Mononuclear Leukocyte β_2-Adrenergic Receptors and Adenylate Cyclase Sensitivity in Insulin-dependent Diabetes Mellitus

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SUMMARY

Patients with insulin-dependent diabetes mellitus (IDDM) have been found to have a heightened hyperglycemic response to epinephrine. To determine if patients with IDDM have increased sensitivity of cellular β_2-adrenergic receptor-effector systems, we assessed β_2-adrenergic receptors and adenylate cyclase sensitivities to isoproterenol in partially purified mononuclear leukocyte (MNL) plasma membranes from 10 patients with IDDM (without adrenergic neuropathy) and 10 matched nondiabetic controls. MNL β_2-adrenergic receptor densities (B_{max} = 48 ± 8 fmol [^{3}H] DHA/mg protein in IDDM, 44 ± 3 fmol [^{3}H] DHA/mg protein in controls) and binding affinities (apparent K_D = 0.3 ± 0.07 nM in IDDM, 0.3 ± 0.04 nM in controls) did not differ. Further, MNL adenylate cyclase activities were not significantly different either at baseline (325 ± 86 pmol/mg protein/15 min in IDDM, 275 ± 49 pmol/mg protein/15 min in controls) or in response to isoproterenol (842 ± 229 pmol/mg protein/15 min in IDDM, 608 ± 86 pmol/mg protein/15 min in controls). Thus, the data do not support the presence of a generalized alteration of β-adrenergic receptors or adenylate cyclase sensitivity in IDDM. To the extent that MNL β_2-adrenergic receptors and adenylate cyclase activities reflect those of extravascular catecholamine target cells, these findings suggest that the heightened hyperglycemic response to epinephrine exhibited by patients with IDDM is not due to increased sensitivity of cellular β_2-adrenergic receptor-effector systems and is best attributed to the altered hormonal milieu of the insulin-deficient state. DIABETES *32*:825–829, September 1983.

Epinephrine, the hormone of the adrenal medullae, increases plasma glucose by stimulating hepatic glucose production and limiting glucose utilization.[1] These are the result of both direct and indirect hyperglycemic actions and are mediated by both α- and β-adrenergic receptors.[1-3] Indirect actions include α-adrenergic restraint of insulin secretion.[2,3] β-Adrenergic stimulation of glucagon secretion occurs[1,2,4,5] but glucagon does not appear to normally play an important role in mediating the hyperglycemic response to epinephrine.[1,5] Direct stimulation of hepatic glucose production is mediated predominantly through β-adrenergic mechanisms in humans;[3,6] limitation of glucose utilization may be mediated exclusively through β-adrenergic mechanisms.[3,6] During sustained hyperepinephrinemia glucose production normally increases and then declines to approximate baseline rates, although epinephrine continues to support glucose production.[1-3] In contrast, glucose utilization is limited persistently during sustained hyperepinephrinemia.[1-3]. Thus, hyperglycemia persists during prolonged epinephrine elevations.

Shamoon et al.[7] found that intravenous infusions of epinephrine resulted in greater degrees of hyperglycemia in patients with insulin-dependent diabetes mellitus (IDDM) than in nondiabetic controls. This occurred despite intravenous infusions of insulin in doses sufficient to produce normal plasma glucose concentrations and kinetics before epinephrine infusions. It was the result of a somewhat greater initial increase and a clearly more sustained increase in hepatic glucose production in the patients with IDDM. Theoretically, this heightened hyperglycemic response to epinephrine could be due to the altered hormonal milieu of the insulin-deficient state or increased sensitivity of cellular adrenergic receptor-effector systems per se in patients with IDDM. To test the latter, we examined β_2-adrenergic receptors and adenylate cyclase sensitivity to the adrenergic agonist isoproterenol in partially purified plasma membranes of mononuclear leukocytes obtained from patients with IDDM and from matched nondiabetic controls.

