Effects of Experimental Diabetes on Adrenergic and Cholinergic Receptors of Rat Myocardium

R. SANDERS WILLIAMS, THOMAS F. SCHAIBLE, JAMES SCHEUER, AND REBECCA KENNEDY

SUMMARY

To more fully characterize the alterations in myocardial adrenergic and cholinergic receptors induced by the diabetic state, we investigated the binding characteristics of (−) [^{3}H] dihydroalprenolol to beta adrenergic receptors (bAR), [^{3}H] prazosin to alpha adrenergic receptors (aAR), and [^{3}H] quinuclidinyl-benzilate to muscarinic cholinergic receptors (MCR) in myocardial membranes derived from rats 8 wk after treatment with streptozotocin. We also studied an equal number of animals from three control groups: free-eating nondiabetics, pair-weighted nondiabetics, and streptozotocin-treated animals treated daily with insulin.

Diabetic hearts demonstrated 27% fewer bAR ($P < 0.01$) and 31% fewer aAR ($P < 0.01$) than free-eating controls, without changes in MCR, and without changes in antagonist affinity, agonist affinity, or agonist slope factor (pseudo-Hill coefficient) for any class of receptors. Food restriction had no effect on receptor characteristics, and treatment of diabetic rats with insulin prevented any downregulation of cardiac bAR or aAR.

The parallel decrease in both bAR and aAR suggests that streptozotocin-induced hypothyroidism is not the primary causative factor of bAR downregulation in this model, since hypothyroidism produces upregulation of aAR. Furthermore, the lack of change in cardiac MCR suggests that the adrenergic receptor alterations are not the result of nonspecific abnormalities of protein synthesis in the diabetic heart. Further studies are required to establish the physiologic significance of these receptor alterations, but these data support the hypothesis that altered adrenergic receptor properties may underlie, at least in part, the chronotropic and inotropic abnormalities of cardiac performance that are associated with the diabetic state. DIABETES 32:881–886, October 1983.

Portions of the data included here were presented in abstract form at the national meeting of the AFCR/ASCI/AAP in Washington, D.C., in May 1982, and at the annual scientific sessions of the American Heart Association in Dallas, Texas, in November 1982.

From the Departments of Medicine and Physiology, Duke University Medical Center, Durham, North Carolina; and Montefiore Hospital, Albert Einstein College of Medicine, Bronx, New York.

Address reprint requests to R. Sanders Williams, M.D., Box 3945, Duke University Medical Center, Durham, North Carolina 27710.

Received for publication 31 December 1982.

Abnormalities of cardiac function are widely prevalent among human diabetics, as evidenced by both the occurrence of clinically detectable congestive heart failure, and by the noninvasive assessment of cardiac performance in asymptomatic subjects.[1,2] Furthermore, the abnormal cardiovascular physiology associated with human diabetes appears to occur even in the absence of myocardial infarction or other clinical manifestations of coronary atherosclerosis.[1–3]

Animal models have been previously employed to investigate the underlying mechanisms of the cardiac abnormalities associated with diabetes mellitus, and several possible pathophysiologic links at the biochemical level have been established.[4–12] ATP-ase activity of contractile proteins is reduced in diabetic rats[4,5] concomitantly with reductions in mechanical parameters of ventricular systolic performance in isolated hearts.[6] In addition, subnormal chronotropic, inotropic, and biochemical responsiveness to adrenergic stimulation has been observed in hearts of diabetic rats in several laboratories,[7–9] paralleling reductions in peak heart rate and oxygen consumption during exercise in intact animals.[10] One prior report has suggested that these abnormalities could be attributable to a reduction in the density of myocardial beta adrenergic receptors (bAR).[11] We undertook the experiments described here to more fully characterize the effects of experimental diabetes mellitus on all the major classes of cardiac receptors for neurotransmitters, and to provide further clarification about the specific aspects of the diabetic state that contribute to alterations in receptor characteristics.

