PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 181, 345–350 (1986)

Ventricular Performance in Diabetic Rabbits with Norepinephrine Cardiomyopathy[1] (42263)

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Abstract. The purpose of this investigation was to examine the effects of norepinephrine cardiomyopathy (NE-CM) on left ventricular (LV) performance in diabetic rabbits. Diabetes mellitus was produced in 11 rabbits by giving them alloxan monohydrate, 120 mg/kg. Cardiomyopathy was produced in five animals by a 90-min infusion of norepinephrine (2 μg/min/kg). Left ventricular contractility and pump function (VF) were examined 2 days later. The effects of hypercapnia and inotropic responsiveness to NE were also determined. VF was assessed by means of left ventricular function curves obtained with constant mean aortic pressure and heart rate and quantified by determining stroke volume (SV) at a left ventricular pressure of 10 cm H_2O (SV_{10}). Mean SV_{10} was 1.22 ± 0.08 ml in control diabetics but averaged only 0.95 ± 0.08 ml in diabetics with NE-CM ($P < 0.05$). NE-CM markedly reduced LV *dP/dt* max responses to NE infusion but the increments in SV_{10} did not differ. Hypercapnia caused significantly greater ventricular depression in NE-CM than in control diabetic rabbits ($P < 0.001$). The depressive effect of hypercapnia can be countered in part by the administration of NE in both groups, but differential depression in VF to hypercapnia was persistent between the two groups.

It is widely recognized that diabetes mellitus is associated with increased morbidity and mortality from cardiac disease. Although microvascular damage and accelerated atherosclerosis of the coronary arteries are important pathological factors (1), other mechanisms may be involved. Intrinsic abnormalities of diabetic myocardium have recently been uncovered by various experimental stresses (2–5). The basis for the appearance of a proposed diabetic cardiomyopathy is unresolved, however. Alterations in cardiac high energy phosphate production and utilization (1, 6, 7), depression in myofibrillar Ca-ATPase activity (8), and changes in cardiac sarcoplasmic reticular calcium transport (9) have been suggested to explain observed reductions in mechanical performance.

Administration of norepinephrine (NE) results in dose-related myocardial damage in rabbits (10). These morphological changes are accompanied by substantial impairment of left ventricular performance when studied with afterload curves (11), or with standard ventricular function curves (12). Earlier studies from our laboratory have shown that insulin substantially reduces myocardial responsiveness to NE in both isolated (13) and intact (14) heart preparations from several species. Pretreatment of animals with insulin also greatly reduces the extent of NE-induced myofiber injury (10) and associated depression of ventricular function (11). It is possible that insulin deficiency, as in type I diabetes mellitus, may potentiate myocardial injury from excessive catecholamine stimulation. The present study was designed to assess the effect of NE cardiomyopathy on left ventricular performance in alloxan diabetic rabbits.

Methods. Studies were performed on 11 diabetic adult New Zealand white rabbits of both sexes. The animals were made diabetic by injection of alloxan monohydrate (Sigma), 120 mg/kg into an ear vein. The alloxan was prepared as a 10% solution dissolved in sterile saline immediately before use. All rabbits were allowed to ingest glucose in water for the next 24 hr and then were provided with food (Purina Rabbit Chow), and water *ad libitum*. The diabetic state of each animal was monitored daily by testing ear vein blood for hyperglycemia using a semiquantitative Dextrostix and

[1] Supported in part by Grants HL-20401 and HL-08659 from the National Institutes of Health.

0037-9727/86 $1.50

Glucometer (Ames) method. Following a period of 8 weeks stabilization, these animals had nonfasting blood glucose values above 300 mg/dl. Five rabbits were made cardiomyopathic by intravenous infusion of norepinephrine (Levophed, Winthrop, 2 μg/kg/min for 90 min). The method for generation of NE cardiomyopathy has been described in detail elsewhere (10, 12). In brief, the rabbits were anesthetized with intravenous pentobarbital sodium (30 mg/kg). Polyethylene catheters were placed in a femoral artery for monitoring hemodynamic conditions, and in a femoral vein for infusion of NE. At the end of the infusion, the incisions were surgically closed and the animals were returned to their cages for recovery.

