β-Adrenergic Receptors and Adenylate Cyclase Activity in Diabetic Rat Fat Cells

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SUMMARY

Our study describes the effect of norepinephrine on adenylate cyclase activity in adipocytes from control and diabetic rats. The results show that diabetes induced an increase in both basal and norepinephrine-stimulated adenylate cyclase activity. This higher activity was not suppressed when the animals were treated for 2 days with the β-blocking agent propranolol. On the other hand, adipocytes from control animals treated with propranolol showed a higher adenylate cyclase activity (basal and in response to norepinephrine).

β-Adrenergic receptors were examined in adipocytes from control and diabetic rats with and without treatment with propranolol. The results show a higher β-receptor density in adipocytes from diabetic animals. When the animals were treated with propranolol, the β-blocker induced a higher receptor density in adipocytes from control animals without affecting the already increased receptor density in diabetic preparations.

The data suggest that adenylate cyclase activity in response to norepinephrine in adipose tissue is increased during at least a certain period of the diabetic state. This increase in adenylate cyclase activity is accompanied with an increase in β-receptor density, but in contrast to control animals, this receptor density is not further increased with propranolol treatment.

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Diabetes is associated with many metabolic alterations of the adipose tissue. For example, an elevation in intracellular cyclic AMP (cAMP) content in adipocytes from diabetic rats has been reported.[1] The cAMP level at any time reflects the balance between its rate of production through adenylate cyclase and its destruction through the enzyme phosphodiesterase. From the literature, many reports have led to opposite conclusions concerning the influence of diabetes on the cAMP-dependent lipolytic process in the adipocyte. For instance, it has been shown that diabetes induces either an increase[2] or a decrease[3,4] in adenylate cyclase activity. On the other hand, phosphodiesterase activity was also found decreased[2,5–7] or increased.[3,8] In the diabetic state, due to insulin deficiency, the effect of hormones inducing an increase in adenylate cyclase activity might be predominant. Among these, catecholamine's site would induce an increase in adenylate cyclase activity and, consequently, a rise in cAMP via interaction with the β-receptor.

Zumstein et al.[3,9] reported that the sensitivity and maximal response of adenylate cyclase to catecholamines were enhanced in adipocytes from diabetic rats. On the other hand, La Casa et al.[4] found an increased sensitivity of adenylate cyclase to isoproterenol stimulation but a decrease in the maximal response. Similar results were obtained by others with perifused adipocytes from diabetic rats.[10]

The purpose of our study was to examine adenylate cyclase activity in response to norepinephrine in adipocytes from diabetic rats with and without treatment with propranolol. In addition, experiments were designed to study the number and affinity of β-adrenergic receptors.

MATERIALS AND METHODS

Animals. Male Wistar rats (180–190 g) maintained on a standard laboratory diet and water ad libitum were used in our study. The diabetic group was obtained by the intravenous injection of streptozocin (40 mg/kg). The animals were used 10 days after the injection, their blood glucose concentration was 400–600 mg/100 ml, and they did not show a significant loss of body weight. When treated with propranolol, they were given two doses (3 mg/kg body wt) in the tail vein 48 and 24 h before the experiment. The animals were decapitated, and adipocytes were obtained by enzymatic digestion of epididymal fat pad according to the procedure of Rodbell.[11]

Adenylate cyclase assay. Adenylate cyclase activity was measured in a cell membrane–enriched fraction of fat cells. Each experiment was performed with adipocytes obtained

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from pooled adipose tissue from four animals. Briefly, adipocytes, after isolation, were washed twice with Krebs-Ringer bicarbonate buffer containing 3% bovine albumin, pH 7.4, and once with 0.25 M sucrose, 3 mM ATP, 1 mM EDTA, and 10 mM Tris-HCl, pH 7.4 (medium 1). The fat cells were then resuspended in medium 1 and homogenized at room temperature in a glass Potter-Elvehjem–type homogenizer fitter with a Teflon pestle. The homogenate was centrifuged at 20,000 × *g* for 15 min at 4°C. The pellet was washed by resuspension in medium 1, which was ice cold, and then centrifuged at the same speed and time. The final pellet was used directly in the assay for adenylate cyclase activity or frozen and maintained at −70°C until assayed. Just before the assay, the pellet was suspended in 1 mM EDTA and 1 mM Tris-acetate, pH 7.4. Protein concentrations were determined by the method of Lowry et al.,[12] with bovine albumin as standard. The membrane fraction (30 μg protein) was incubated at 37°C in the presence of 0.5 mM [α-^{32}P]ATP (~100 cpm/pmol), 2 mM $MgCl_2$, 0.2 mM 1-methyl-3-isobutylxanthine and cAMP, 5 mM creatine phosphate, 0.5 U creatine phosphokinase, and 50 mM Tris-HCl buffer, pH 7.5, in a final volume of 0.1 ml, with and without norepinephrine (10^{-7}–10^{-4} M). After 10 min, the reaction was terminated by adding 0.1 ml of stopping solution;[13] cAMP formed was determined by the use of Dowex 50 and alumina columns.[13]

