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STREPTOZOTOCIN-INDUCED DIABETES AND HORMONE SENSITIVITY OF ADENYLATE CYCLASE IN RAT MYOCARDIAL SARCOLEMMA, AORTA AND LIVER*

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Abstract—Adenylate cyclase activity was investigated in myocardial sarcolemma, aorta particulate fractions, and liver plasma membranes from control and 5-day streptozotocin-induced diabetic rats. The basal adenylate cyclase activity was increased in heart sarcolemma from diabetic rats, whereas the extent of stimulation by glucagon, dopamine, isoproterenol, epinephrine, sodium fluoride and forskolin was decreased markedly. The decreased responsiveness was associated with a decrease in V_{max} but not in the activation constant. In contrast, GTP stimulated adenylate cyclase in control and diabetic myocardial sarcolemma to the same extent. In addition, the basal adenylate cyclase activity was not altered significantly in aorta particulate fraction of liver plasma membranes from diabetic rats, but the stimulation of adenylate cyclase by catecholamines and forskolin (in the case of aorta) and by adenosine, glucagon, NaF and forskolin (in the case of liver) was diminished markedly. These data suggest that, in streptozotocin-induced diabetes, the responsiveness of adenylate cyclase to various hormones and agents (fluoride and forskolin) which act through receptor-independent mechanisms is decreased.

The adenylate cyclase/cAMP system is believed to be one of the biochemical mechanisms participating in the regulation of cardiovascular functions [1, 2]. A decreased myocardial performance associated with a decreased number of β-adrenergic receptors in myocardium has been shown in diabetic cardiomyopathy [3–7]. A striking increase in cyclic AMP levels has been reported in alloxan-diabetic rat hearts by Chaudhuri and Shipp [8], whereas Das [9] did not observe any change in cyclic AMP levels in hearts from streptozotocin-induced diabetic rats. There are some recent studies which indicate a decrease in the responsiveness of myocardial adenylate cyclase to catecholamines [10, 11] in streptozotocin- or alloxan- [12] induced diabetic rats; however, Ingebretsen *et al.* [13] have not been able to detect any change in adenylate cyclase sensitivity to catecholamines, although a reduction in the number of β-receptors was reported in alloxan-induced diabetic rat hearts [13]. Alterations in adenylate cyclase activity and its responsiveness to various hormones have also been demonstrated in other tissues such as liver [14, 15], skeletal muscle [16], cerebrum, cerebral microvessels and retina [17]. Since hormone-responsive adenylate cyclase is composed of three components (receptor, guanine nucleotide regulatory protein and catalytic subunit [18]), any change in the adenylate cyclase activity in diabetes may be attributed to an alteration in the functions of any of these components. The present studies were undertaken to investigate whether short-term streptozotocin-induced diabetes is associated with changes in adenylate cyclase activity and, if so, whether the changes are confined to only basal adenylate cyclase activity or also involve alterations in the responsiveness of various hormones, guanine nucleotides and agents such as NaF and forskolin that activate adenylate cyclase by receptor-independent mechanisms [19].

The present report demonstrates that diabetes altered the responsiveness of various hormones and agents such as NaF and forskolin in heart sarcolemma without any change in the stimulation of adenylatecylase by guanine nucleotides. Preliminary reports of this work have been presented [20, 21].

MATERIALS AND METHODS

Materials. Streptozotocin was purchased from the Sigma Chemical Co., St. Louis, MO. All other chemicals were obtained as in Ref. 22. Male Sprague–Dawley rats (180–200 g) were used. Diabetes was induced by intraperitoneal injection of streptozotocin (70 mg/kg body wt) in 0.9% NaCl to rats starved for 24 hr as described previously [23]. The control rats were injected with 0.9% NaCl. The rats were fed *ad lib.* on Purina lab chow. Blood glucose levels were monitored 5 days after the injection using a dextrometer (Ames). Streptozotocin-injected rats with blood glucose levels in excess of 350 mg/dl were considered to be diabetic and used in this study.

Membrane preparations. Rats were decapitated, and liver, heart, and brain were immediately processed to isolate liver plasma membranes according to Pilkis *et al.* [14], heart sarcolemma according to Anand-Srivastava *et al.* [24], and striatal membranes according to Anand-Srivastava and Johnson [25]. The aorta were dissected out, immediately frozen in

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liquid nitrogen, and subsequently pulverized to a fine powder with a percussion mortor cooled in liquid nitrogen and stored at −70° until assayed. Aorta particulate fraction was prepared as described previously [26].

Adenylate cyclase determination. Adenylate cyclase activity was determined by measuring [^{32}P]-cAMP formation from [α-^{32}P]ATP as described previously [25]. Under the assay conditions used, adenylate cyclase activity was linear with respect to protein concentration and time of incubation.

Protein was determined essentially as described by Lowry *et al.* [27] with crystalline bovine serum albumin as standard.

