The Adrenergic β-Receptor Adenylate Cyclase System in Heart and Lymphocytes from Streptozotocin-Diabetic Rats

In Vivo and In Vitro Evidence for a Desensitized Myocardial β-Receptor

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SUMMARY

The myocardial β-receptor adenylate cyclase system was investigated in short-term streptozotocin-diabetic rats. Earlier reports of a decreased sensitivity of the myocardium to isoproterenol (ISO) in these animals were elucidated by measuring the in vivo production of cAMP after ISO. A substantial decrease was seen in diabetic animals compared with controls and starved animals, and thyroxine treatment, known to sensitize the myocardium to catecholamines, did not normalize the response. The desensitization was retained in a membrane fraction in such a way that ISO was unable to increase the cAMP production while stimulation via the nucleotide-binding protein (with NaF or GTP) leads to a normal cAMP response. As the β-adrenergic receptor number and affinity turned out to be identical in control and diabetic animals, a functional uncoupling of the myocardial β-receptor from productive adenylate cyclase activation seems thus to exist in experimental diabetes. It is unlikely that it has anything to do with the thyroid status of the animals, but the possibility of a catecholamine-induced desensitization cannot be excluded. The phenomenon is not universal as the β-receptor-adenylate cyclase system is normal in isolated spleen lymphocytes. Whether the described phenomenon obtained in an animal study has any relevance for the increased incidence of heart failure in human diabetes mellitus is not known at present. DIABETES *32*:1110–1116, December 1983.

Recently we have demonstrated an abnormal myocardial response to the strong β-adrenergic stimulator, isoproterenol (ISO), in streptozotocin-diabetic rats. These animals show a protection against the cardiotoxic effect of high doses of ISO when given in vivo.[1] The myocardial calcium uptake after ISO is, moreover, diminished in the isolated heart perfusion system.[2] Combination of the β-receptor with a hormone agonist stimulates the adenylate cyclase to form cAMP, which in turn promotes the transport of calcium through the sarcolemma. Abnormalities in this system leading to a decreased myocardial calcium uptake have recently been proposed to be essential for the development of the failing heart in human beings.[3] This is a situation that occurs more frequently in diabetic patients,[4] even when the increase of coronary vascular disease is taken into account.[5] Experimental studies have delivered biochemical as well as hemodynamic evidence for a decreased myocardial sensitivity to catecholamines in short-term diabetic rats.[6,7] We therefore suggest that these alterations are due to functional changes in the sarcolemma in experimental diabetes and that the molecular basis might be a β-receptor desensitization. The β-receptor adenylate cyclase system consists of a receptor located on the outer cell membrane surface and at the inner side a nucleotide regulatory component and the catalytic unit of the adenylate cyclase.[8] The nucleotide regulatory component requires the binding of GTP and is responsible for mediating the effect of a hormone (i.e., ISO) to the adenylate cyclase. Experimental studies on this β-adrenergic system have centered on the desensitizing effect of catecholamines and the modulating influence of thyroid hormone levels. Moreover, some but not all reports have shown parallel changes in the β-adrenergic system in heart and lymphocytes.[9,10]

The purpose of the present investigation was (1) to investigate the thyroid status and the in vivo myocardial cAMP accumulation in response to ISO in control, diabetic, and starved rats, and to analyze the influence of thyroxine treatment; (2) to get more detailed information on the mechanism of decreased sensitivity of cardiac β-adrenoceptors by describing the binding characteristics of these receptors as well as the in vitro stimulation of isolated membrane fractions; and (3) to examine the β-receptor function in lymphocytes from control and diabetic rats using an in vitro surface stimulation technique.

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MATERIAL AND METHODS

Animals. Female Wistar rats, 3–4 mo old, weighing 180–250 g were divided into the following groups: (1) nondiabetics, (2) diabetics, (3) starved controls, (4) thyroxine-treated controls, and (5) thyroxine-treated diabetics. Diabetes was induced by an intraperitoneal injection of streptozotocin (70 mg/kg, Upjohn, Kalamazoo, Michigan) and animals with a non-fasting blood glucose 5 days later of 300–450 mg/dl (glucose-oxidase method) and with constant glucosuria (Diastix, Ames, United Kingdom) were used 8 days after the injection of streptozotocin. Serum thyroxine (T_4) and serum triiodothyronine (T_3) were analyzed by radioimmunoassay.[11,12] Serum albumin was estimated from the total serum protein and a serum electrophoresis.