METHODS

Treatment of animals. Male Wistar rats from the same shipment, weighing 180–200 g, were randomly allocated such

that half the animals received an injection of streptozotocin (50 mg/kg dissolved in 0.05 M citrate, pH 4.5) into the tail vein, and the other half were not injected. After 4 days, blood samples were withdrawn from the tail vein of conscious restrained rats to ensure the successful induction of diabetes in the streptozotocin group, and animals with blood glucose less than 300 mg/dl were excluded from further study.

At this point the diabetic animals were subdivided into two groups: a treated diabetic (TD) group that received daily insulin injections (3–5 U/day; PZI, Eli Lilly, Indianapolis, Indiana) adjusted to maintain morning blood glucose levels at approximately 100 mg/dl; and untreated diabetics. Both groups were fed ad libitum. The nondiabetic animals were also subdivided into two groups: those fed ad libitum, and a second group (pair-weighted) whose food intake was restricted (about 40%) so as to maintain their rate of growth equal to that of the untreated diabetic group.

Two separate sets of animals were treated in this fashion. The first set (experiment A) consisted of 16 animals in each of the four treatment groups; their hearts were used for analysis of bAR. The second set (experiment B) consisted of 15–19 animals in each treatment group; their hearts were used for analysis of alpha adrenergic (aAR) and muscarinic cholinergic receptors (MCR), and for sialic acid measurements.

Eight weeks after streptozotocin injection animals were killed under ether anesthesia and the hearts were removed, weighed, frozen, and stored at −80°C until biochemical studies were performed.

Blood glucose was measured using hexokinase and glucose-6-phosphate dehydrogenase.[13]

Preparation of cardiac membrane homogenates and radioligand binding experiments. Individual hearts were weighed, then thawed and rinsed in 0.9% NaCl at 0°C. Membrane homogenates were prepared by the method of Baker and Potter.[14] Radioligand binding experiments were performed as described in previous publications from this laboratory.[15] Briefly, approximately 900 mg of membrane protein were incubated in a 0.9-ml assay volume containing 50 mM Tris HCl, 16.7 mM $MgCl_2$, pH 7.4, in the presence of either varying concentrations of radioligand (saturation curves) or in the presence of a fixed radioligand concentration and varying concentrations of a competing unlabeled ligand (competition curves) such as (−) isoproterenol (Sigma, St. Louis, Missouri), or (−) norepinephrine (Sigma). (−) [^{3}H] Dihydroalprenolol (DHA) (Amersham, Arlington Heights, Illinois; specific activity 80 Ci/mmol) was used to identify bAR; [^{3}H] prazosin (PRAZ) (Pfizer, New York, New York; specific activity 33 Ci/mmol) was used to identify aAR; and [^{3}H] quinuclidinylbenzilate (racemic) (QNB) (New England Nuclear, Boston, Massachusetts; specific activity 40 Ci/mmol) was used to identify MCR. Competitive binding assays of agonist binding to MCR labeled with QNB were not performed due to an inadequate number of hearts to complete the studies. Incubations were conducted either for 20 min at 25°C (DHA and PRAZ) or for 60 min at 37°C (QNB). Nonspecific binding for DHA, PRAZ, and QNB was defined as the residual binding in the presence of 10^{-5} M (±) propranolol (Sigma), 10^{-5} M phentolamine (Ciba-Geigy), or 10^{-6} M unlabeled (−) QNB (New England Nuclear). We quantitated protein by the method of Lowry et al.,[16] and sialic acid by the method of Warren.[17]

Data analysis. Body weight, heart weight, blood glucose, membrane protein yield, and membrane sialic acid content were compared by one-way analysis of variance. Saturation curves were analyzed by a nonlinear least-squares curve fitting procedure employing a generalized model for complex ligand-receptor interactions and performed using an iterative program in PL/1 on a PDP 11/45 computer. The details of this analytic approach have been published previously.[18] Competition curves were analyzed by a four-parameter generalized model for dose-response relationships as previously described.[19] The statistical significance of differences between treatment groups in receptor characteristics determined from the curve-fitting procedures was assessed by F ratio test comparing the residual variance between the observed data and the fitted curves when each treatment group was analyzed independently, and the residual variance between the observed data and the hypothetical curves assuming the null hypothesis for each parameter estimate (e.g., K_D DHA in diabetic membranes equals K_D DHA in control membranes).