METHODS

Subjects. Ten patients with insulin-dependent diabetes mellitus (IDDM) and 10 matched nondiabetic controls consented

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Received for publication 28 October 1982 and in revised form 16 March 1983.

to participate in this study, which was approved by the Washington University Human Studies Committee. The mean (±SD) duration of diabetes in the patients was 12.0 ± 7.0 yr (range 1.5–25.5 yr). None had nephropathy (serum creatinine < 1.3 g/dl; urine protein < 0.3 g/24 h), four had retinopathy (background in three, proliferative in one), and two had clinical evidence of somatic neuropathy (absent reflexes at the knees and ankles). Clinical characteristics of the patients with IDDM and the nondiabetic controls are summarized in Table 1. As shown, the patient and control groups were matched with respect to sex, age, weight, and height.

Study protocol. Patients with IDDM and nondiabetic controls were admitted to the Washington University Clinical Research Center. On the morning of the study, after an overnight fast and with the subject remaining supine, the resting heart rate and blood pressure were recorded, an intravenous sampling needle was inserted into an antecubital vein and 150 ml of blood was removed for mononuclear leukocyte isolation, membrane preparation, and measurement of β_2-adrenergic receptors and adenylate cyclase sensitivity as described below. Immediately thereafter, 150 ml of 0.9% sodium chloride was infused intravenously. Forty-five minutes later an electrocardiogram was attached and variation in heart rate during deep breathing was determined according to the method of Hilsted et al.[8] Subsequently, plasma samples for norepinephrine and epinephrine were drawn through the sampling needle, and heart rates and blood pressures were recorded, 10 min before, just before, and 2, 5, and 10 min after the subject assumed the standing position. The study being completed, the controls were discharged and the patients were given their usual doses of insulin, fed, and discharged.

Analytical methods. Mononuclear leukocyte (MNL) β_2-adrenergic receptors were assessed with methods previously described from our laboratory,[9,10] as recently modified. MNL were isolated from heparinized whole blood by the method of Böyum.[11] Partially purified MNL plasma membranes were prepared from the MNL fraction by a modification of the method of Davies and Lefkowitz.[12] The MNL fraction (80–85% lymphocytes) was incubated in hypotonic buffer (25 mM Tris HCl, pH 7.0, with 2.5 mM $MgCl_2$) at 4°C for 10 min, sonicated three times (30 s each), and homogenized (100 strokes) with a motor-driven homogenizer (3000 rpm) with a teflon pestle. The homogenate was then centrifuged at 500 × *g* for 10 min with centrifugation of the resultant supernatant at 30,000 × *g* for 15 min to yield a pellet enriched in MNL plasma membranes. An aliquot of the MNL membrane preparation was then frozen in a dry-ice/ethanol bath and stored at −70°C for subsequent measurement of β_2-adrenergic receptors, generally the following day but always less than 5 days after the sample was obtained. As in our earlier studies with MNL homogenates,[10] preliminary studies showed that such storage for up to 10 days had no effect on β_2-adrenergic receptor density or affinity.

MNL β_2-adrenergic receptors were assessed with the antagonist [^{3}H]dihydroalprenolol ([^{3}H]DHA)[9,10] using 10^{-5} M (−)isoproterenol to define nonspecific binding. Ascorbic acid, 10 mM, was present in all tubes. This binding system, employing partially purified MNL plasma membranes, has been extensively characterized in our laboratory: specific [^{3}H]DHA binding exhibits rapid association and dissociation and is saturable, stable over time, temperature-dependent with respect to rate but not maximum binding, stereospecific [(−) propranolol displaces more potently than (+)propranolol; (−)isoproterenol displaces more potently than (+)isoproterenol], and agonist-specific (isproterenol > epinephrine > norepinephrine). The latter pattern of displacement by agonists is typical of binding to a β-adrenergic receptor of the β_2 subtype as is the finding that the nonselective β-adrenergic antagonist propranolol displaces [^{3}H]DHA binding more effectively than does the relatively selective β_1-adrenergic antagonist metoprolol. Specific [^{3}H]DHA binding is to an apparently homogenous class of receptors (linear Scatchard plots) and is of high affinity with apparent dissociation constants of ~0.5 nM. Maximum [^{3}H]DHA specific binding (B_{max}) and apparent dissociation constants (K_D), both estimated by Scatchard analysis, are used in this article as measures of MNL β_2-adrenergic receptor density and binding affinity, respectively.