In vivo studies. Rats were made hyperthyroid by twice-daily subcutaneous injections of thyroxine (1 mg/kg) for 6 days before death. The injections were given at 10 a.m. and 8 p.m. Tail blood taken at 3 p.m. the day before death was analyzed for serum T_4 and serum T_3. Starved control rats were kept with no food but with water ad libitum for 6 days. At termination the animals were given a subcutaneous injection of (−)−ISO (30 mg/kg, Boehringer-Mannheim). The ISO was dissolved in distilled water with sodium pyrosulfite as antioxidant and spectral analysis showed no sign of oxidation products from ISO in this solution. Forty-five minutes later the animals were anesthetized with pentobarbital (30 mg/kg). Exactly 60 min after the ISO injection the hearts were excised, freed of large vessels, and immediately frozen in liquid nitrogen. Hearts were stored for less than 1 wk at −20°C and were then powdered in a percussion mortar under continuous addition of liquid nitrogen and transferred to 4 ml of ice-cold 50 mM phosphate buffer (pH 5.5) with 4 mM theophylline and 9 mM mercaptoethanol. Further homogenization of this solution was performed in an ice bath using a Potter Elverhjelm homogenizer with a motor-driven teflon pestle. After 10 strokes, aliquots were taken for later estimation of protein content using a Lowry technique with bovine albumin as a standard,[13] while 250 μl was transferred to prewarmed assay tubes and kept in boiling water for 5 min. After a 3-min centrifugation (1000 g, Beckman Microfuge, Beckman Instruments, Fullerton, California), 50 μl in duplicate from supernatant was frozen down (−20°C) for later estimation of cAMP, using a cAMP assay kit (Amersham, United Kingdom). The recovery of this procedure was 90 ± 4% (SD) (N = 3), and the interassay coefficient of variations was 3% (N = 5).

Preparation of heart membranes. Heart ventricles were frozen, powdered, and homogenized as described above with the exception of the use of the following buffer during the homogenization: 0.25 sucrose, 5 mM Tris/HCl (pH 7.4), 1 mM $MgCl_2$. After filtration through nylon mesh the solution was centrifuged 750 × *g* for 10 min and the supernatant recentrifuged 48,000 × *g* for 15 min at 4°C. The final pellet representing a crude membrane fraction was suspended in 2 ml sucrose buffer, resulting in a protein concentration of 3–5 mg/ml (similar in control and diabetic animals), and frozen at −70°C until estimation of β-adrenergic receptor number and adenylate cyclase activity.

β-Adrenergic receptor binding assay. Receptor binding was performed according to the method of Williams et al.[14] using [^{3}H]dihydroalprenolol ([^{3}H]DHA, specific activity 90.0 Ci/mmol, New England Nuclear Corp., Boston, Massachusetts). Aliquots of membrane suspension (approximately 200 μg protein) were incubated with 0.5–20.0 nM [^{3}H]DHA in a total volume of 250 μl at 23°C for 30 min. After the incubation was stopped by adding 2 ml of ice-cold sucrose buffer, vacuum filtration was performed through Whatman GF/C glass fiber filters (Whatman Inc., New Jersey). These filters were then dried, scintillation liquid added, and counted. Specific binding was defined as the binding not displaced by 10^{-5} M (±) propranolol (Sigma Chemical Co., St. Louis, Missouri) and constituted 89–42% (range) (lowest to highest level of [^{3}H]DHA of the total counts bound).

Adenylate cyclase assay. Approximately 75 μg of heart membrane protein was incubated in triplicate in a total volume of 300 μl at 30°C for 15 min. Final concentrations in the incubation mixture were 75 mM Tris/HCl (pH 7.5), 6 mM $MgCl_2$, 8 mM KCl, 7.5 mM theophylline, 3 mM dithiotreitol, 0.7 mM EGTA [ethyleneglycol-bis (β-amino ethyl ether)-N,N′-tetraacetic acid], 1 mM ATP (adenosine triphosphate), 0.1 mM ascorbate, 2.5 mM creatinine phosphate, 0.075 mg/ml creatinine kinase, 0.4 mg/ml bovine serum albumin. In certain assays the following agents were included: 10^{-5} M GTP (guanosine triphosphate), 10^{-5} M GTP plus 10^{-5} ISO, 60 μM 5′ guanylimidodiphosphate [Gpp(NH)p], 7 mM NaF. The reaction was terminated by 5 min of boiling and cAMP determined as described above.

Isolation of lymphocytes. The spleen was gently homogenized in 5 ml Tris Balanced Salt Solution (BBS) with 10% newborn calf serum (NBCS) (Gibco Europe). After nylon mesh filtration, the homogenate was centrifuged 3000 × *g* for 5 min at 23°C. The pellet, resuspended in 2 ml BSS + NBCS, was then layered on 3 ml Lymphoprep (Nyegaard, Norway) and centrifuged 1200 × *g* (swing-out rotor) for 10 min at 23°C. The lymphocyte band was aspirated and washed twice in 2 ml of BSS + NBCS. The cell concentration in the final preparation was counted in a hemocytometer and adjusted to 10^7 cells/ml. The yield was 3–4 × 10^7 cells per spleen, and based on morphologic and histochemical criteria more than 90% of the cells were lymphocytes in preparations from both control and diabetic animals (N = 3).