RESULTS

The morphologic measurements and glucose levels produced by each of the treatment regimens are detailed in Table 1. Diabetic rats were uniformly severely hyperglycemic (range: 317–492 mg/dl), while glucose levels in insulin-treated animals were uniformly less than 190 mg/dl. Five of

TABLE 1
Body weight, heart weight, and blood glucose within each treatment group

Treatment group	Diabetic	Control	Pair-weighted	Treated diabetic
Experiment A				
N	16	16	16	16
Body weight (g)	303 ± 33†	448 ± 27*	308 ± 15†	457 ± 24*
Heart weight (g)	0.81 ± 0.10†	1.01 ± 0.08*	0.72 ± 0.04†,‡	1.04 ± 0.06*
Blood glucose (mg/dl)	409 ± 51†	108 ± 16*	92 ± 7*	80 ± 38*
Experiment B				
N	15	18	17	19
Body weight (g)	222 ± 41†	396 ± 32*	230 ± 8†	379 ± 18*
Heart weight (g)	0.61 ± 0.08†	0.92 ± 0.07*	0.54 ± 0.02†,‡	0.90 ± 0.04*
Blood glucose (mg/dl)	395 ± 44†	112 ± 10*	99 ± 9*	96 ± 42*

Results shown as mean ± SD.
*$P < 0.001$ versus diabetic; †$P < 0.001$ versus control; ‡$P < 0.01$ versus diabetic.

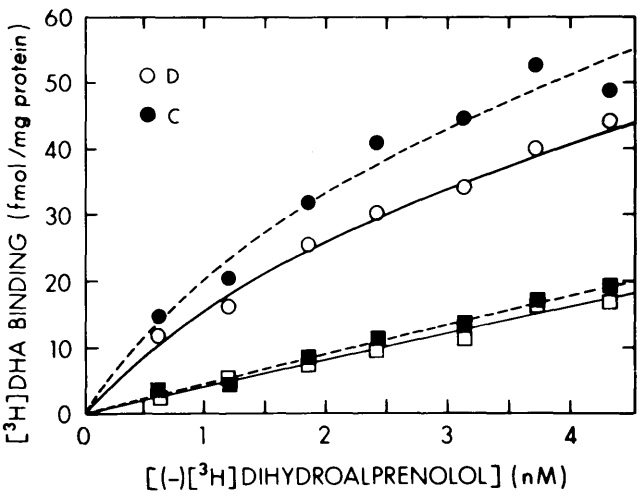

FIGURE 1. Binding of (−) [^{3}H] dihydroalprenolol (DHA) to cardiac bAR from free-eating control (closed symbols) and diabetic (open symbols) rats. Data points represent the means of duplicate determinations in seven experiments, each using one heart from each group. Circles depict total binding at each concentration of DHA and squares depict binding in the presence of 10^{-5} M (±) propranolol (nonspecific binding). Lines indicate the computer-derived best fit of the binding data as described in the text. Similar binding data from insulin-treated and pair-weighted animals were used to calculate the parameter estimates listed in Table 2, but the actual binding data are not shown to conserve space.

35 animals showed glucose levels less than 50 mg/dl at the time of death, suggesting that hypoglycemia was occasionally produced by the insulin regimen. Insulin treatment maintained normal growth, and heart weights in the insulin-treated group were identical to free-eating controls. Diabetics demonstrated a significant increase in heart mass relative to pair-weighted nondiabetic animals.

Membranes from diabetic animals demonstrated reduced specific binding of DHA to bAR in comparison with either of the three control groups at all concentrations of radioligand studied (Figure 1), reflecting a reduced receptor number without significant changes in receptor affinity for DHA (Table 2). There was a trend toward an increased beta receptor number in insulin-treated diabetics in comparison with either free-eating or food-restricted nondiabetic controls, but there was a greater variance in the parameter estimates from the treated diabetic group, and this difference did not reach statistical significance (Table 2).