Binding assay. For the binding assay, adipocytes from pooled adipose tissue from six animals were used. The adipocytes were washed twice with Krebs-Ringer phosphate buffer with 3% bovine albumin, pH 7.4, and once with medium 1. The fat cells were then resuspended in medium 1 and homogenized at room temperature. The homogenate was centrifuged at 15,000 × *g* for 15 min at 4°C. The pellet was washed by resuspension in ice-cold incubation buffer (75 mM Tris-HCl, 12.5 mM $MgCl_2$, 1.5 mM EDTA, pH 7.65, at 37°C). The suspension was transferred to another ice-cold centrifuge tube to eliminate the remainder of fat and was centrifuged at the same speed and time. This procedure was repeated three more times. Membrane protein (100 μg) was incubated with [^{3}H]dihydroalprenolol ([^{3}H]DHA; 10–120 nM) in a total volume of 150 μl incubation buffer for 12 min with shaking at 37°C. A parallel set of incubations containing 10 μM propranolol was used to determine nonspecific binding. The nonspecific binding was, in general, 30–40% of the total counts bound.

At the end of incubation, 4 ml of ice-cold incubation buffer was added to each tube to terminate the reaction. The contents of the tubes were rapidly filtered under vacuum through Whatman GF/C glass filter paper. The filters were then washed with 3 ml of ice-cold incubation buffer, and the radioactivity retained by the filter was determined for each sample by scintillation spectroscopy. Specific binding of [^{3}H]DHA was defined as the difference between total binding and the amount bound in the presence of 10 μM propranolol. Dissociation constants (K_d) and maximum [^{3}H]DHA binding (B_{max}) were obtained from Scatchard analysis. The adjustment of the experimental data to a linear function was obtained by a least-squares program.[14]

Materials. [^{3}H]DHA and [α-^{32}P]ATP were prepared by New England Nuclear (Boston, MA). ATP, cAMP, creatine phosphokinase, creatine phosphate, and norepinephrine bitartrate were obtained from Sigma (St. Louis, MO). Propranolol was from Ayerst (New York). Streptozocin was kindly provided by Dr. P. W. O'Connell from Upjohn (Kalamazoo, MI).

RESULTS

The effect of norepinephrine (10^{-7}–10^{-4} M) on adenylate cyclase activity in fat cell membranes from control and diabetic rats was investigated. Norepinephrine stimulated adenylate cyclase activity in fat cell membranes prepared from the two groups (Fig. 1*A*). However, the dose dependence for norepinephrine-stimulated adenylate cyclase is shifted to the left in fat cell membranes from diabetic animals. Figure 1*A* also shows that basal adenylate cyclase activity is higher in diabetic fat cell membranes. On the other hand, stimulation of adenylate cyclase by fluoride was the same in fat cell membranes from both control (82 ± 10 pmol · min^{-1} · mg^{-1} protein) and diabetic rats (87 ± 12 pmol · min^{-1} · mg^{-1} protein). To study whether the higher response to norepinephrine observed with membranes from diabetic rats can be suppressed by blocking the β-receptor site, we conducted a new series of experiments with adipocytes from control and diabetic rats with and without treatment with propranolol for 2 days. The results show that adenylate cyclase activity in adipocytes from control rats treated with propranolol presents a higher response to norepinephrine compared with control rats (Fig. 1*B*). It was also observed that propranolol treatment induces a higher basal adenylate cyclase activity. However, adenylate cyclase activity, either basal or in response to norepinephrine, in adipocyte membranes from diabetic rats was not affected by treatment with propranolol (Fig. 1*C*).

