separate experiments.

reduced in liver cells of diabetic animals. Although CDR is lower in each subcellular fraction of diabetics, the differences are significant only in the nuclear ($p < .02$) and soluble ($p < .02$) fractions.

DISCUSSION

Insulin lowers cAMP concentrations in stimulated liver and fat cells (8,9), by apparently activating a low Km PDE in these cells (10,11). During diabetes PDE activity becomes depressed, but is restored with insulin therapy (2,12,13). Data from isolated liver cells reported here show that PDE activity is not only decreased in diabetes, but that the enzyme appears to redistribute from the particulate to the soluble fraction of the cells. Furthermore, there is a reduction in the amount of CDR present in the cells.

There are several possibilities for the loss of PDE activity during diabetes. First, there may be less PDE enzyme protein in the cells (14,15). Since PDE activity in the soluble fraction of diabetic cells was higher than in control cells and insulin can stimulate PDE activity in short term experiments (data not shown, also ref. 10), it is not likely that decreased protein synthesis is the major factor changing PDE activity in acute diabetes.

Alterations of regulatory factors could contribute to the depression of PDE activity in diabetes. Several investigators have been involved in the identification, purification, and regulation of CDR and PDE (16,17,18), and preliminary studies have shown that CDR activity is decreased in fat cells of diabetic rats (19). Herein we show that the CDR content is significantly depressed in liver cells of diabetic rats particularly in the nuclear and soluble fractions. It is still unknown if the low CDR levels in diabetic cells are responsible for the reduction in PDE activity since CDR is present in excess of PDE concentrations (6,16). Also in various tissues inhibitors of PDE have been identified which lower the Vmax of PDE without altering the Km (20-23). We are presently looking for inhibitors of PDE in liver and alterations of inhibitor content in diabetic livers.

Finally, PDE may be redistributed between the particulate and the soluble components of cells as a result of diabetes. PDE and CDR translocation have been shown to occur between the subcellular compartments of heart (24), brain (25) and adrenal medulla (26) in other conditions.

Shifts or translocation of PDE from particulate to soluble compartments could produce major metabolic alterations. Pilkis et al (27) have argued that the supernatant enzyme is apparently responsible for most of the hydrolysis of cAMP. This seems unlikely since cAMP levels in fat and liver cells are elevated during diabetes (28) at a time when there is more PDE in the soluble fraction (Table I). Smoake et al (29) have recently shown that liver membranes containing an insulin sensitive PDE also contain a CDR-dependent PDE, CDR, and adenylate cyclase. Localization of an insulin-sensitive PDE in membranes containing adenylate cyclase is probably the most significant factor controlling cyclic nucleotide metabolism. Reduction of PDE from particulate fractions may be due to loss of CDR or other control factors such as Ca^{2+} or inhibitors from the sites

of regulation. These modulations could account for the elevation of intracellular cAMP in fat and liver cells and the lowering of total PDE activity.

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