Hormonal stimulation of cAMP production in lymphocytes. Incubation was performed with 530 μl lymphocyte preparation supplemented with 10 mM theophylline. After 3 min 80 μl was transferred to prewarmed assay tubes containing 80 μl of the previously described phosphate buffer (0 value). Then ISO was added (final concentration 10^{-5} M) and aliquots taken 2, 4, and 6 min later. Cyclic AMP content was assayed as described above. As it has been shown that the receptor-mediated cAMP response in lymphocytes can be amplified by increasing the membrane fluidity, e.g., by using alcohol,[15] the same procedure was followed in parallel experiments in the presence of 2% ethanol during the incubation.

Statistics. All data are presented as the mean ± SEM. Differences were evaluated by Student's *t* test (paired or unpaired as appropriate), with a $2P < 0.05$ level of significance.

RESULTS

In vivo studies. Decreased serum levels of thyroid hormones have been reported in streptozotocin-diabetic rats.[16,17] As seen from Table 1, our results confirm this finding,

TABLE 1
Serum values, heart protein, and body weight in the in vivo experiment

	N	Body weight (g)	Blood glucose (mg/dl)	Serum protein (g/L)	Serum albumin (g/L)	Serum T_4 (μg/dl)	Serum T_3 (ng/ml)	Heart protein (mg)	Heart protein/body weight (mg/g)
Control	8	238 ± 11	128 ± 5	65.1 ± 1.2	30.9 ± 0.7	3.6 ± 0.3	0.75 ± 0.1	62 ± 3	0.27 ± 0.01
Diabetes	9	218 ± 5	361 ± 14†	54.2 ± 2.3†	24.2 ± 2.3†	2.3 ± 0.4*	0.26 ± 0.03†	55 ± 2	0.25 ± 0.008
Starvation	9	177 ± 11†	120 ± 3	—	—	3.1 ± 0.3	0.61 ± 0.05	55 ± 4	0.31 ± 0.01
Thyroxine treatment									
Control	10	222 ± 6	136 ± 4	—	—	40.6 ± 3.8	14.1 ± 1.1	88 ± 3	0.40 ± 0.02
Diabetes	10	196 ± 7	363 ± 16	—	—	39.4 ± 3.2	8.7 ± 0.8†	76 ± 3	0.39 ± 0.01

Data are mean ± SEM.
*$2P < 0.05$, †$2P < 0.01$ compared with respective controls.

showing a significant decrease in T_4 ($2P < 0.05$). This decrease was, however, paralleled by a decrease in serum albumin ($2P < 0.01$), the serum protein to which T_4 is mainly bound in rats,[18] implying that the rats were not necessarily hypothyroid. The T_3 level was clearly lowered in diabetes ($2P < 0.01$). Body and heart weight decreased to the same extent after 1 wk of diabetes, leaving their ratio unchanged, while 6 days of starvation led to an excessive decrease in body weight. Extensive thyroxine treatment for 6 days induced a substantial increase in serum T_4 in both control and diabetic rats when measured halfway between the last two injections. Serum T_3 also increased significantly, although a significantly lower level was seen in the diabetic animals when compared with the thyroxine-treated control rats ($2P < 0.01$). These findings are in agreement with the reported diminished ability to generate T_3 from T_4 in the liver from streptozotocin-diabetic rats.[19] It can be concluded that thyroxine-treated control and diabetic rats showed the same degree of hyperthyroidism, which is further supported by the fact that both groups had overt signs of hyperthyroidism with motoric hyperactivity and diarrhea. Thyroxine treatment resulted in a marked increase in heart protein in controls (62 ± 3 to 88 ± 3 mg, $2P < 0.001$) and in diabetic (55 ± 2 to 76 ± 3 mg, $2P < 0.001$). The heart protein/body weight ratio also increased ($2P < 0.00001$ in both controls and diabetics), indicating a true cardiac hypertrophy of approximately the same extent. Thyroxine treatment thus seems to overcome the catabolic state of diabetes in this respect.

TABLE 2
Amount of myocardial cAMP (pmol/mg protein)

	Control	P	Diabetes	Starvation
Basal	2.68 ± 0.13 (N = 5)	NS	2.64 ± 0.26 (N = 5)	—
	$P < 0.01$		NS	
Isoproterenol	5.13 ± 0.47 (N = 8)	<0.001	2.95 ± 0.18 (N = 9)	4.87 ± 0.40 (N = 9)
	NS		NS	
Isoproterenol (thyroxine treated)	4.82 ± 0.28 (N = 10)	<0.001	3.36 ± 0.17 (N = 10)	—

Data represent the in vivo content, basal as well as 60 min after a subcutaneous injection of isoproterenol (30 mg/kg) without and with previous thyroxine treatment (1 mg/kg twice daily for 6 days) (mean ± SEM).