To determine whether β-adrenergic-receptor alterations may have influenced the adenylate cyclase activity in response to agonist stimulation, β-receptor characteristics were examined in fat cell membrane preparations from control and diabetic rats with and without treatment with pro-

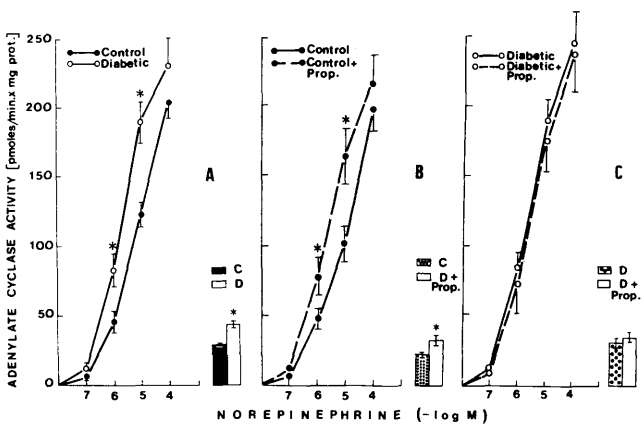

FIG. 1. Effect of norepinephrine on adenylate cyclase activity in fat cell membranes. *A*: control and diabetic rats. *B*: control with and without treatment with propranolol. *C*: diabetic with and without treatment with propranolol. Fat cell membranes (30 μg of protein) were incubated for 10 min at 37°C with [α-^{32}P]ATP in absence or presence of norepinephrine. Adenylate cyclase activity was measured as described in MATERIALS AND METHODS. Results are expressed as pmol cAMP formed · min^{-1} · mg^{-1} protein. Basal activity (in *bars*) was subtracted from each value obtained in presence of indicated concentration of norepinephrine. *Points* represent means ± SE of 7 experiments performed in duplicate. *$P < .05$, statistically significant difference from control.

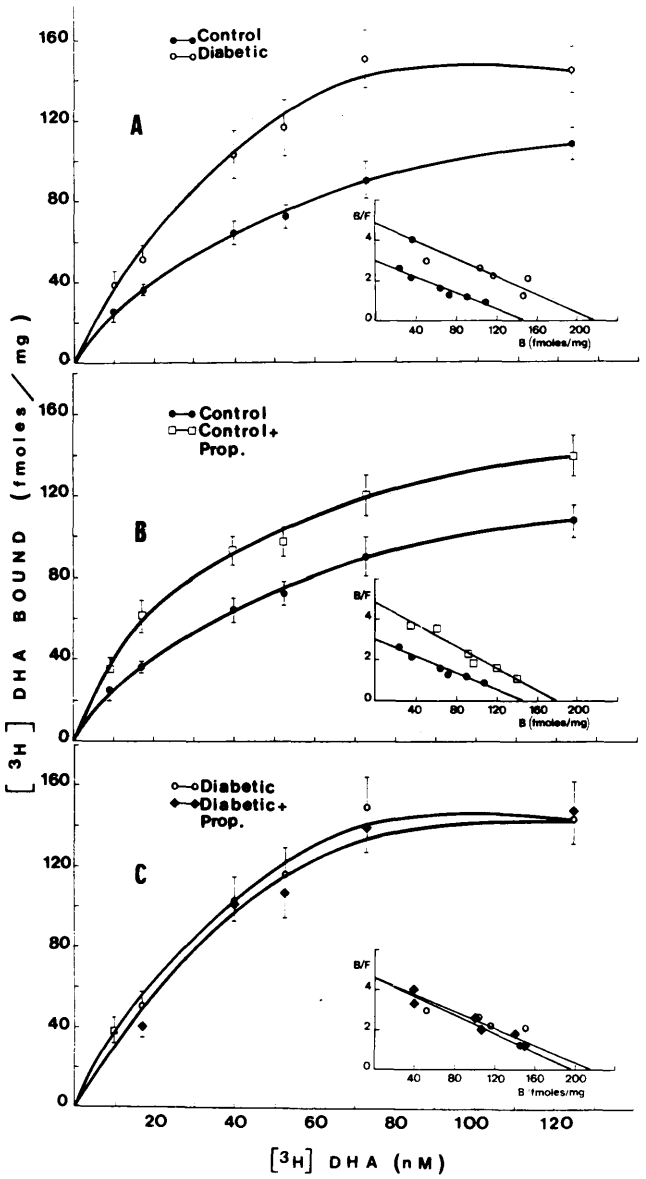

FIG. 2. [^{3}H]dihydroalprenolol ([^{3}H]DHA) binding to fat cell membranes as function of concentration of radioligand in incubation mixture. *A*: control and diabetic rats. *B*: control with and without treatment with propranolol. C: diabetic with and without treatment with propranolol. [^{3}H]DHA (10–120 nM) was incubated with 100 μg protein of fat cell membrane for 12 min at 37°C. At end of period, binding was measured as described in MATERIALS AND METHODS. Values are means ± SE of the mean from 8 separate experiments. *Inset* shows Scatchard analysis of results.

pranolol. As shown in Fig. 2*A*, [^{3}H]DHA specifically bound to diabetic membrane preparations more than to control membranes. A similar effect was found when membranes from control animals treated with propranolol were compared with control membranes (Fig. 2*B*). Propranolol treatment did not affect [^{3}H]DHA binding to diabetic preparations (Fig. 2*C*). Scatchard analysis of the data from these figures was carried out (*inset* in each figure) and the data summarized in Table 1. The results demonstrate a statistically significant increase in β-receptor densities in fat cell membranes prepared from either diabetic or control animals treated with propranolol. Propranolol treatment did not modify the increased density of β-receptors in fat cell membranes from diabetic rats. The dissociation constants were the same for all the preparations.

