Abnormal Myocardial Calcium Uptake in Streptozocin-diabetic Rats

Evidence for a Direct Insulin Effect on Catecholamine Sensitivity

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SUMMARY

Myocardial calcium uptake after isoproterenol (ISO) in the isolated, perfused heart was investigated at 24-h intervals after the injection of streptozocin (STZ) in rats. After 4 days, when hyperglycemia had persisted for 3 days, myocardial calcium uptake in response to this strong β-adrenergic agonist fell significantly to the level of unstimulated hearts, which also was the level of propranolol-pretreated hearts exposed to ISO. Insulin, when given in vivo 60–90 min before perfusion, led to a complete normalization of this ISO response in diabetic rats (duration 8 days), while in vitro addition of insulin to the perfusate (0.1 U/ml) significantly increased, while not completely normalizing, the ISO-induced myocardial calcium uptake. Insulin, therefore, has a direct effect on this β-adrenergic response in diabetic rats and streptozocin in itself does not cause the desensitization. Considering the essential role of this calcium transport for the electromechanical coupling in the heart, such metabolically induced changes in catecholamine sensitivity might hypothetically have relevance for the increased incidence of heart failure in diabetes. DIABETES 1985; 34:287–90.

Cardiac complications such as pump failure and sudden death are often seen in diabetic patients.[1] Epidemiologic and clinical studies indicate that this cannot solely be explained by an increased tendency for atherosclerosis.[2,3] Preclinical abnormalities in left ventricular function in diabetic children have been described.[4] The existence of a specific diabetic heart disease, termed diabetic cardiopathy, has been proposed based on diabetic micro- and macroangiopathy as well as diabetic neuropathy.[5] Whether these causes should be complemented with a metabolically induced muscle cell dysfunction is still a matter of speculation, although there are recent experimental[6] as well as clinical studies[7,8] indicating this. Moreover, the occurrence of pump failure in diabetic patients admitted with myocardial infarction correlates with the acute metabolic status of the patients.[9,10]

Reduced mechanical response to myocardial stress has been reported in clinical[11] and experimental studies.[12] Using the short-term diabetic rat as a model for a possible muscle cell dysfunction, we have focused on a decreased catecholamine-induced myocardial calcium uptake[13] and a β-adrenergic desensitization[14] in experimental diabetes. In consideration of the essential role of the calcium transport through the sarcolemma for the electromechanical coupling in the heart, this model could hypothetically be a metabolically induced, electrophysiologic equivalent for this reduced mechanical response. The present report deals with this abnormal calcium uptake during strong β-adrenergic stimulation with respect to the time course for its induction and reversion in experimental diabetes. A direct sensitizing effect of insulin on this catecholamine response in the diabetic heart is suggested.

MATERIALS AND METHODS

Animals. Female Wistar rats weighing 200–300 g were used when they were 3–4 mo old. Diabetes was induced by an intraperitoneal (i.p.) injection of streptozocin (STZ, Upjohn, Kalamazoo, Michigan) (75 mg/kg). Within 24 h, persisting hyperglycemia appeared (BM Glycemie Stix with reflectance meter, Boehringer, Mannheim, FRG) as well as constant glucosuria (Diastix, Ames, United Kingdom). None of the animals showed ketonuria (Ketostix, Ames). At appropriate times after the STZ, animals were taken for the heart perfusions. Unless otherwise indicated, the diabetes duration was 8 days.

Heart perfusion. After receiving 250 IU heparin i.p., the rats were anesthetized with pentobarbital (30 mg/kg), opened, and the hearts excised, washed in cold saline until beating ceased, and mounted via the aorta in a Langendorff perfusion apparatus. The perfusion medium consisted of a Krebs bicarbonate buffer as earlier described.[13] When retrograde perfusion was started, spontaneous contractions

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TABLE 1
Myocardial calcium uptake during in vivo heart perfusion

	Control	Diabetes
Basal	125.54 ± 4.67 (4)	114.34 ± 4.84 (4)
P	0.01	NS
ISO	163.44 ± 7.49 (10)	124.17 ± 4.64 (7)
P	0.007	
ISO + propranolol	124.90 ± 7.62 (5)	—
ISO + in vivo insulin	—	150.30 ± 4.82* (5)