The ability to accumulate cAMP in the heart after ISO in the various groups can be seen in Table 2. It is seen that ISO induces a highly significant increase in control animals 60 min after the injection of ISO, while the content in the diabetic hearts at that time does not differ from the basal level. This does not exclude, of course, that there has been a significant increase in cAMP within the first 60 min in these animals as shown by Ingebretsen et al.[6] Starvation induces metabolic changes in the myocardium, which in some respect can be compared with those induced by diabetes,[20] but this control group showed the same amount of cAMP after ISO as their fed controls. Hyperthyroidism did not alter the ISO-stimulated cAMP content in either control or diabetic rats, indicating that an excessive level of T_4 in serum leading to myocardial hypertrophy is unable to normalize the decreased sensitivity to catecholamines in experimental diabetes.

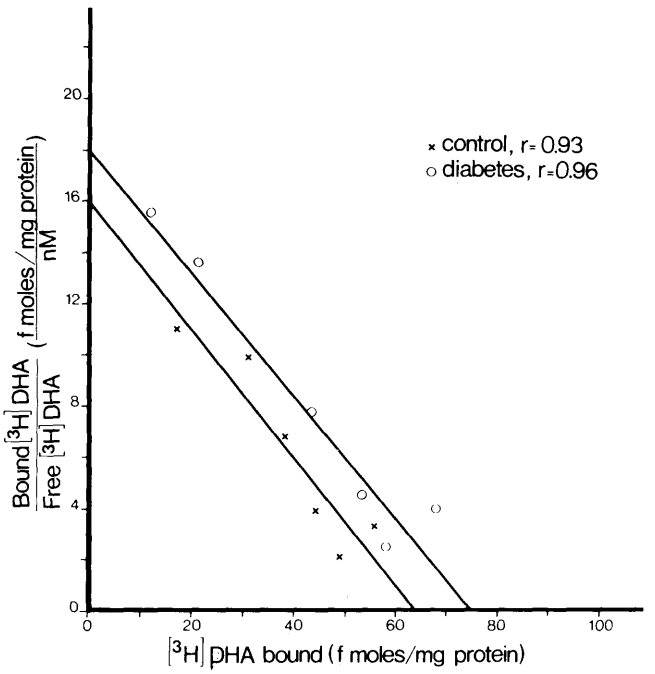

FIGURE 1. Scatchard plot of the [³H]DHA binding to a myocardial membrane fraction from control and diabetic rats. These experiments are representative of three experiments from each group.

TABLE 3
Number and affinity of cardiac β-adrenergic-receptors

	Control	Diabetes
Number of [^{3}H]DHA binding sites (fmol/mg protein)	68.5 ± 4.1	73.8 ± 1.5
K_d of [^{3}H]DHA (nM)	3.7 ± 0.2	4.8 ± 0.7
Number of experiments	3	3

All results are expressed as mean ± SEM.

In vitro studies. Using the Scatchard analysis (Figure 1) the number (the x intercept) and dissociation constant (negative reciprocal of the slope) of the [^{3}H]DHA binding sites in the membranes can be estimated.[14] The figure shows representative experiments performed on membrane fractions pooled from five animals in each group. Each point is the mean of triplicate determinations. From Figure 1 and Table 3 it is seen that the number of binding sites is unchanged in these short-term diabetic animals; moreover, no significant alteration in the affinity for this agonist could be detected. These results therefore indicate that the first step in catecholamine action, i.e., the interaction of agonist with the available β-adrenergic receptors, is intact in the diabetic animal.