DISCUSSION

Our study describes the adenylate cyclase activity in response to norepinephrine in adipose tissue from control and diabetic rats. The results show a higher response to norepinephrine in fat cell membranes prepared from diabetic rats. These results agree with those previously reported by Zumstein et al.[3,9] This higher response parallels an increase in β-receptor density. Because fluoride stimulated adenylate cyclase activity to the same extent in both control and diabetic rat fat cell membranes, it can be assumed that the catalytic site of adenylate cyclase is not affected in the diabetic preparations.

The effects of catecholamines on their receptors have been studied extensively in animals. It has been shown in several laboratories that the catecholamine responsiveness of some systems is regulated by adaptive changes in the number of β-adrenergic receptors.[15–22] The increase in β-receptor density in the diabetic fat cell membranes could be explained as an adaptive change due to a lower plasma level of catecholamines. This upregulation of β-adrenergic receptors has been shown in submaxillary glands of chronically reserpine-treated rats in which plasma catecholamine concentration is decreased[22] and in rat cardiac tissue after chronic guanethidine treatment.[19] It has been reported that tissue catecholamine levels in rats with severe diabetes are increased at 6 wk as well as at 13 wk.[23] The investigators suggest that there might be a catecholamine accumulation, which is later accompanied by an impairment of catecholamine secretion in diabetic rats. A resulting lower plasma catecholamine level in diabetic rats would then perhaps result in an increase in receptor density, as was found in our experiments with fat cell membranes prepared from diabetic rats.

In diabetes mellitus, different changes in catecholamine's response have been reported: some investigators found it decreased,[24,25] others reported it increased,[26] and still others both increased and decreased.[27] The different findings suggest that plasma catecholamine level depends on the severity of the diabetic state. This could explain the discrepancy in our results, along with the results reported by Zumstein et al.[3,9] and those reported by other investigators. For instance, La Casa et al.[4] found a decrease in the number of β-adrenergic receptors in diabetic rat adipocytes, but no change in their affinity. However, the same report shows that the dose-response curves of isoproterenol-stimulated adenylate cy-

TABLE 1
β-Adrenergic-receptor density (B_{max}) and dissociation constant (K_d) for [^{3}H]DHA binding to fat cell membranes

Group	B_{max} (fmol/mg)	K_d (nM)
Control	146 ± 8	49 ± 6
Diabetic	216 ± 20*	47 ± 11
Control with propranolol	178 ± 11*	36 ± 5
Diabetic with propranolol	199 ± 15*	44 ± 16

Values are means ± SE and were obtained from the experiments described in Fig. 2, *A–C*.
*$P < .05$ vs. control.

clase activity revealed an increased sensitivity to isoproterenol.

Short-term treatment with propranolol failed to suppress the increased adenylate cyclase activity in response to norepinephrine observed in fat cell membranes from diabetic rats. On the other hand, the same propranolol treatment induced an increase in adenylate cyclase activity in response to norepinephrine as well as in receptor density in fat cell membranes from control animals. The increased sensitivity to β-agonist induced by propranolol has been reported in other tissues after chronic high-dose propranolol treatment.[15,18] Our data show that two intravenous injections of 3 mg/kg propranolol (one each 24 h) induce the same effect observed by others with longer treatment and higher doses.[18] The increase in β-receptor density induced by propranolol in fat cell membranes from control rats is not shown in fat cell membranes from diabetic animals, i.e., the already increased β-receptor number is no longer affected by propranolol treatment.

Our findings suggest that adenylate cyclase activity in response to norepinephrine in adipose tissue is increased during at least a certain period of the diabetic state. The increased response to norepinephrine could be partly explained by an increase in the number of β-adrenergic receptors. We cannot rule out the possibility that the insulin deficiency present in the diabetic state may also induce a higher adenylate cyclase activity in response to catecholamines. Thus, the increased lipolysis associated with diabetes is probably the consequence of increased sensitivity of lipolysis to catecholamines.

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