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

ASHOK K. SRIVASTAVA and MADHU B. ANAND-SRIVASTAVA†
Clinical Research Institute of Montreal, Montreal, Quebec H2W 1R7, Canada

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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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† Canadian Heart Foundation Scholar.

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.

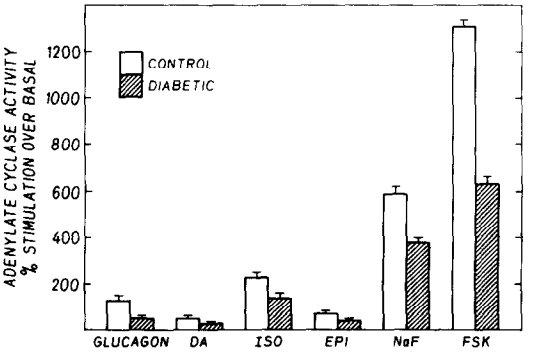

Fig. 1. Effects of various agonists on myocardial adenylate cyclase activity from control (□) and diabetic (▨) rats. Adenylate cyclase activity was determined in the absence or presence of 1 μM glucagon, 100 μM dopamine (DA), 50 μM isoproterenol (ISO), 50 μM epinephrine (EPI), 10 mM sodium fluoride (NaF), 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.

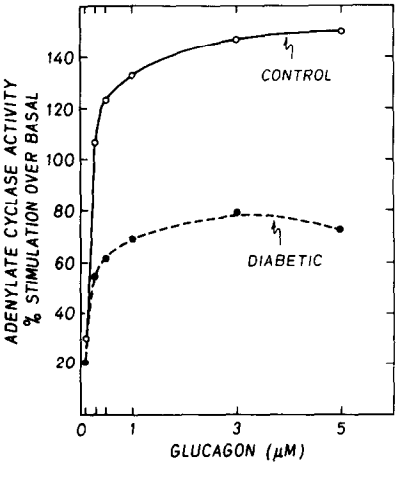

Fig. 2. Effect of various concentrations of glucagon on myocardial adenylate cyclase from control (○——○) and diabetic (●——●) rats. Adenylate cyclase activity was measured as described in Materials and Methods. Values are the means of triplicate determinations from one of three separate experiments. Six 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.

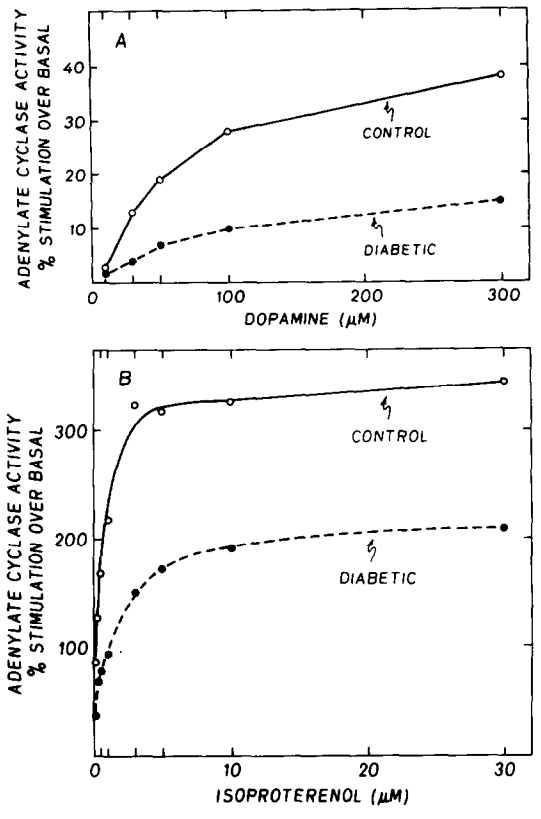

Fig. 3. Effects of various concentrations of dopamine (A) and isoproterenol (B) on myocardial adenylate cyclase from control (○——○) and diabetic (●——●) rats. Adenylate cyclase activity was measured as described in Materials and Methods. Values are the means of triplicate determinations from one of three separate experiments. Six animals from each group were utilized for each experiment. The basal adenylate cyclase activities in control and diabetic rats were 61 ± 5 and 123 ± 3 pmoles cAMP (mg protein · 10 min)$^{-1}$ respectively (A) and 73 ± 4 and 135 ± 8 pmoles cAMP (mg protein · 10 min)$^{-1}$ respectively (B).