To further illustrate the decreased response to ISO in diabetic rats, the adenylate cyclase activity in isolated heart membranes was investigated in response to various stimulants. As GTP is required for the coupling of the response from the receptor to the adenylate cyclase and as various unknown amounts of endogenous GTP conceivably were present in such a membrane fraction,[8] the ISO-induced production of cAMP was expressed as the amount produced by ISO plus GTP as a percentage of the cAMP-produced GTP alone. Figure 2 shows these results. In the presence of GTP, ISO is not able to increase the activity of the adenylate cyclase in diabetic hearts (1.2 ± 5.7%), while a 50.0 ± 8.7% increase is seen among the control animals. The difference between these responses is highly significant ($2P < 0.001$). The adenylate cyclase can be stimulated by other routes than via the receptor. The nucleotide-binding protein is able to activate the catalytic unit of the adenylate cyclase by binding GTP or its non-hydrolyzable analogue, 5′ guanylimidodiphosphate [Gpp(NH)p]. Moreover, the binding of fluoride to the nucleotide-binding component leads to a strong stimulation considered to represent the maximal activity of the system.[8] In Figure 3 the adenylate cyclase activity after such stimulants is shown. Addition of Gpp(NH)p (6×10^{-5} M) and NaF (7×10^{-3} M) leads to significant increases in the activity when compared with the H_2O value in both controls and diabetics; however, neither the basal level nor the stimulants [GTP, Gpp(NH)p, NaF] showed any difference between control and diabetes. An altered cAMP degradation due to changes in phosphodiesterase activity in diabetes mellitus cannot, therefore, account for our results, which are further supported by the fact that all incubations were performed in the presence of the phosphodiesterase inhibitor, theophylline. The activity of the myocardial adenylate cyclase in experimental diabetes thus seems intact when stimulated via the nucleotide-binding component while the receptor-mediated activation seems defective.

The results from the ISO-induced cAMP accumulation in

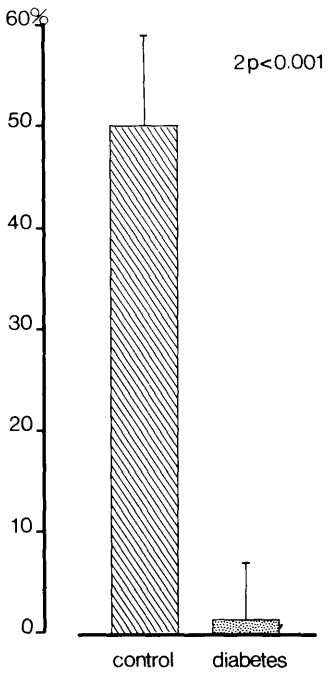

FIGURE 2. ISO-induced increase in myocardial adenylate cyclase activity in the presence of GTP (10^{-6} M). Values are estimated as pmol/min/mg protein and expressed as percentage increase above the activity of GTP alone in each animal. Data represent mean ± SEM in the two groups.

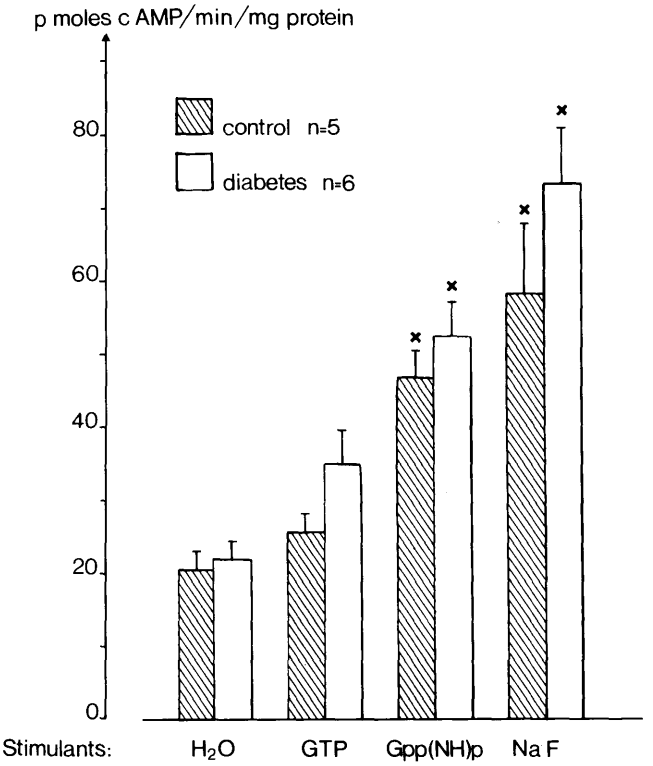

FIGURE 3. Myocardial adenylate cyclase activity. Values are from 15-min incubation of crude membrane fraction with various stimulants. x: $2P < 0.02$ compared with H_2O values. No significant difference is seen between controls and diabetics by any of the stimulants.