Values are expressed as nmol/g protein/min. Number of experiments is indicated in parentheses. ISO: isoproterenol, 10^{-4} M.
*2P = 0.005 compared with the ISO response in diabetes.

appeared within seconds and, after an initial 5-min washout, 40 ml of buffer was recirculated with the addition, as appropriate, of the following substances: insulin (Actrapid, Novo, Denmark, final concentration 0.1 U/ml) or propranolol (ICI, UK, final concentration 10^{-4} M) followed 5 min later by ^{45}Ca (5 μCi/40 ml). After 10 min of labeling, perfusion was changed to cold (1.5°C) washout with a calcium-free buffer containing EGTA (0.5 mM). It has earlier been shown that 20 min of cold washout clears the vascular and interstitial phases of radiocalcium so that the remaining ^{45}Ca trapped within the cells can be used as a measure of the myocardial calcium uptake during the labeling period.[13] At termination, the hearts were homogenized in 5% trichloroacetic acid, centrifuged, and the supernatant counted in a scintillation counter. The protein content was determined using the Lowry technique with bovine albumin as standard.[15] By division with the specific activity of the buffer, the myocardial calcium uptake could be expressed as nmol/g protein/min perfusion.

Distribution of calcium uptake in individual heart segments. The distribution of the increased calcium uptake after isoproterenol (ISO) was analyzed in a 2-mm-thick transectional slice from the midventricular part of the hearts after perfusion. The slice was subdivided into anterior and posterior parts of the endocardial as well as epicardial myocardium of the left ventricle, and into the septum and right ventricle. The myocardial calcium uptake was determined in these individual segments as described above.

Statistics. Student's *t*-test for unpaired samples was used with a 2P = 0.05 level of significance. Data are presented as mean ± SEM.

RESULTS

ISO increases the myocardial calcium uptake in control rats (from 125.54 ± 4.67 to 163.44 ± 7.49 nmol/g/min, 2P = 0.01), while no significant increase is seen in diabetic hearts (from 114.34 ± 4.84 nmol/g/min to 124.17 ± 4.64 nmol/g/min) (Table 1). The basal uptake did not differ in control and diabetic hearts. β-Adrenergic blockade, by in vivo injection of 5 mg/kg i.p. of propranolol 60 min before perfusion, together with the addition of this β-blocker to the perfusate abolished the ISO-induced calcium uptake in control rats (124.90 ± 7.62 nmol/g/min), indicating a true β-receptor-mediated response. Table 2 shows the calcium distribution in the individual myocardial segments. It can be seen that all four areas within the free wall of the left ventricle, as well as the septum and the right ventricle, respond equally with respect to ISO-induced calcium uptake.

Time-graded collections of flow showed no differences in any of the experimental groups. Increasing the pump rate, effecting an increased flow from 5.56 ± 0.24 ml/min to 9.01 ± 0.18 ml/min, did not change the basal myocardial calcium uptake (125.54 ± 4.67 [N = 4] to 122.62 ± 11.5 [N = 5]). Therefore, a differential effect on coronary blood flow by any of the interventions cannot explain the changes in myocardial calcium uptake.

Concerning the time course for the induction of this phenomenon after establishment of the diabetic state, Figure 1 shows the ISO-induced calcium uptake in groups of animals investigated at 24-h intervals after the injection of STZ. In the same figure, the blood glucose levels are indicated. It appears that 96 h after the STZ or after 72 h of established hyperglycemia, a significant fall in ISO-induced calcium uptake is seen (2P = 0.03).

Turning to the reversibility experiments, acute in vivo insulin administration (4 IU i.p.) 60–90 min before heart perfusion with a consequent fall in blood glucose from 18.3 ± 0.9 to 5.7 ± 0.7 mmol/L, led to a normalization of the ISO-induced calcium uptake (150.30 ± 4.82 nmol/g/min, 2P = 0.005 compared with the ISO response in diabetes) (Table 1). To see if this was a direct insulin effect on the myocardial cells or mediated via intermediate insulin effects in vivo, insulin was added to the perfusate in four groups of animals: controls plus and minus ISO, and diabetics plus and minus ISO (Figure 2). Insulin did not affect basal or ISO-induced calcium uptake significantly in control animals, although there was a tendency for exaggerated ISO response after exposure to insulin. However, in diabetic hearts, ISO then led to a moderate but significant increase (117.18 ±