Adenylate cyclase activity in the MNL plasma membrane preparations was measured, on the day the sample was obtained, with the method of Salomon et al.[13] based on the formation of cyclic [^{32}P]AMP from [α-^{32}P]ATP. Preliminary studies showed that MNL plasma membrane adenylate cy-

TABLE 1
Clinical characteristics of patients with insulin-dependent diabetes mellitus and nondiabetic controls

	IDDM	Controls	P
Number	10	10	—
Sex (female/male)	8/2	8/2	—
Age (yr)	24.7 ± 1.3	23.8 ± 1.2	NS
Weight (kg)			
Females	62.7 ± 2.6	56.5 ± 2.6	NS
Males	63.5 ± 1.4	72.5 ± 1.4	NS
Height (cm)			
Females	162.2 ± 2.9	168.2 ± 3.0	NS
Males	169.5 ± 0.8	180.3 ± 1.6	NS
Fasting plasma glucose (mg/dl)	138 ± 18	81 ± 1	<0.01
Hemoglobin A_1 (%)	8.2 ± 0.6	—	—
Resting heart rate (bpm)	76 ± 3	66 ± 3	<0.05
Respiratory variation in heart rate (bpm)	15 ± 2	21 ± 2	<0.05
Resting blood pressure (mm Hg)			
Systolic	108 ± 1	107 ± 2	NS
Diastolic	66 ± 2	68 ± 2	NS

clase activity is stimulated by agonists in the potency sequence isoproterenol > epinephrine > norepinephrine and that isoproterenol-stimulated adenylate cyclase is more effectively antagonized by the nonselective β-adrenergic antagonist propranolol than by the relatively selective β_1-adrenergic antagonist metoprolol. Further, the selective β_2-adrenergic partial agonist zinterol[14] both inhibits isoproterenol-stimulated adenylate cyclase activity and stimulates basal adenylate cyclase activity in this MNL membrane preparation. These findings are typical of adenylate cyclase stimulation mediated by a β_2-adrenergic receptor. In the present study, MNL plasma membrane adenylate cyclase activity was measured at 30°C in the absence of (−)isoproterenol and in the presence of 10^{-9} to 10^{-4} M (−)isoproterenol over 15 min. Ascorbic acid, 10 mM, was present in all assays.

Plasma norepinephrine and epinephrine concentrations were measured with a single isotope derivative method[15] employing 50-μl samples. Plasma membrane protein was measured with the method of Lowry et al.[16]

Statistical methods. Data were compared with *t* tests for unpaired or paired data when appropriate. Scatchard plots were subjected to linear regression analysis to define the slopes and intercepts. Data are expressed as the mean ± SE except where specified.

RESULTS

Autonomic neural function. As shown in Table 1, mean resting heart rate was significantly higher and respiratory variation in heart rate significantly lower in the patients with IDDM. These indicate some degree of impairment of parasympathetic function in the patients as a group, although only two patients had clearly abnormal[8] respiratory variation in heart rate (less than 10 bpm). There were no significant differences in blood pressure or plasma norepinephrine and epinephrine concentrations, in either the supine or standing positions, between the two groups (Figure 1). Thus, there was no detectable sympathetic neural impairment in the patients, although the sensitivity of the approach used is not great and mild impairment cannot be categorically excluded.