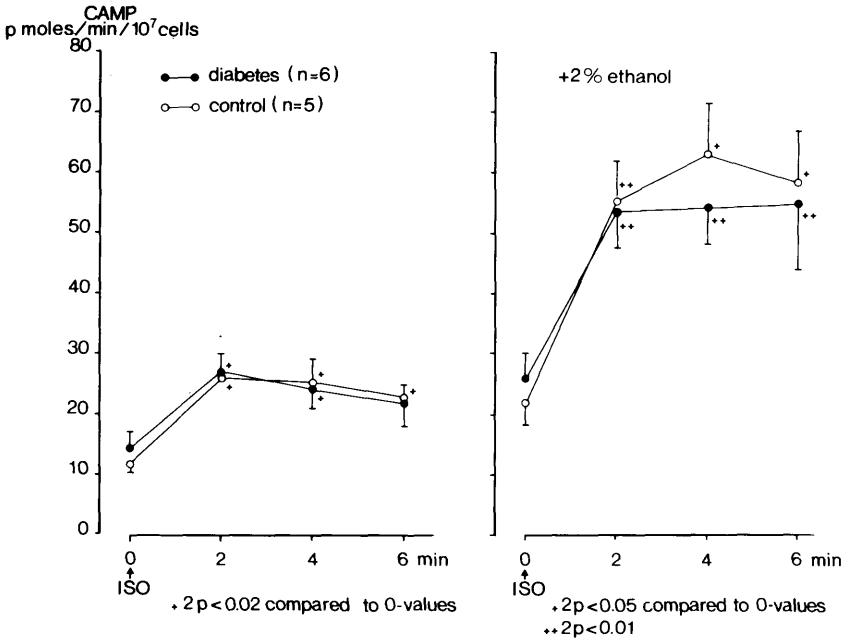

FIGURE 4. ISO-stimulated cAMP production in spleen lymphocytes. No significant difference between diabetic and control rats is seen with or without the addition of ethanol. +: 2P < 0.05; ++: 2P < 0.01 compared with 0 values.

spleen lymphocytes is seen in Figure 4. Stimulation, again performed in the presence of theophylline, showed no significant difference between control and diabetic rats. The amplifying effect of 2% ethanol on receptor-mediated activation of the adenylate cyclase was seen in both control and diabetic rats, and again no significant difference in the response was seen at any reported point of time.

DISCUSSION

The present report on a β-receptor desensitization in the myocardium of streptozotocin-diabetic rats is in agreement with our two earlier observations of an in vivo protection against cardiotoxic doses of ISO[1] and decreased myocardial calcium uptake in response to ISO in diabetic rats.[2] The present report characterizes the defect further, showing a decreased ability to produce cAMP after ISO both in vivo and in vitro. This implies that the defect is retained within the sarcolemma during the isolation of the membrane fraction. The possibility that a toxic effect of streptozotocin itself should account for the findings seems unlikely as we have earlier shown that insulin treatment normalizes the response.[1]

Among the factors known to influence the activity of the β-receptor adenylate cyclase system is the thyroid status. In hypothyroid rats responsiveness of the adenylate cyclase system in the left atrium has been reported to be decreased.[21] In radioligand binding studies, Williams et al.[14] have documented a doubling of the number of myocardial β-receptors after thyroxine treatment. Whether this increase is accompanied by an increase in ISO-stimulated adenylate cyclase activity was, however, not documented. The decreased levels of serum T_4 and T_3 in streptozotocin-diabetic rats have been observed earlier.[16,17] This does not necessarily indicate that these animals are, in fact, hypothyroid. As for the low serum T_4, an abnormally low concentration of serum albumin could explain the results. The concentration of free hormone might still be normal. This is further confirmed by the reported normal level of TSH in rats with experimental diabetes.[22] The low serum T_3 is often seen in acute and chronic diseases in animals as well as man. To see whether thyroxine administration could normalize the catecholamine sensitivity of the β-receptor adenylate cyclase system in diabetes, control and diabetic rats were treated with high doses of thyroxine for 6 days. This procedure did not increase the ability to produce cAMP in response to ISO in the controls and did not normalize the desensitization in the diabetic hearts, although both groups showed marked myocardial hypertrophy. As the dose of thyroxine is comparable to the one in the experiments of Williams et al. showing that thyroxine administration markedly increases the number of β-adrenergic receptors, it is not conceivable that the desensitization is due to a mechanism regulated in part by thyroid hormones. This possibility was also directly approached in our study by estimating the number and affinity of the binding sites for [^{3}H]DHA. No alterations were seen in diabetic animals. This is opposed to another radioligand binding study[23] showing a 28% decrease in the number of β-receptors in the myocardium of streptozotocin-diabetic rats. In this study the duration of diabetes was 2 mo and no information about the condition of the animals (i.e., weight loss, blood glucose) was delivered. The animals might therefore have been sicker than in our study.

The in vivo findings are in agreement with the results from the in vitro adenylate cyclase assay. The fact that the basal (H_2O) activity, as well as activity due to stimulation of the nucleotide-binding protein [Gpp(NH)p, NaF], remained intact does not support the possibility of a differential yield of functional membrane from control and diabetic hearts. As the assays were performed in the presence of ATP, it seems unlikely that the findings are due to an ATP depletion in the diabetic hearts. If the marked decrease in cAMP response is explained by a decrease in the number of β-adrenergic receptors, one would expect the radioligand binding ex-

periments in the present study to have delivered such evidence, even though the results were obtained in pooled material from several animals in each group.