As expected for studies performed in washed membranes in the absence of exogenous guanine nucleotide, competition curves for (−) isoproterenol were shallow (Figure 2) with slope factors significantly less than 1.0 (Table 2), indicating the complex ligand-receptor interaction involving two classes of binding affinities that is typical for beta adrenergic agonists in competitive binding assays.[18] There were no significant differences between the four treatment groups in either the slope factor or the half-maximal inhibitory concentration (EC_{50}) for (−) isoproterenol binding to bAR. The EC_{50} can be construed as an "average" affinity, and in view of the lack of apparent differences in either the position or shape of the (−) isoproterenol competition curves between the treatment groups, we did not perform further mathematical modeling on these curves to quantify the separate low-affinity and high-affinity components of agonist binding in these membranes.

TABLE 2
Myocardial receptor characteristics within each treatment group

Treatment group	Diabetic	Control	Pair-weighted	Treated diabetic
Membrane protein yield (mg/g heart wet weight)	29 ± 2	26 ± 2	31 ± 2	25 ± 2
Sialic acid content (μg/mg protein)	10.6 ± 1.2	9.5 ± 1.0	8.7 ± 0.8	8.5 ± 0.8
Beta adrenergic receptors labeled with (−) [^{3}H] dihydroalprenolol				
Receptor number (Bmax) (fmol/mg protein)	39 ± 5†	53 ± 6*	52 ± 9*	77 ± 12*
Antagonist affinity (K_D DHA) (nM)	2.3 ± 0.6	2.3 ± 0.6	2.5 ± 1.0	3.9 ± 1.1
Agonist slope factor [(−) isoproterenol]	0.62 ± 0.12	0.60 ± 0.09	0.70 ± 0.11	0.65 ± 0.10
Agonist affinity (EC_{50}) (nM)	78 ± 24	51 ± 15	70 ± 11	75 ± 20
Alpha adrenergic receptors labeled with [^{3}H] prazosin				
Receptor number (Bmax) (fmol/mg protein)	36 ± 2†	52 ± 3*	50 ± 4*	48 ± 3*
Antagonist affinity (K_D prazosin) (nM)	0.16 ± 0.01	0.16 ± 0.02	0.14 ± 0.02	0.15 ± 0.03
Agonist slope factor [(−) epinephrine]	1.07 ± 0.24	1.08 ± 0.18		
Agonist affinity (EC_{50}) (μM)	9.5 ± 2.1	7.9 ± 1.3		
Muscarinic cholinergic receptors labeled with [^{3}H] quinuclidinylbenzilate				
Receptor number (Bmax) (fmol/mg protein)	150 ± 18	147 ± 5	123 ± 21	127 ± 14
Antagonist affinity	0.19 ± 0.05	0.14 ± 0.06	0.14 ± 0.01	0.14 ± 0.14

Results shown as mean ± SE.
*$P < 0.01$ versus diabetic; †$P < 0.01$ versus control.

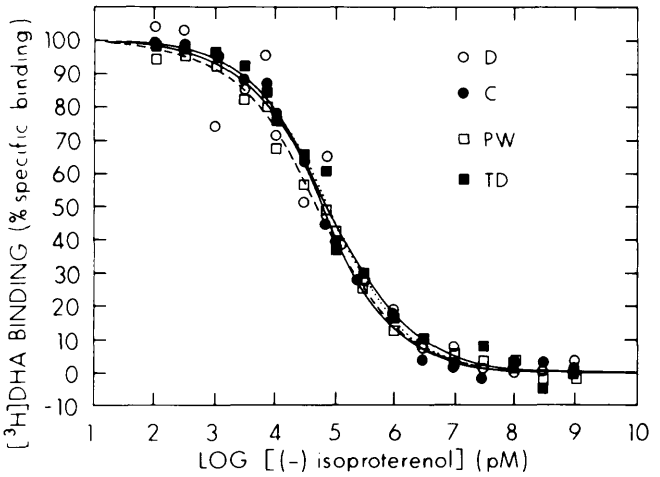

FIGURE 2. Competition by (−) isoproterenol for bAR labeled by DHA (3.0 nM) in myocardial homogenates from diabetic (open circles), control (solid circles), pair-weighted (open squares), and insulin-treated diabetic (solid squares) rats. Data points represent the means of duplicate determinations in five experiments, each using one heart from each group. Lines depict the computer-derived best fit of the binding data as described in the text.