β_2-adrenergic receptors. As shown in Figure 2, β_2-adrenergic receptor densities (B_{max} = 48 ± 8 fmol/mg protein in IDDM, 44 ± 3 fmol/mg protein in controls) and binding affinities (apparent K_D = 0.3 ± 0.07 nM in IDDM, 0.3 ± 0.04 nM in controls) were not significantly different in MNL preparations from the two groups.

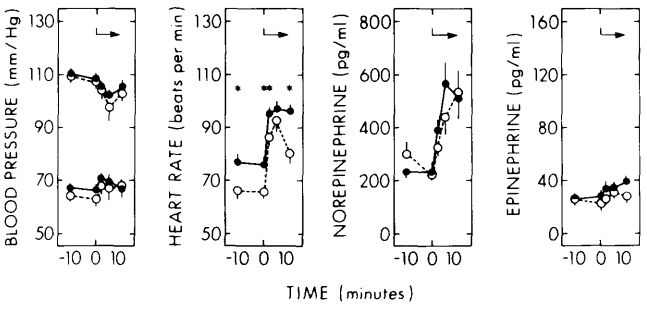

FIGURE 1. Mean (±SE) blood pressures (systolic above, diastolic below), heart rates, plasma norepinephrine concentrations, and plasma epinephrine concentrations in 10 patients with insulin-dependent diabetes mellitus (IDDM, solid circles) and 10 matched nondiabetic subjects (controls, open circles) in the supine position and after assumption of the standing position (arrows). Asterisks denote significant differences between the patient and control values.

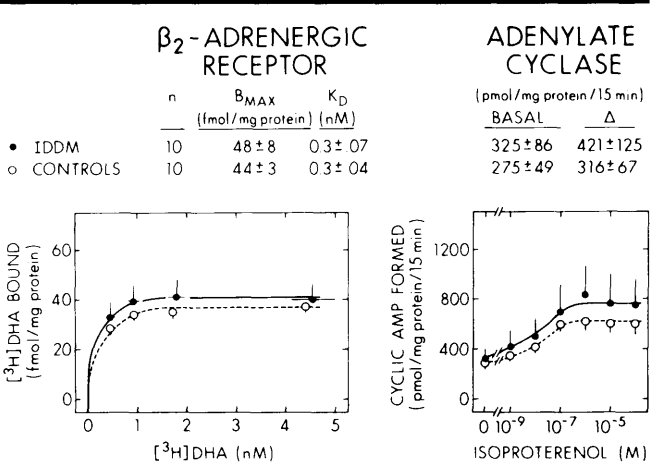

FIGURE 2. Left: Mean (±SE) specific binding of [^{3}H]dihydroalprenolol ([^{3}H]DHA), at the indicated mean (±SE) [^{3}H]DHA concentrations, to partially purified MNL plasma membranes from 10 patients with insulin-dependent diabetes mellitus (IDDM, solid circles and continuous curve) and from 10 matched nondiabetic subjects (controls, open circles and interrupted curve). Mean (±SE) β_2-adrenergic receptor densities (B_{max}) and binding affinities (apparent dissociation constants, K_D), determined by Scatchard analysis, are shown above the binding curves. Right: Mean (±SE) adenylate cyclase activities and the responses to isoproterenol in vitro in partially purified MNL plasma membranes from patients with IDDM and matched controls (symbols same). Basal adenylate cyclase activities and the increments (△) in response to 10^{-6} M isoproterenol are shown above the dose-response curves.