TABLE 2
Distribution of myocardial calcium uptake in heart segments

	Left ventricle					
	Anterior epi	Anterior endo	Posterior epi	Posterior endo	Septum	Right ventricle
Basal (5)	136.4 ± 16.1	117.8 ± 7.5	125.8 ± 14.0	112.7 ± 5.7	120.6 ± 11.9	112.8 ± 13.6
Isoproterenol (5)	157.7 ± 11.0	166.9 ± 28.0	151.6 ± 13.3	155.1 ± 13.8	161.4 ± 20.3	161.2 ± 20.3

Distribution of myocardial calcium uptake (nmol/g protein/min) in individual heart segments from a 2-mm-thick slice (midventricular portion) from hearts without (basal) and with stimulation with isoproterenol. Epi and endo designate the epicardial and endocardial, respectively, part of the myocardium. Number of experiments is indicated in parentheses.

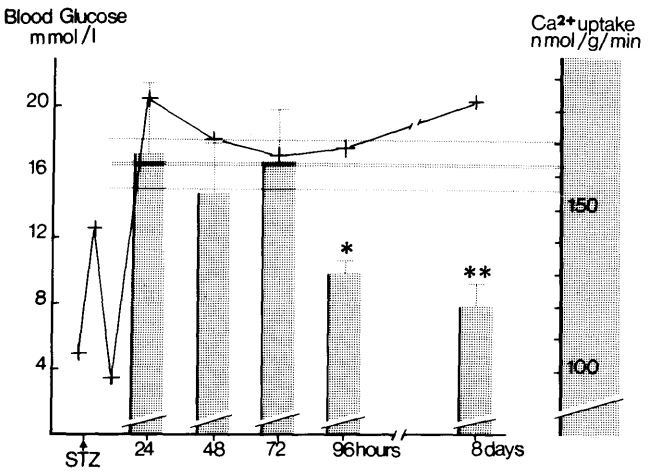

FIGURE 1. Time course after STZ for the induction of abnormal myocardial calcium uptake. Left ordinate indicates the blood glucose (mmol/L, solid line). Right ordinate depicts the myocardial calcium uptake after stimulation with isoproterenol, and in the columns are the values from groups of animals investigated at the points of time after the injection of STZ indicated on the abscissa. Stippled lines show the mean ± SEM of the isoproterenol-induced calcium uptake in control rats (N = 10). *2P = 0.03 and **2P = 0.001 compared with this value. Each group consists of 4–8 animals.

6.17 to 138.65 ± 6.42 nmol/g/min, 2P = 0.04) in calcium uptake, indicating a direct insulin effect on this β-receptor-mediated calcium transport into the myocardial cells.

DISCUSSION

The present radiolabeling method for estimating the myocardial calcium uptake in in vitro, perfused rat hearts has reproduced our earlier observation that STZ-diabetic rats have a marked decreased myocardial calcium uptake in response to ISO. Moreover, this decrease has now been shown to appear in the rats 4 days after the injection of STZ, when hyperglycemia has persisted for 3 days. These 4 days could indicate the time needed for the myocardial cells to adjust to the diabetic metabolism and contradicts a direct effect of hyperglycemia per se on this β-adrenergic response. Further evidence that this is an acute metabolic phenomenon can be seen from the fact that acute insulin treatment normalizes the response. A possible acute toxic effect of STZ in itself cannot, therefore, explain this catecholamine insensitivity. Control experiments on the effect of flow changes have excluded a possible differential ISO effect on coronary flow in explaining differences between calcium uptake in control and diabetic hearts.

Diabetic rats are protected against the necrotic effect of large doses of ISO when given in vivo.[16] These lesions appear as patchy areas mainly located in the subendocardium, and an excessive calcium influx is held responsible for their appearance.[17] This in vivo finding is, therefore, consistent with the present in vitro observation obtained in the isolated, retrograde-perfused, nonworking heart. The increase in calcium uptake in control hearts was equally distributed throughout the myocardium and no preferential accumulation was seen, e.g., in the subendocardium. The defect in the diabetic myocardium seems, therefore, to be an equally distributed metabolic abnormality located in the myocytes. Any potential modifying effect by the performance of external work (working heart preparation) has not been examined and could be of interest.