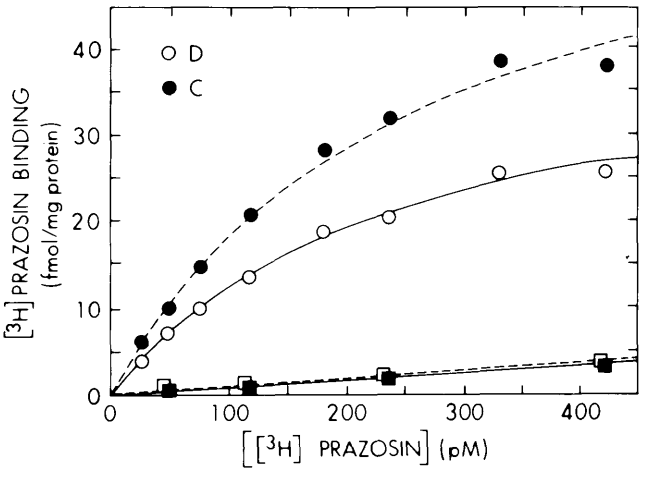

FIGURE 3. Binding of [^{3}H] prazosin to cardiac aAR from control and diabetic rats. Symbols are explained in the legend to Figure 1, except that data points are derived from duplicate determinations in six experiments and nonspecific binding was defined as residual binding in the presence of 10^{-5} M phentolamine.

In a similar fashion to DHA binding, cardiac membranes from diabetic animals demonstrated reduced specific binding of PRAZ than membranes from either control group (Figure 3 and Table 2), again indicating a reduced receptor number without changes in receptor affinity for the radioligand, or for the agonist (−) epinephrine. In contrast, we could discern no significant effects of experimental diabetes on QNB binding to myocardial MCR (Figure 4 and Table 2).

Diabetes did not alter the yield of membrane protein per gram of initial cardiac mass produced by our homogenization procedure, nor did it significantly alter the sialic acid content of the membranes relative to the protein concentrations (Table 2).

DISCUSSION

The magnitude of downregulation of cardiac bAR number that we observed in diabetic rats is nearly identical to that noted previously by Savarese and Berkowitz,[11] and also approximates the magnitude of the reduction in isoproterenol-induced cyclic AMP accumulation and protein kinase activity reported by Ingebretsen et al.[7] in a slightly different experimental model (96 h of insulin withdrawal in alloxan-treated rats). Our new observation that competitive binding curves for isoproterenol are not altered by the diabetic state provides support for the conclusion that the reduction in bAR number, rather than changes in the interaction of the receptor with the guanine nucleotide regulatory protein, accounts for the reported reductions in hormone-sensitive adenylate cyclase activity, since other interventions that alter receptor-cyclase coupling do perturb the shape of agonist competition curves.[20,21]

Our current data also contribute new information regarding the specific aspects of the diabetic state that produce bAR downregulation in the myocardium. First, since no receptor alterations are observed in our pair-weighted or insulin-treated diabetic group, we can conclude that bAR downregulation in diabetic rats is attributable neither to diminished caloric intake and altered growth rates, nor to toxic effects of streptozotocin distinct from its diabetogenic activity. Streptozotocin has been reported to produce mild hypothyroidism in rats,[4] which could contribute to downregulation of cardiac bAR.[22] However, our observation that cardiac aAR in diabetic hearts changed in a direction opposite to the effects produced by hypothyroidism[22] supports the conclusion that hypothyroidism does not contribute substantially to bAR downregulation in this model. Our data also indicate that reduced bAR number is not a laboratory artifact produced by nonuniformity of the homogenization techniques used in these broken cell preparations, since the yields of membrane protein and the content of the sarcolemmal membrane marker sialic acid were similar in all treatment groups.