Adenylate cyclase. MNL plasma membrane adenylate cyclase activity increased from 275 ± 49 pmol/mg protein/15 min in the absence of (−)isoproterenol to 608 ± 86 pmol/mg protein/15 min ($P < 0.001$) in the presence of 10^{-6} M (−)isoproterenol in samples from nondiabetic controls. Similarly, in samples from patients with IDDM, MNL adenylate cyclase increased from 325 ± 86 pmol/mg protein/15 min in the absence of (−)isoproterenol to 842 ± 229 pmol/mg protein/15 min ($P < 0.01$) in the presence of 10^{-6} M (−)isoproterenol. As shown in Figure 2, there were no significant differences in MNL adenylate cyclase activities between samples from patients with IDDM and those from nondiabetic controls either in the absence of (−)isoproterenol or in the presence of 10^{-9} to 10^{-4} M (−)isoproterenol. To further address the issue of cyclase sensitivity to agonist, the concentration of isoproterenol required to produce half-maximal stimulation of MNL adenylate cyclase activity was determined from plots of the dose-response curves for each patient and each control. Again, there was no significant difference ($4.2 \pm 1.2 \times 10^{-8}$ M in IDDM; $1.6 \pm 0.4 \times 10^{-8}$ M in controls).

DISCUSSION

There is considerable evidence consistent with the assumption that the regulation of β-adrenergic receptors on circulating leukocytes faithfully reflects that of β-adrenergic receptors on extravascular catecholamine target cells. Tohmeh and Cryer[9] found that isoproterenol-induced increments in mononuclear leukocyte (MNL) β-adrenergic receptor densities are paralleled by increments in the cardiac

chronotropic response to isoproterenol in vivo in normal human subjects. Fraser et al.[17] found MNL β-adrenergic receptor densities to be inversely related to plasma and urinary norepinephrine and epinephrine in normal humans consuming diets ranging from 10 to 400 mmol of sodium per day. Further, they found MNL β-adrenergic receptor densities and cardiac chronotropic sensitivities to isoproterenol in vivo to be correlated. β-adrenergic receptor densities on circulating cells are decreased by administration of agonists,[18] which is known to decrease the adenylate cyclase responsiveness of lymphocytes to agonists in vitro and to decrease the bronchodilator response to agonists in vivo. Further, MNL β-adrenergic receptor densities are increased in patients with hypoadrenergic states who exhibit increased sensitivity to agonists in vivo.[19] Importantly, in rats infused with propranolol, Aarons and Molinoff[20] found parallel changes in β-adrenergic receptor density in lymphocytes, heart, and lung. Thus, considerable data support the premise that MNL β_2-adrenergic receptor characteristics reflect those of adrenergic receptors in extravascular catecholamine target tissues. However, other data suggest that this relationship may not be invariable. Tashkin et al.[21] found administration of the β_2-adrenergic agonist terbutaline to normal humans to be associated with decrements in MNL β-adrenergic receptors and cyclic AMP responses to isoproterenol in vitro but not with decrements in the bronchodilator responses to subcutaneous terbutaline. However, the bronchodilator response to inhaled isoproterenol was diminished. Also, there are reports of divergent effects of hormones on β-adrenergic receptors. Davies and Lefkowitz[12] reported that cortisone administration to humans resulted initially in a decrease in MNL, but an increase in polymorphonuclear, β-adrenergic receptor densities, although the changes were small. There is substantial evidence that thyroid hormone excess results in increased β-adrenergic receptors and adenylate cyclase sensitivity in rat myocardium[22–26] whereas these are decreased in rat hepatocytes.[27] However, the data concerning the effect of thyroid hormone excess on MNL β-adrenergic receptors are less clear-cut. MNL β-adrenergic receptor densities have been reported to be unchanged by thyroid hormone administration to rats,[26] not different from those of euthyroid controls in thyrotoxic humans,[28,29] increased in short-term, triiodothyronine-induced thyrotoxicosis in normal humans,[10] and increased in spontaneously thyrotoxic humans compared with the same patients after return to euthyroidism.[30]