The finding of an abnormally low response to β-receptor-stimulated adenylate cyclase activity in diabetes might hypothetically be compared with the situation where the myocardial β-receptor is desensitized due to acute exposure to ISO.[24] Results obtained from studies of chick embryo ventricles also showed a decreased receptor-mediated activation of the adenylate cyclase, leaving the NaF and Gpp(NH)p stimulation intact. These results, however, are opposed to the findings of Tse et al., who used the rat heart after chronic exposure to ISO, and reported a decreased basal and NaF-stimulated adenylate cyclase activity, together with the β-receptor desensitization.[25]

Whether it is reasonable to compare the present phenomenon with catecholamine-induced desensitization of the myocardial β-receptor is not known. Moreover, one has to be careful when trying to deduce from in vitro experiments in animals to human studies. However, such deductions are made plausible by the report of Neubauer and Christensen of a markedly decreased content of norepinephrine in the myocardium of long-term diabetic patients.[26] This concentration was comparable to the one in patients with heart failure, a situation known to deplete myocardial catecholamine stores,[27] but the contributing damage of the adrenergic system due to additional diseases in these patients of Neubauer and Christensen is unknown. A myocardial catecholamine depletion seems not directly consistent with a β-receptor desensitization but the possibility of a more specific change in the sarcolemma of diabetic animals must, however, be considered and one might speculate as to an increased catecholamine turnover rate with the consequence that the β-receptor is in some way permanently desensitized. It is not known presently what factors in the diabetic metabolism are responsible for the described phenomenon.

Concomitant changes in the activity of the adenylate cyclase in lymphocytes and cardiac sensitivity to ISO have been described in human beings.[28] This led us to investigate the response in spleen lymphocytes from diabetic animals. The results showed a normal response of the adenylate cyclase to ISO. This indicates that the receptor defect is not universal as would be expected if it was a stress-induced desensitization due to increased amounts of circulating catecholamines.

A decreased catecholamine sensitivity has recently been described in failing human hearts.[3] A reduction in β-receptor density, in the ability to produce cAMP in response to ISO and in ISO-stimulated muscle contractions was reported in the myocardium from patients with end-stage heart failure. Studies in young asymptomatic diabetic patients with diabetes of short duration show a decreased myocardial contractility during the stress induced by exercise, while myocardial function under basal conditions was unaltered.[29,30] Although this does not provide specific evidence of a defect in the β-adrenergic pathway it still shows the same pattern as in the present investigation, namely a normal basal level and a demasked defect during stimulation. The present finding, induced by the diabetic state, is obtained in an animal study and might be species specific; however, the fact that alterations in the β-receptor adenylate cyclase system with functional consequences for the myocardium also exist in diabetic patients cannot be excluded.

REFERENCES

[1] Gøtzsche, O.: Lack of cardiotoxic effect of isoproterenol in streptozotocin diabetic rats. Virchows Arch. [Pathol. Anat.] 1982; 397:83–91.

[2] Gøtzsche, O.: Decreased myocardial calcium uptake in response to isoproterenol in streptozotocin diabetic rats. Lab. Invest. 1983; 48:156–61.

[3] Bristow, M. R., Ginsburg, R., Minobe, W., Cubicciotti, R. S., Sagemann, W. S., Lurie, K., Billingham, M. E., Harrison, D. C., and Stinson, E. B.: Decreased catecholamine sensitivity and β-adrenergic-receptor density in failing human hearts. N. Engl. J. Med. 1982; 309:206–11.

[4] Partamian, J. O., and Bradley, R. F.: Acute myocardial infarction in 258 cases of diabetes. N. Engl. J. Med. 1965; 273:455–61.

[5] Kannel, W. B.: Role of diabetes in cardiac disease: conclusions from population studies. *In* Diabetes and The Heart. Zoneraich, S., Ed. Springfield, Charles C. Thomas, 1978:97–112.

[6] 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.

[7] Foy, J. M., and Lucas, P. D.: Comparison between spontaneously beating atria from control and streptozotocin-diabetic rats. J. Pharm. Pharmacol. 1978; 30:558–62.

[8] Rodbell, M.: The role of hormone receptors and GTP-regulatory proteins in membrane transduction. Nature 1980; 284:17–22.

[9] Colucci, W. S., Alexander, R. W., Williams, G. H., Rude, R. E., Holman, B. L., Konstam, M. A., Wynne, J., Mudge, G. H., and Braunwald, E.: Decreased lymphocyte beta-adrenergic-receptor density in patients with heart failure and tolerance to the beta-adrenergic agonist pirbuterol. N. Engl. J. Med. 1981; 305:185–90.

