Adrenergic Receptors and Adenylate Cyclase Activity in Hepatocytes of the Streptozotocin-Diabetic Rat

SUMIO SHIMA, HIROYUKI FUKASE, AND NOBU AKAMATSU

Department of Biochemistry, St. Marianna University School of Medicine, Kawasaki 213, Japan

Abstract. Effects of short and long exposure to the diabetic state induced by an injection of streptozotocin to young female rats on glucagon- and catecholamine-sensitive adenylate cyclase activity and adrenergic receptors of hepatic membranes have been studied. The short period of exposure to the diabetic state exhibited an increase in the sensitivity of the enzyme to isoproterenol without changes in the affinity and the number of β-adrenergic receptors. The increased response of adenylate cyclase activity to isoproterenol was accompanied with a greater GTP-induced lowering of the affinity to the β-adrenergic agonist in diabetic membranes than in the controls. The chronic diabetic state produced a decrease in the adenylate cyclase activity to hormonal or non-hormonal stimuli with a fall in the number of α- and β-adrenergic receptors. These results suggest that the observed effects of the diabetic state on hormonally sensitive adenylate cyclase activities and their receptor binding sites of the hepatic membranes would vary depending on the duration and/or severity of the diabetic state experimentally induced.

Key words: Adrenergic receptor, Adenylate cyclase, Hepatic membrane, Diabetes.

(Hormone-sensitive adenylate cyclase activity in the liver of rats having models of diabetes mellitus has been variously reported as increased [1-4], decreased [5-8] or unchanged [9] in comparison with nondiabetic control animals. These discrepancies seem to result from the degree and/or duration of the diabetic conditions experimentally induced. The experiments [1-4, 11] reporting the up-regulation of glucagon- or epinephrine sensitive adenylate cyclase activity of the liver from diabetic rats are appear to have been done without perfusion of the liver. On the other hand, in most of the experiments [5-7] showing down-regulation of the hormone-sensitive enzyme in the diabetic liver, the liver was perfused to eliminate the contamination by blood cells.

Recently, the reduction of hepatic α-adrenergic receptors with no differences in the β-adrenergic receptors has been reported in the liver from the genetically diabetic mouse C57 [10]. Concerning adipocytes from diabetic rats, there are also conflicting reports of an increase [11, 12] or decrease [13] in β-adrenergic receptor-adenylate cyclase activity. Moreover, a reduction in the number of β-adrenergic receptors [14], the related adenylate cyclase activity [15] and α-adrenergic receptors [16] has been observed in heart membranes from diabetic rats.

In hepatocytes from female rats, catecholamines have been reported to react both with α- and β-adrenergic pathways which activate phosphorylation and glycogenolysis via Ca++ or cyclic AMP, respectively [17].

The present experiments were designed to investigate changes in adrenergic receptors and adenylate cyclase activity in relation to the duration and/or severity of the diabetic state experimentally induced.
Materials and Methods

Chemicals

The following drugs were generously given: 1-isoproterenol (Nicken Chemicals, Tokyo, Japan), propranolol (Japan ICI Pharma, Osaka, Japan), cyclic AMP (Daiichi Seiyaku, Tokyo, Japan), phenolamine (Japan Ciba Geigy, Tokyo, Japan). Glucagon and 5'-guanylyl imidodiphosphate (p[NH]ppG) were obtained from Calbiochem-Hoechst, Tokyo, Japan, and Streptozotocin was purchased from Calbiochem-Hoechst, Tokyo, Japan.

Animals and animal treatments

Female rats weighing 50-60 g, of Donryu strains (Nippon Rats Co., Tokyo, Japan) received intravenous injections containing 50 mg/kg of streptozotocin or citrate buffer, pH 4.0, alone, and were killed by decapitation 3 or 15 days later. Plasma glucose concentrations were determined in fasting blood taken at the time of sacrifice by the glucose oxidase method [18].

Adenylate cyclase assay

Hepatocytes were prepared by collagenase perfusion of the liver by the procedure of Berry and Friend [19], as modified by Tolbert et al. [20]. Adenylate cyclase activity was assayed by incubation of the membrane from the hepatocytes [21, 22] in a total volume of 0.6 ml of 40 mM Tris-HCl buffer [pH 7.4] containing 4 mM MgCl₂, 10 mM theophylline, 2 mM ATP, 100 μg/ml pyruvate kinase, and 5 mM phosphoenolpyruvate for 10 min, as previously described [23, 24]. Cyclic AMP formed during incubation was measured by a competitive binding assay with a protein purified from rabbit skeletal muscle up to the DEAE-cellulose column step [25].