Regarding cardiac aAR and diabetes, Miller et al.[23] observed enhanced epinephrine-stimulated cardiac phosphorylase kinase activity in the presence of propranolol in al-

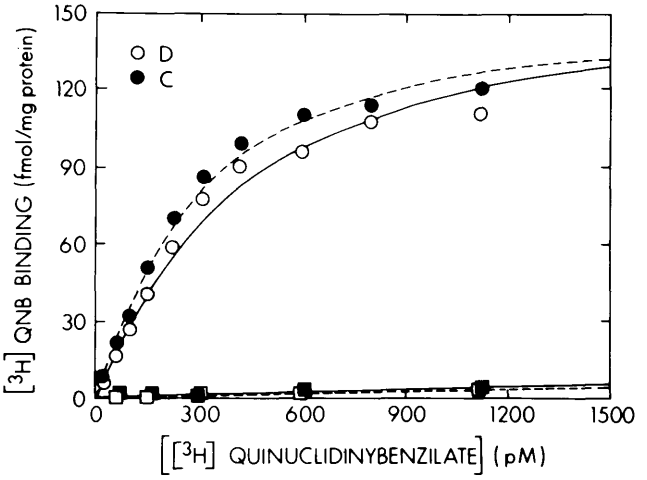

FIGURE 4. Binding of [^{3}H] quinuclidinylbenzilate (QNB) to cardiac MCR from control and diabetic rats. Symbols are explained in the legend to Figure 1, except that data points are derived from six experiments and nonspecific binding was defined as residual binding in the presence of 10^{-6} M unlabeled (−) QNB.

loxan-treated rats and hypothesized that this phenomenon could represent augmented aAR modulation of phosphorylase activity. Our observation that downregulation of cardiac aAR parallels bAR downregulation and that agonist binding to aAR is unaltered in diabetic rats does not exclude diabetes-induced augmentation of aAR-mediated processes. However these data indicate that any such changes are not mediated at the receptor level, but must necessarily involve intracellular biochemical events distal to the hormone-receptor interaction.

The absence of diabetes-induced changes in cardiac MCR density, as contrasting with the results for adrenergic receptors, permits two further conclusions. First, it suggests that downregulation of cardiac aAR and bAR is not a nonspecific phenomenon due to generalized protein catabolism or to generalized alterations of membrane lipids. Second, it suggests that subnormal physiologic responsiveness to acetylcholine in diabetic animals as reported by Foy and Lucas,[8] is not mediated through perturbation of the cardiac MCR.

The experiments presented here do not address the physiologic or clinical significance of the receptor alterations that we observed. Neither do they exclude the possibility of additional effects of diabetes on biochemical events occurring later in the cascade between the agonist-receptor interaction and the ultimate physiologic effects of hormonal stimulation of the heart. Indeed, the work of other investigators suggests that postreceptor events at the level of phosphorylase activation are induced by diabetes.[7,23] However, our current data do provide a greater degree of resolution regarding the effects of diabetes on the heart at the biochemical level, and are compatible with the hypothesis that receptor alterations may provide a molecular mechanism for at least some of the abnormalities of cardiac physiology that accompany the diabetic state. It is of interest that similar reductions in bAR number to those noted here in diabetic rats have been recently reported in hearts taken from human subjects undergoing cardiac transplantation for congestive heart failure for cardiomyopathies produced by etiologies other than diabetes.[24]