[10] Scarpace, P. J., and Abrass, I. B.: Thyroid hormone regulation of rat heart, lymphocyte and lung β-adrenergic receptors. Endocrinology 1981; 108:1007–1011.

[11] Weeke, J., and Ørskov, H.: Evaluation of thyroid function. *In* Recent Advances in Clinical Biochemistry. Vol. 1. Alberti, K. G. M. M., Ed., Edinburgh, Churchill Livingstone, 1978:111–28.

[12] Weeke, J., and Ørskov, H.: Ultrasensitive radioimmunoassay for direct determination of free triiodothyronine concentration in serum. Scand. J. Clin. Lab. Invest. 1975; 35:237–44.

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

[14] Williams, T. T., Lefkowitz, R. J., Watanabe, A. M., Hathaway, D. R., and Besch, H. R.: Thyroid hormone regulation of β-adrenergic receptor number. J. Biol. Chem. 1977; 252:2787–89.

[15] Hynie, S., Lanefelt, F., and Fredholm, B. B.: Effect of ethanol on human lymphocyte levels of cyclic AMP in vitro: potentiation of the response to isoproterenol, prostaglandin E_2 or adenosine stimulation. Acta Pharmacol. Toxicol. 1980; 47:58–65.

[16] Fein, F. S., Kornstin, L. B., Strobeck, J. E., Capasso, J. M., and Sonnenblick, E. M.: Altered myocardial mechanics in diabetic rats. Circ. Res. 1980; 47:922–33.

[17] Dillmann, W. H.: Influence of thyroid hormone administration on myosin ATPase activity and myosin isoenzyme distribution in the heart of diabetic rats. Metabolism 1982; 31:199–204.

[18] Refetoff, S., Robin, N. I., and Fang, V. S.: Parameters of thyroid function in serum of 16 selected vertebrate species: a study of PBI, serum T_4, free T_4 and the pattern of T_4 and T_3 binding to serum proteins. Endocrinology 1970; 86:793–805.

[19] Balsam, A., Ingbar, S. H., and Sexton, F.: The influence of fasting, diabetes and several pharmacological agents on the pathways of thyroxine metabolism in rat liver. J. Clin. Invest. 1978; 62:415–24.

[20] Randle, P. J., Newsholme, E. A., and Garland, P. B.: Regulation of glucose uptake by muscle. Effects of fatty acids, ketone bodies and pyrovate, and of alloxan diabetes and starvation, on the uptake and metabolic fate of glucose in rat heart and diaphragm muscles. Biochem. J. 1964; 93:652–65.

[21] Brodde, O. E., Schümann, H. J., and Wagner, J.: Decreased responsiveness of the adenylate cyclase system on left atria from hypothyroid rats. Mol. Pharmacol. 1980; 17:180–86.

[22] Las, M. S., and Surks, M. I.: Dissociation of serum triiodothyronine concentration and hepatic nuclear triiodothyronine-binding capacity in streptozotocin diabetic rats. Endocrinology 1981; 109:1259–63.

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

[24] Marsch, J. D., Barry, W. H., Near, E. J., Alexander, R. W., and Smith, T. W.: Desensitization of chick embryo ventricle to the physiological and biochemical effects of isoproterenol. Circ. Res. 1980; 47:493–501.

[25] Tse, J., Powell, J. R., Baste, C. A., Priest, R. E., and Kuo, J. F.: Isoproterenol-induced cardiac hypertrophy: modifications in characteristics of β-adrenergic receptor, adenylate cyclase, and ventricular contraction. Endocrinology 1978; 105:246–55.

[26] Neubauer, B., and Christensen, N. J.: Norepinephrine, epinephrine,

and dopamine contents of the cardiovascular system in long-term diabetics. Diabetes 1976; 25:6–10.

[27] Chidsey, C. A., Braunwald, E., Morrow, A. G., and Mason, D. T.: Myocardial norepinephrine concentration in man. N. Engl. J. Med. 1963; 269:653–58.

[28] Jennings, G. L., Bobik, A., Esler, M. D., and Korner, P. I.: Relationship between maximum chronotropic response to isoproterenol in man and generation of cyclic adenosine monophosphate by lymphocytes. Circ. Res. 1980; 46 (Suppl. I):I49–50.

[29] Abenavoli, T., Rubler, S., Fisher, V. J., Axelrod, H. I., and Zuckerman, K. P.: Exercise testing with myocardial scintigraphy in asymptomatic diabetic males. Circulation 1981; 63:54–64.

[30] Karlefors, R.: Hemodynamic studies in male diabetics. Acta Med. Scand. 1966; 180 (Suppl. 449):45–80.