To determine whether the diminished stimulation of adenylate cyclase by glucagon and catecholamines in diabetic rats was associated with a decrease in the number of receptors or an increase in the adenylate cyclase activation constant, the effects of various concentrations of glucagon (Fig. 2) and catecholamines (Fig. 3, A and B) on adenylate cyclase were studied in diabetic and control rats. Glucagon (Fig. 2), isoproterenol (Fig. 3B) and dopamine (Fig. 3A) stimulated adenylate cyclase in a concentration-dependent manner in both diabetic and control rats. However, the percent stimulation by these agents at all concentrations was higher in control rats than in diabetic rats. At 1 μM, glucagon stimulated adenylate cyclase by about 130% in control rats which was decreased to about 70% in diabetic rats. Similarly, about 30% stimulation observed at 100 μM dopamine in control rats was decreased to about 10% in diabetic rats, and about 300% stimulation observed at 10 μM isoproterenol in control rats was decreased to about 180% in diabetic rats. The observed diminished stimulation of adenylate cyclase by glucagon, dopamine and isoproterenol was associated with a decrease in V_{max} but not in the activation constant, suggesting that the number of hormone receptors is decreased in diabetic rats.

Effects of agonists on adenylate cyclase from rat aorta, liver plasma membranes and brain striatum. To investigate whether diabetes also affects adenylate cyclase and its responsiveness to various hormones and agents in other tissues, the effects of various agonists on adenylate cyclase were studied in aorta particulate fraction and in liver plasma membranes; the results are shown in Figs. 4 and 5. Epinephrine, norepinephrine, isoproterenol, dopamine and forskolin all stimulated adenylate cyclase activity in the aorta particulate fraction to various degrees in both control and diabetic rats, but the extent of stimulation by these hormones (VHormones/VBasal) was decreased markedly in diabetic rats. In addition, basal adenylate cyclase activity was not altered significantly in diabetic rats. A similar decrease in NECA- (an adenosine analog that is not deaminated by adenosine deaminase), glucaon-, NaF- and forskolin-stimulated adenylate cyclase activities was observed in liver plasma membranes from diabetic rats (Fig. 5); however, basal adenylate cyclase

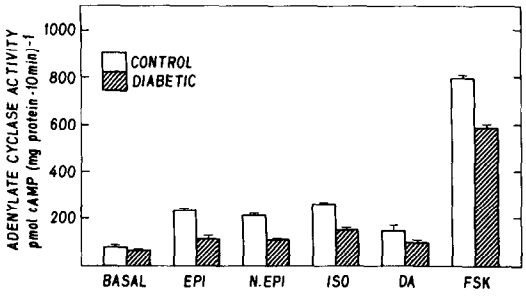

Fig. 4. Effects of various agonists on adenylate cyclase activity of aorta particulate fractions from control (□) and diabetic (▨) rats. Adenylate cyclase activity was determined in the absence or presence of 50 μM epinephrine (EPI), 50 μM norepinephrine (N.EPI), 50 μM isoproterenol (ISO), 100 μM dopamine (DA), and 50 μM forskolin (FSK). Values are the means ± S.E.M. of triplicate determinations from one of three separate experiments. Twelve animals in each group were utilized for each experiment.

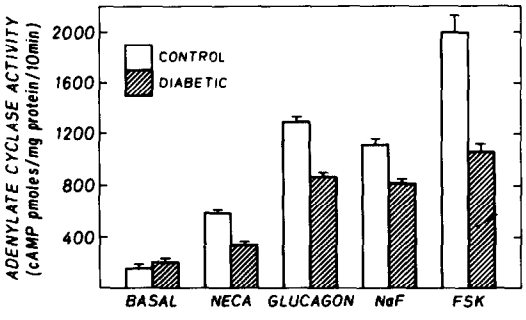

Fig. 5. Effects of various agonists on the adenylate cyclase activity of liver plasma membranes from control (□) and diabetic (▨) rats. Adenylate cyclase activity was determined in the absence or presence of 10 μM *N*-ethyl carboxamide adenosine (NECA), 1 μM glucagon, 10 mM sodium fluoride (NaF) and 50 μM forskolin (FSK). Values are the means ± S.E.M. of triplicate determinations from one of three separate experiments. Six animals in each group were utilized for each experiment.

activity was not altered significantly. In addition, we also studied adenylate cyclase activity in rat brain striatum from control and diabetic rats; no significant difference was observed in basal or hormone responsive adenylate cyclase activities (data not shown).