Finally, whereas our data help to define the aspects of experimental diabetes that produce adrenergic receptor downregulation, the ultimate cause of this phenomenon remains unknown, and must be resolved by further investigations. It seems feasible that agonist-induced downregulation[25] of both aAR and bAR may ensure from chronic elevations in the concentration of endogenous catecholamines to which the heart is exposed in diabetics,[26] but we did not obtain plasma catecholamine measurements to directly address the correlation between agonist concentrations and receptor density in the animals. A more intriguing possibility is that adrenergic receptors undergo nonenzymatic glucosylation in the face of chronic hyperglycemia in a manner similar to that described for numerous other peptides,[27] or that other glucosylated peptides interfere with normal receptor function, producing subsequent alterations either in the ligand binding characteristics, in the position of the receptor in the membrane lipid milieu, or in the catabolism of adrenergic receptors. There is no current evidence for such a hypothesis, but diabetes-induced abnormalities in other types of receptors have been attributed to peptide glucosylation,[28] and newer techniques for purification of adrenergic receptors and for photoaffinity labeling of the receptor peptide[29,30] may permit this hypothesis to be addressed directly in future studies.

ACKNOWLEDGMENTS

Dr. Williams is supported by NHLBI Young Investigator's Award HL25146. This work was also supported by grants from the Kaiser Family Foundation, from the Pepsico Foundation, and by USPHS Grant HL21482.

REFERENCES

[1] Regan, T. J., Lyon, M. M., Ahmed, S. S., Levinson, G. E., Oldewurtel, H. A., Ahmed, M. R., and Haider, B.: Evidence for cardiomyopathy in familial diabetes mellitus. J. Clin. Invest. 1977; 60:885–89.

[2] Sanderson, J. E., Brown, D. J., Rivellese, A., and Kohner, E.: Diabetic cardiomyopathy? An echocardiographic study of young diabetics. Br. Med. J. 1978; 1:404–407.

[3] Ledet, T.: Diabetic cardiomyopathy: quantitative histologic studies of the heart from young juvenile diabetics. Acta. Pathol. Microbiol. Scand. 1976; 84:421–28.

[4] Malhotra, A., Penpargkul, S., Fein, F. S., Sonnenblick, E. H., and Scheurer, J.: The effect of streptozotocin-induced diabetes in rats on cardiac contractile proteins. Circ. Res. 1981; 49:1243–50.

[5] Pierce, G. N., and Dhalla, N. S.: Cardiac myofibrillar ATPase activity in diabetic rats. J. Mol. Cell. Cardiol. 1981; 13:1063–69.

[6] Penpargkul, S., Schaible, T., Yipintsoi, T., and Scheuer, J.: The effect of diabetes on performance and metabolism in rat hearts. Circ. Res. 1980; 47:911–21.

[7] Ingebretsen, W. R., Peralta, C., Monsher, M., Wagner, L. K., and Ingebretsen, C. G.: Diabetes alters the myocardial cAMP-protein kinase cascade system. Am. J. Physiol. 1981; 240:H375–82.

[8] Foy, J. M., and Lucas, P. D.: Effect of experimental diabetes, food deprivation and genetic obesity on the sensitivity of pithed rats to autonomic agents. Br. J. Pharmacol. 1976; 57:229–34.

[9] Namm, D. H., and Mayer, S. E.: Effects of epinephrine on cardiac cyclic 3′, 5′-AMP, phosphorylase kinase and phosphorylase. Mol. Pharmacol. 1968; 4:61–69.

[10] Murphy, R. D., Vailas, A. C., Tipton, C. M., Matthes, R. D., and Edwards, J. G.: Influence of streptozotocin-induced diabetes and insulin on the functional capacity of rats. J. Appl. Physiol. 1981; 50:482–86.

[11] Savarese, J. J., and Berkowitz, B. A.: B-adrenergic receptor decrease in diabetic rat hearts. Life Sci. 1979; 25:2075–78.

[12] Fein, F. S., Strobeck, J. E., Malhotra, A., Scheuer, J., and Sonnenblick, E. H.: Reversibility of diabetic cardiomyopathy with insulin in rats. Circ. Res. 1981; 49:1251–61.

[13] Bergmeyer, H. U., Bernt, E., Schmidt, F., and Stock, H.: Determination with hexokinase and glucose-6-phosphate dehydrogenase. *In* Methods of Enzymatic Analysis. Vol. 3. Bergmeyer, H. U., Ed. New York, Academic Press, 1974:1196–1201.