RESULTS

Effect of GTP on adenylate cyclase from heart sarcolemma. Table 1 shows the effect of various concentrations of GTP on myocardial adenylate cyclase activity in control and streptozotocin-induced diabetic rats. Basal adenylate cyclase activity in diabetic rats was higher (~45%) than in control rats. GTP, which has been shown to regulate adenylate cyclase activity by interacting with guanine nucleotide regulatory protein [18], stimulated myocardial adenylate cyclase in a concentration-dependent manner. Although the activity was always higher in diabetic rats in the absence or presence of GTP, the extent of stimulation was not significantly different in both groups. These data suggest that the guanine nucleotide regulatory protein may not be affected by the diabetic state. The increased enzyme activity in diabetic rats was not due to the decrease in protein, because the protein content in control (C) and diabetic (D) heart was not significantly different (C = 101 ± 6 mg/g heart, D = 104 ± 6 mg/g heart). However, the increased enzyme activity in diabetic rats may be due to the higher levels of circulating catecholamines [28].

Effect of some agonists on adenylate cyclase in heart sarcolemma from control and diabetic rats. To investigate if the responsiveness of adenylate cyclase to various agonists is also altered in diabetic rats, the effects of some hormones and agents on adenylate cyclase were studied and the results are shown in Fig. 1. Glucagon, dopamine, isoproterenol and epinephrine all stimulated myocardial adenylate cyclase

Table 1. Effect of GTP on adenylate cyclase activity of myocardial sarcolemma from control and diabetic rats*

GTP (μM)	Adenylate cyclase activity [pmoles cAMP (mg protein · 10 min)$^{-1}$]	
	Control	Diabetic rats
None	87 ± 2	124 ± 3
0.5	92 ± 3	135 ± 9
1.0	99 ± 2	142 ± 4
5.0	100 ± 5	144 ± 3
10.0	113 ± 4	160 ± 6
20.0	135 ± 4	209 ± 9

* Adenylate cyclase activity was determined as described in Materials and Methods. Values represent the mean ± S.E.M. of triplicate determinations from one of three separate experiments.

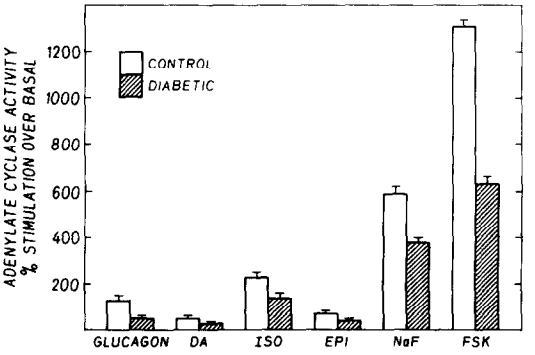

Fig. 1. Effects of various agonists on myocardial adenylate cyclase activity from control (□) and diabetic (▨) rats. Adenylate cyclase activity was determined in the absence or presence of 1 μM glucagon, 100 μM dopamine (DA), 50 μM isoproterenol (ISO), 50 μM epinephrine (EPI), 10 mM sodium fluoride (NaF), and 50 μM forskolin (FSK) as described in Materials and Methods. Values are the means ± S.E.M. of triplicate determinations from one of three separate experiments. Six animals from each group were utilized for each experiment. Basal adenylate cyclase activities in control and diabetic rats were 90 ± 16 and 162 ± 4 pmoles cAMP (mg protein · 10 min)$^{-1}$ respectively.

to various degrees in control and diabetic rats; however, the extent of stimulation by these agonists was decreased markedly in diabetic rats. For example, glucagon stimulation was decreased by about 60% whereas dopamine, isoproterenol and epinephrine-stimulated adenylate cyclase activities were inhibited by about 50, 40 and 40% respectively. In addition, the stimulation by F^- and forskolin, which activate adenylate cyclase by receptor-independent mechanisms, was also decreased by about 35 and 50%, respectively, in diabetic rats.

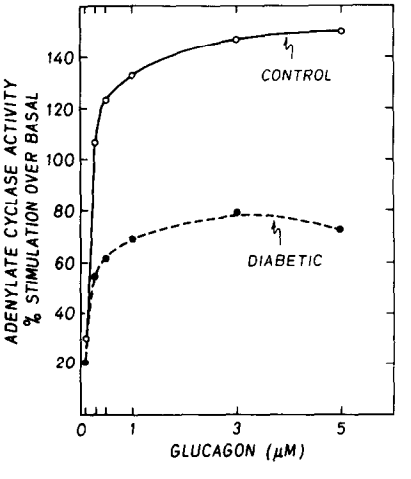

Fig. 2. Effect of various concentrations of glucagon on myocardial adenylate cyclase from control (○——○) and diabetic (●——●) rats. Adenylate cyclase activity was measured as described in Materials and Methods. Values are the means of triplicate determinations from one of three separate experiments. Six animals from each group were utilized for each experiment. Basal adenylate cyclase activities in control and diabetic rats were 93 ± 4 and 151 ± 7 pmoles cAMP (mg protein · 10 min)$^{-1}$ respectively.