Receptor-binding assay

Samples of the membrane preparation were assayed for β-adrenergic receptor binding with [³²H]dihydroalprenolol (DHA). Membranes were incubated with [³²H]DHA (1 to 20 nM) in a buffer (50 mM tris-HCl, pH 7.4 and 2 mM MgCl₂) for 10 min at 30°C [26]. α-Adrenergic receptor binding was performed with [³¹H]prazosin as described by Geynet et al. [27]. Incubation of membranes with 0.2–10.0 nM [³¹H]prazosin in 50 mM Tris-HCl buffer, pH 7.4, containing 10 mM MgCl₂, 1 mM sodium (–) ascorbate and 1 mM pyrocatechol was carried out for 30 min at 25°C. Protein concentrations were measured by the method of Lowry et al. [28], with bovine serum albumin as standard.

Results

Rats became diabetic 3 days after an intravenous injection of 50 mg/kg of streptozotocin. The increase in body weight was much less in diabetic rats than in controls (Table 1). A decrease in basal and fluoride-stimulated adenylate cyclase activity of hepatic membranes was apparent 5 days after injection of streptozotocin (Fig. 1). Figure 2 also shows that basal, fluoride, p[NH]ppG and Mn-

Table 1. Characteristics of experimental animals

<table>
<thead>
<tr>
<th>Animal preparation</th>
<th>No. of animals</th>
<th>Body weight at sacrifice (g)</th>
<th>Plasma glucose (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16</td>
<td>mean ± SEM</td>
<td>mean ± SEM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>111.5 ± 10.7</td>
<td>127 ± 16</td>
</tr>
<tr>
<td>Diabetic-3 days</td>
<td>12</td>
<td>97.6 ± 7.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>287 ± 23&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic-15 days</td>
<td>10</td>
<td>62.2 ± 2.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>323 ± 21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Female rats weighing 50 to 60 g were injected intravenously with Streptozotocin in citrate buffer, pH 4.0 (50 mg/kg B.W) 3 or 15 days before the sacrifice. Significantly different from control (a, P<0.05; b, P<0.01). Results are mean±SEM.
Fig. 1. Effects of Streptozotocin-induced diabetic mellitus on adenylate cyclase activities of hepatic membranes. Hepatic membranes from rats treated with streptozotocin were assayed for adenylate cyclase activity as described in "Materials and Method" with (closed circle) or without (opened circle) 10 mM sodium fluoride. Results shown are means ± SEM for four incubations. a, b. Significantly different from control (0 day) (P<0.05).

Fig. 2. Effects of sodium fluoride, p[NH]ppG and MnCl₂ on adenylate cyclase activity in membranes derived from livers of rats after 3 (shaded bar) and 15 (closed bar) days of streptozotocin administration as compared to controls (opened bar). Adenylate cyclase activity was assayed as described under Materials and Methods. Values are means ± SEM for four incubations. a, Significantly different from control (P<0.05).

Fig. 3. Effects of glucagon on adenylate cyclase activity in hepatic membranes of rats after 3 (closed circle) and 15 (triangle) days of streptozotocin administration as compared to controls (opened circle). Adenylate cyclase activity was assayed with increasing concentrations of glucagon as described under Materials and Methods. Values represent means ± SEM for four incubations. a, Significantly different from control (P<0.05).

Stimulated enzyme activity was decreased 15 days after the induction of diabetes. No changes in the enzyme activity were observed within 3 days after the streptozotocin administration (Figs. 1 and 2). In the early period (3 days) of the diabetic state, the maximal activity attained by isoproterenol and glucagon was the same in control and diabetic rats (Figs. 3 and 4). The dose-response relation, however, of isoproterenol-stimulated adenylate cyclase activity shifted to the left more than ten fold in the 3-day-diabetic animals (Fig. 4).

The stimulation of the enzyme by fluoride, p[NH]ppG and Mn was similar in hepatic membranes from both control and the 3-day-diabetic rats (Figs. 2 and 3). Binding affinity and capacity for β-adrenergic receptor evaluated with a Scatchard analysis of [3H]DHA binding were also the same in the control and the 3-day-diabetic rats (Fig. 5). As shown in Fig. 6-B, regulatory effects of GTP in lowering the binding affinity of the agonist to the β-adrenergic receptors were greater in membranes from 3-day-diabetic rats than in controls.

After a longer duration (15 days) of the diabetic state, stimulation by glucagon, isoproterenol, fluoride, p[NH]ppG and Mn of adenylate cyclase...
Fig. 4. Effects of isoproterenol on adenylate cyclase activity in hepatic membranes of rats after 3 (closed circle) and 15 (triangle) days of streptozotocin administration as compared to controls (opened circle). Adenylate cyclase activity was assayed with increasing concentrations of isoproterenol as described under Materials and Methods. Values are means ± SEM for four incubations. a, b, Significantly different from control ($P<0.05$).

Fig. 5. Scatchard analysis of $[^3H]$DHA binding to hepatic membranes from rats after 3 (closed circle) and 15 (triangle) days of streptozotocin administration as compared to controls (opened circle). Conditions for the binding assay were as described in Materials and Methods. The ratio $B/F$ of bound $[^3H]$DHA (f mol/mg protein) to free DHA (nM) is plotted as a function of bound DHA (f mol/mg protein). Points are the means of triplicate incubations.