[14] Baker, S. P., and Potter, L. T.: Biochemical studies of cardiac B-adrenoreceptors, and their clinical significance. Circ. Res. 1980; 46:138–42.

[15] Williams, R. S., and Lefkowitz, R. J.: Alpha-adrenergic receptors in rat myocardium: identification by binding of [^{3}H] dihydroergocryptine. Circ. Res. 1978; 43:721–27.

[16] Lowry, O. H., Rosebrough, H. J., Farr, A. L., and Randall, R. J.: Protein measurements with the Folin phenol reagent. J. Biol. Chem. 1951; 193:265–75.

[17] Warren, L.: The thiobarbituric acid assay of sialic acid. J. Biol. Chem. 1959; 234:1971–75.

[18] Kent, R. S., DeLean, A., and Lefkowitz, R. J.: A quantitative analysis of beta-adrenergic receptor interactions: resolution of high and low affinity states of the receptor by computer modeling of ligand binding data. Mol. Pharmacol. 1980; 17:14–23.

[19] DeLean, A., Munson, P. J., and Rodbard, D.: Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiologic dose response curves. Am. J. Physiol. 1978; 234:E97–102.

[20] Stiles, G. L., Stadel, J. M., DeLean, A., and Lefkowitz, R. J.: Hypothyroidism modulates beta adrenergic receptor-cyclase interactions in rat reticulocytes. J. Clin. Invest. 1981; 68:1450–55.

[21] Williams, R. S., and Bishop, T.: Enhanced receptor-cyclase coupling and augmented catecholamine-stimulated lipolysis in exercising rats. Am. J. Physiol. 1982; 243:E345–51.

[22] Williams, R. S., and Lefkowitz, R. J.: Thyroid hormone regulation of alpha-adrenergic receptors: studies in rat myocardium. J. Cardiovasc. Pharmacol. 1979; 1:181–89.

[23] Miller, T. B., Praderio, M., Wolleben, C., and Bullman, J.: A hypersensitivity of glycogen phosphorylase activation in hearts of diabetic rats. J. Biol. Chem. 1981; 256:1748–53.

[24] Bristow, M. R., Ginsberg, R., Minobe, W., Cubicciotti, R. S., Sagemen, W. S., Lurie, K., Billingham, M. E., Harrison, D. C., and Stinson, E. B.: Decreased catecholamine sensitivity and B-adrenergic receptor density in failing human hearts. N. Engl. J. Med. 1982; 307:205–11.

[25] Motulsky, H. J., and Insel, P. A.: Adrenergic receptors in man: direct identification, physiologic regulation, and clinical alterations. N. Engl. J. Med. 1982; 307:18–29.

[26] Paulson, D. J., and Light, K. E.: Elevation of serum and ventricular norepinephrine content in the diabetic rat. Res. Commun. Chem. Pathol. Pharmacol. 1981; 33:559–62.

[27] Schnider, S. L., and Kohn, R. R.: Effects of age and diabetes mellitus on the solubility and non-enzymatic glucosylation of human skin collagen. J. Clin. Invest. 1981; 67:1630–35.

[28] Summerfield, J. A., Vergall, J., and Jones, E. A.: Modulation of a glycoprotein recognition system on rat hepatic endothelial cells by glucose and diabetes mellitus. J. Clin. Invest. 1982; 69:1337–47.

[29] Homcy, C. J., Graham, R. M., Haber, E., and Hess, H.: Characterization of the purified mammalian A- and B-adrenergic receptors reveals homologous molecular properties. Clin. Res. 1982; 30:482A.

[30] Lavin, T., Nambi, R., Heald, S., Jeffs, P., Shorr, R., Strohsacker, M., Benovic, J., Heinsimer, J., Lefkowitz, R. J., and Caron, M.: Molecular properties of B_1 and B_2 adrenergic receptors revealed by photoaffinity labelling. Clin. Res. 1982; 30:491A.