Insulin resistance with respect to stimulation of glucose uptake has been reported in the myocardium from alloxan-diabetic rats.[18] Still, in vitro insulin administration to isolated, perfused, diabetic rat hearts normalizes the decreased performance in response to anoxia and increased work.[19] In nondiabetic animals, a positive inotropic effect of insulin has been reported in several species[20,21] and, in the rabbit, insulin has been shown to attenuate the inotropic action of norepinephrine[21] and to have a protective effect against catecholamine-induced myocardial lesions.[22] The present study shows that insulin added to the perfusate, especially after acute in vivo administration, has a direct normalizing effect on the decreased β-adrenergic-mediated calcium uptake. In these insulinopenic animals, a direct sensitizing effect of insulin is seen as opposed to what appears as an antagonistic effect between insulin and catecholamines in the nondiabetic heart. The mechanism behind this insulin effect is not known, but the earlier reported uncoupling of the myocardial β-adrenergic receptor from productive adenylate cyclase activation in these rats[14,23] might be of relevance. This enzyme-receptor complex located in the sarcolemma is known for its lability, showing marked short-term changes in sensitivity.[24] Whether insulin could have direct effects on this transmembrane signaling or affect the neuronal reuptake of catecholamines[25] in such a way that the β-receptor is sensitized is not known at present.

Among other reported abnormalities in myocardial cell function in experimental diabetes are depressed cardiac muscle performance,[26] defective sarcoplasmic reticular function,[27] changes in the myosin isoenzyme distribution with

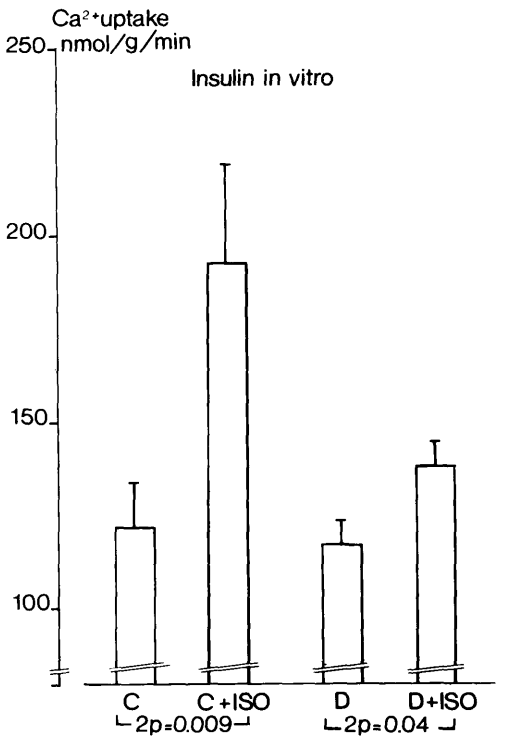

FIGURE 2. In vitro insulin effect on the isoproterenol-induced myocardial calcium uptake. Each group consists of 5–8 experiments.

lowering of the calcium ATPase activity of cardiac actomyosin,[28] and abnormalities in the calcium binding of the myocardial sarcolemma.[29] The present phenomenon located in the sarcolemma seems to appear and reverse somewhat faster than these abnormalities, but whether a causal relationship still exists is not known. Information about intracellular free calcium concentrations in experimental diabetes is limited. Still, changes in myocardial phosphorylase activity could be explained by an intracellular calcium build-up.[30]

Calcium movements are closely associated with contraction, cellular metabolism, and integrity,[31] and the possibility that the present insulin effect on calcium transport across sarcolemma also has relevance for human hearts cannot be excluded. To what extent optimal metabolic regulation of diabetic patients during episodes of myocardial stress (e.g., acute myocardial infarction) influences the incidence of pump failure also deserves further investigation.

REFERENCES

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

[2] 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.

[3] 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.

[4] Friedman, N. E., Levitsky, L. L., Edidin, D. V., Vitullo, D. A., Lacina, S. J., and Chiemmongkoltip, P.: Echocardiographic evidence for impaired myocardial performance in children with type I diabetes mellitus. Am. J. Med. 1982; 73:846–50.

[5] Ledet, T., Neubauer, B., Christensen, N. J., and Lundbaek, K.: Diabetic cardiopathy. Diabetologia 1979; 16:207–209.

[6] Reinilä, A., and Åkerblom, H. K.: Ultrastructure of heart muscle in short-term diabetic rats. The influence of insulin treatment. Abstract. Acta Endocrinol. 1983; 257 (Suppl.):57.