Fig. 6. Isoproterenol competition curves for $[^3H]$DHA binding in hepatic membranes from rats after 3 (B) and 15 (C) days of streptozotocin administration as compared to controls (A). Membranes were incubated in the presence (closed circle) or the absence (opened circle) of 0.1 mM GTP in the binding assay as described in Materials and Methods. Maximum specific binding to membranes in the absence of isoproterenol was taken as 100%. Values shown represent the means ± SEM of triplicate incubations.

Fig. 7. Scatchard analysis of $[^3H]$prazosin binding to hepatic membranes from rats after 3 (closed circle) and 15 (triangle) days of streptozotocin administration as compared to controls (opened circle). Conditions for the binding assay were as described in Materials and Methods. The ratio $B/F$ of bound $[^3H]$prazosin (f mol/mg protein) to free prazosin (nM) is plotted as a function of bound prazosin (f mol/mg protein). Points are the means of triplicate incubations.
activity was greatly reduced as compared to control rats (Figs. 1, 2, 3 and 4). Binding capacity for β-adrenergic receptors was also reduced in membranes from the 15 day diabetic rats (Fig. 5). Alteration of the β-adrenergic receptor affinity for the competition curves with addition of GTP was similar in both control and 15-day-diabetes (Fig. 6-C). The amount of α-adrenergic receptors was greatly reduced in membranes from the 15-day-diabetic rats, while no changes were observed in the 3-day-diabetic rats (Fig. 7).

Discussion

The present experiments described the different responses in adrenergic receptors and adenylate cyclase activity to the β-adrenergic agonist, isoproterenol in hepatic membranes of rats, depending on the duration and/or severity of the diabetic state. Effects of the diabetic state of short duration (less than 3 days) on the hepatic adrenergic receptors and adenylate cyclase activity have not been investigated so far.

In the 3-day-diabetic state, higher sensitivity to β-adrenergic agonist, isoproterenol, of adenylate cyclase activity was observed without any increase in the number of β-adrenergic receptor sites. This increased response to isoproterenol of adenylate cyclase activity in the short-term diabetic membranes was accompanied with a greater decrease in the affinity of β-adrenergic agonist binding in the presence of GTP than in the control membranes. These results suggest that the diabetic state of short duration facilitates the interaction of the receptor, guanine nucleotide stimulatory proteins (Ns) and/or the catalytic subunit. The up-regulation of β-adrenergic receptors has been demonstrated in the submaxillary gland from chronically reserpine-treated rats with decreased plasma catecholamine concentrations [29] and in rat cardiac tissues after chronic guanethidine treatment [30]. Zumstein et al. [11] reported enhanced sensitivity and maximal response of adenylate cyclase activity to catecholamine in adipocytes from diabetic rats. Solomon et al. [31] also reported increased sensitivity to catecholamines in lipolysis in per fused adipocytes from diabetic rats. On the other hand, a decrease in the number of β-adrenergic receptors and in basal and isoproterenol stimulated adenylate cyclase activity has been reported in streptozotocin-treated rat adipocytes [13].

Recently, de Cingolani [12] found hyperresponsiveness to β-adrenergic stimulation of adenylate cyclase activity with increased numbers of β-adrenergic receptor in diabetic rat adipocytes. This investigator suggests that a lower plasma catecholamine level in diabetic rats might result in an increase in β-adrenergic receptors and their response in fat cell membranes prepared from diabetic rats.

In the present experiments, the sensitivity of adenylate cyclase activity to β-adrenergic agonist is increased during early exposure to the diabetic state. As de Cingolani [12] stated, the increase in adenylate cyclase activity in response to catecholamines during the diabetic state could be explained as an adaptive change due to a lower plasma level of catecholamine in diabetic rats. The increased sensitivity of the diabetic enzyme to β-adrenergic agonist could be partly accounted for an increase in the GTP-induced dissociation rate of the agonist coupling to the β-adrenergic receptor, resulting in more efficient facilitation of the coupling function to the guanine nucleotide stimulatory (Ns) mechanism in diabetic membranes than in controls. Moreover, in the experiments on the diabetic state of short duration (3 days), the possibility of toxic and/or stressing effects of streptozotocin could not be excluded. In the chronic diabetic state longer than 10 days, the adenylate cyclase activity activated hormonally or non-hormonally was decreased as reported previously [6-8, 32]. The density of α- and β-adrenergic receptors was also decreased in hepatic membranes from long-term diabetic rats. Increased hepatic autophagy and accelerated protein degradation have reported in severe diabetes mellitus [33-36]. These generalized increases in proteolysis might account for the decrease in hormonal receptors and adenylate cyclase regulatory and/or catalytic components in hepatic membranes with long-term diabetes [8].
References