The present data demonstrate that β_2-adrenergic receptor densities and binding affinities and adenylate cyclase sensitivities to isoproterenol in partially purified MNL plasma membranes obtained from patients with IDDM are not demonstrably different from those in MNL membranes from matched nondiabetic controls. We have noted marked intraindividual as well as interindividual variation in MNL β_2-adrenergic receptor measurements and suggested that this represents biologic as well as analytical variation.[10] Substantial interindividual variation in MNL adenylate cyclase activities, as well as β_2-adrenergic receptor densities and binding affinities, is apparent in the present data. Given this variation and the fact that mean maximal isoproterenol-stimulated adenylate cyclase activities tended to be higher in MNL membranes from patients with IDDM, one might suggest that the study of a large group of patients might disclose significantly higher maximal adenylate cyclase responses in IDDM MNL membranes. We would point out, however, that mean concentrations of isoproterenol required to produce half-maximal adenylate cyclase stimulation tended to be higher in MNL membranes from patients with IDDM suggesting, if anything, decreased adenylate cyclase sensitivity in IDDM MNL membranes. Thus, the data do not support the presence of a generalized alteration of β-adrenergic receptors or adenylate cyclase sensitivity in IDDM. To the extent that MNL β_2-adrenergic receptors and adenylate cyclase activities reflect those of extravascular catecholamine target cells, as discussed earlier, these findings suggest that patients with IDDM do not have increased sensitivity of cellular β_2-adrenergic receptor-effector systems per se.

It should be noted that the patients with IDDM reported here did not have detectable diabetic adrenergic neuropathy. It is quite possible that patients with adrenergic neuropathy have increased adrenergic receptor densities as has been reported in other hypoadrenergic states.[19]

In normal human subjects, the hyperglycemic response to epinephrine is in large part due to α-adrenergic suppression of insulin secretion.[2] In contrast, since patients with IDDM cannot suppress insulin secretion, it is likely that their hyperglycemic response to epinephrine is largely the result of direct hyperglycemic actions of the hormone. An enhanced glucagon secretory response to epinephrine occurs in patients with IDDM[4,31] and this indirect action contributes to the hyperglycemic response in dogs with uncontrolled (but not well controlled) diabetes.[32,33] Both the direct hyperglycemic actions of epinephrine[3,6] and the glucagon secretory response to epinephrine[1,2,4,5] are mediated predominantly through β-adrenergic mechanisms, as discussed earlier. Thus, in contrast to nondiabetic individuals, the hyperglycemic response to epinephrine is mediated predominantly through β-adrenergic mechanisms in patients with IDDM. This conclusion is supported by in vivo data. It has been shown that the nonselective β-adrenergic antagonist propranolol reduces[4] or virtually obliterates[34] the hyperglycemic response to epinephrine in patients with IDDM. In contrast, propranolol reduces the hyperglycemic response to epinephrine minimally[2] or not at all[34] in normal human subjects in whom α-adrenergic suppression of insulin secretion is in large part responsible for hyperglycemia.[2] Although the β-adrenergic receptor subtype that mediates the direct hyperglycemic actions of epinephrine in man is not known, hepatic β-adrenergic receptors have been reported to be of the β_2 subtype in rats.[35]

In view of the evidence presented here that patients with IDDM do not have increased cellular sensitivity of β_2-adrenergic receptor-effector systems per se, coupled with the evidence that the hyperglycemic actions of epinephrine are mediated through β_2-adrenergic receptors in such patients, the heightened hyperglycemic response to epinephrine exhibited by patients with IDDM[7] is best attributed to the altered hormonal milieu of the insulin-deficient state. We suggest that increased responsiveness of hepatic glucose production to epinephrine in patients with IDDM is the result of hepatic portal venous hypoinsulinemia, the heightened glucagon secretory response, or both. Proof of this hypothesis awaits studies specifically designed to test it.

ACKNOWLEDGMENTS

The authors are grateful to the nursing staff of the Washington University Clinical Research Center for their assistance with the studies reported here and to Theresa Lautner for typing the manuscript.

This work was supported by USPHS grants AM27085, AM20579, and RR00036. Dr. Sérusclat was supported by a grant from the French Ministry of Foreign Affairs during the performance of these studies.

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