[7] Uusitupa, M., Sütonen, O., Aro, A., Korhonen, T., and Pyörälä, K.: Effect of correction of hyperglycemia on left ventricular function in non-insulin-dependent (type II) diabetics. Acta Med. Scand. 1983; 213:363–68.

[8] Friedman, H. S., Sacerdote, A., Bandu, I., Jubay, F., Herrera, A. G., Vasavade, C. C., and Bleicher, S. J.: Abnormalities of the cardiovascular response to cold pressor test in type I diabetes. Correlation with blood glucose control. Arch. Intern. Med. 1984; 144:43–47.

[9] Harrower, A. D., and Clarke, B. F.: Experience of coronary care in diabetes. Br. Med. J. 1076; 1:126–28.

[10] Oswald, G., Corcoran, S., Casburn-Budd, M., and Yudkin, J. S.: The significance of hyperglycemia following acute myocardial infarction. Abstract. Diabetologia 1983; 25 (Suppl. 2):184.

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

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

[13] Gøtzsche, O.: Decreased myocardial calcium uptake after isoproterenol in streptozotocin-induced diabetic rats. Lab. Invest. 1983; 48:156–61.

[14] Gøtzsche, O.: 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. Diabetes 1983; 32:1110–16.

[15] 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.

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

[17] Fleckenstein, A.: Specific inhibitors and promotors of calcium action in the excitation-contraction coupling of heart muscle and their role in the prevention of production of myocardial lesions. *In* Calcium and the Heart. Harris, P., and Poie, L. H., Eds. London, Academic Press, 1971:135.

[18] Randle, P. J., Garland, P. B., Hales, C. N., Newsholme, E. A., Denton, R. M., and Pogson, C. I.: Interactions of metabolism and the physiological role of insulin. Rec. Prog. Horm. Res. 1966; 22:1–48.

[19] Ingebretsen, C. G., Moreau, P., Hawelu-Johnson, C., and Ingebretsen, W. R.: Performance of diabetic rat hearts: effect of anoxia and increased work. Am. J. Physiol. 1980; 239:H614–20.

[20] Lucchesi, B. R., Medina, M., and Kniffen, F. J.: The positive inotropic action of insulin in the canine heart. Eur. J. Pharmacol. 1972; 18:107–15.

[21] Lee, L. C., and Downing, S.: Effects of insulin on cardiac muscle contraction and responsiveness to norepinephrine. Am. J. Physiol. 1976; 230 (Suppl. 5):1360–65.

[22] Downing, S. E., and Lee, J. C.: Effects of insulin on experimental catecholamine cardiomyopathy. Am. J. Pathol. 1978; 93:339–52.

[23] 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.

[24] Marsh, J. D., Barry, W. H., Neer, E. J., Alexander, R. W., and Smith, T. W.: Desensitization of chick embryo ventricles to the physiologic and biochemical effect of isoproterenol. Evidence for uncoupling of the β-receptor-adenylate cyclase complex. Circ. Res. 1980; 47:493–501.

[25] Bhagat, B., Burke, W. J., and Dhalla, N. S.: Insulin-induced enhancement of uptake of noradrenaline in atrial strips. Br. J. Pharmacol. 1981; 74:325–32.

[26] 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.

[27] Penpargkul, S., Fein, F., Sonnenblick, E. H., and Scheuer, J.: Depressed cardiac sarcoplasmic reticular function from diabetic rats. J. Mol. Cell. Cardiol. 1981; 13:303–309.

[28] Dillmann, W. H.: Diabetes mellitus induces changes in cardiac myosin of the rat. Diabetes 1980; 29:579–82.

[29] Pierce, G. N., Kutryk, M. J. B., and Dhalla, N. S.: Alterations in the Ca^{2+} binding by and composition of the cardiac sarcolemma membrane in chronic diabetes. Proc. Natl. Acad. Sci. USA 1983; 80:5412–16.

[30] Vadlamudi, R. V. S. V., and McNeill, J. H.: Effect of experimental diabetes on rat cardiac cAMP, phosphorylase and inotropy. Am. J. Physiol. 1983; 244:H844–51.

[31] Dhalla, N. S., Pierce, G. N., Panagia, V., Singal, P. K., and Beamish, R. E.: Calcium movement in relation to heart function. Basic Res. Cardiol. 1982; 77:117–39.