DISCUSSION

This is the first comparative study investigating the effect of short-term streptozotocin-induced diabetes on adenylate cyclase activity and its hormonal responsiveness in rat heart, aorta, liver and brain.

Data in this paper demonstrate that in streptozotocin-induced diabetes there is not a generalized defect in the adenylate cyclase system, but that some tissues are more susceptible than others. For example, brain striatal adenylate cyclase activity and its responsiveness to various hormones were not affected by the diabetic state, whereas significant alterations in basal and/or hormone-sensitive adenylate cyclase activities were observed in cardiac sarcolemma, aorta and liver particulate fractions from diabetic rats. Furthermore, only in cardiac sarcolemma from diabetic rats was the basal adenylate cyclase activity higher than in control rats, whereas no significant change in basal enzyme activity was observed in aorta or liver. The higher basal adenylate cyclase activity in the absence or presence of various concentrations of GTP in heart sarcolemma, but not in other tissues from diabetic rats tested in the present studies, suggests that heart may be more sensitive to desensitization, due to increased amounts of circulating catecholamines [28], than liver, aorta and brain. Increased cAMP levels in diabetic rat heart have also been reported by other investigators [8]. Our results, however, contrast with the recent report of Ingebretsen *et al.* [13] who did not detect any change in adenylate cyclase activity in alloxan diabetic rats.

The diminished stimulation of adenylate cyclase by various concentrations of dopamine, isoproterenol, and glucagon (Figs. 2, 3A and 3B) in myocardial sarcolemma from diabetic rats, compared to control rats, suggests that the numbers of dopamine receptors, β-adrenergic receptors and glucagon receptors coupled to adenylate cyclase were reduced in diabetic rats. Our results are consistent with earlier reports in the literature [11, 12]. Since we have not performed receptor binding studies, we do not know whether the total number of hormone receptors was also reduced in diabetes. However, Ingebretsen *et al.* [13] have recently shown a decrease in catecholamine receptors in alloxan diabetic rat heart membranes. In our present studies, we have further shown that GTP stimulated myocardial adenylate cyclase in both control and diabetic rats to the same extent, suggesting that guanine nucleotide regulatory protein was not affected in diabetes; hence, the diminished responsiveness of adenylate cyclase to various hormones may not have been due to a defect in the coupling process *per se* but, instead to a reduction in the number of hormone receptors. The decreased stimulation of adenylate cyclase by catecholamines in diabetic heart membranes has also been shown by other investigators [10, 12]; however, Ingebretsen *et al.* [13] have recently shown a decrease in the number of β-adrenergic receptors in diabetic heart membranes without any change in the sensitivity of adenylate cyclase to catecholamine. These apparent discrepancies in enzyme activity and hormonal responsiveness may have been due to differences in membrane isolation technique, different experimental models and also the duration of diabetes. For example, Ingebretsen *et al.* [13] used microsomal vesicles in their studies whereas in the present studies we used sarcolemma enriched membrane fractions. In addition, we used streptozotocin rather than alloxan [13] to induce diabetes in rats.

Forskolin, a positive isotropic and antihypertensive agent [29, 30], has been shown to activate adenylate cyclase in many tissues [31]. In the present studies, the stimulatory effects of NaF and forskolin that activate adenylate cyclase by receptor-independent mechanisms were also inhibited in diabetic rats. Since we did not detect any difference between the stimulatory effects of GTP on adenylate cyclase of diabetic and control rats, an involvement of guanine nucleotide regulatory protein in the diminished stimulation of adenylate cyclase by NaF or forskolin in diabetes seems unlikely; however, from the data, it appears that the catalytic subunit is impaired in diabetes. Since phospholipids have been shown to be required in the expression of NaF- and forskolin-stimulated adenylate cyclase activities [32], any alteration in phospholipid composition of the membrane will result in the loss or diminished responsiveness of adenylate cyclase to these agents. The altered membrane phospholipid composition has been shown recently in diabetes [33]; hence, it may be that the observed decreases in the stimulatory effects of NaF and forskolin on adenylate cyclase in diabetes were the consequence of such changes.

It is concluded from the present studies that, in diabetes, not only was the sensitivity of adenylate cyclase to various hormones altered but also the responsiveness of catalytic subunit was impaired. It can be postulated that the decreased production of cAMP may be responsible for the impaired myocardial and vascular functions in diabetic cardiomyopathy.

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