Two days after the NE infusion the rabbits were again anesthetized and prepared for ventricular function studies. These methods have been described in detail previously (12). After tracheal intubation, the chest was opened along the midline, and ventilation was maintained with a Harvard respiratory pump. Heparin (1000 units) was given intravenously to prevent clotting in the recording catheters and extracorporeal system. The thoracic aorta was cannulated, and aortic flow was measured with a Biotronix extracorporeal transducer and electromagnetic flowmeter system. Aortic flow was then passed through a heat exchanger and returned to the descending aorta and brachiocephalic artery. The left subclavian artery was ligated.

Arterial blood pressure was controlled by means of an adjustable constant pressure reservoir. Cardiac frequency was controlled by electrically pacing the left atrium with a Grass SD-4 stimulator, 5–10% faster than the intrinsic rate. Cardiac output was altered by means

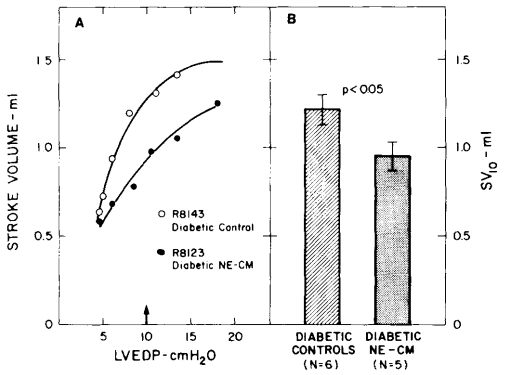

FIG. 1. (A) Left ventricular function curves from a representative diabetic control (open circles) and a diabetic with NE-induced cardiomyopathy. (B) Mean stroke volume at left ventricular end-diastolic pressure of 10 cm H_2O (SV_{10}). Vertical brackets indicate SE. Ventricular function was significantly less in the catecholamine-injured (NE-CM) hearts.

of a pump-operated arteriovenous shunt that permitted blood to be pumped from the aortic circuit to the superior vena cava. The extracorporeal system was primed with freshly drawn heparinized (600 U/100 ml) donor blood. Blood temperature was maintained at 37 ± 1°C with a heat exchanger and was measured with a Yellow Springs probe and telethermometer.

An 18-gauge needle was passed through the apex into the left ventricular cavity and used to obtain pressure traces. Aortic and ventricular pressure measurements were made with Sanborn transducers, using the midlevel of the heart as zero reference. Left ventricular *dP/dt* max was obtained by a differentiating circuit incorporated in the carrier preamplifier. The pressures, aortic flow, heart rate, and left ven-

TABLE I. ARTERIAL pH, BLOOD GAS TENSIONS, AND HEMATOCRIT IN DIABETIC RABBITS

		Eucapnia				Hypercapnia			
	N	pH	PO_2	PCO_2	Hct	pH	PO_2	PCO_2	Hct
Controls	6	7.36 ± 0.02	124.0 ± 12.9	30.8 ± 4.5	31.8 ± 0.08	7.05 ± 0.05	126.4 ± 11.1	69.8 ± 1.3	32.0 ± 0.8
NE-CM	5	7.30 ± 0.05	101.0 ± 5.6	37.0 ± 1.2	32.5 ± 0.9	7.03 ± 0.01	88.8 ± 5.1	64.5 ± 1.1	32.3 ± 0.8

Note. Values are means ± SE. *N*, number of animals. pH in units, arterial O_2 and CO_2 tensions (PO_2 and PCO_2) in Torr, and hematocrit (Hct) in percentage. NE-CM, norepinephrine cardiomyopathy.

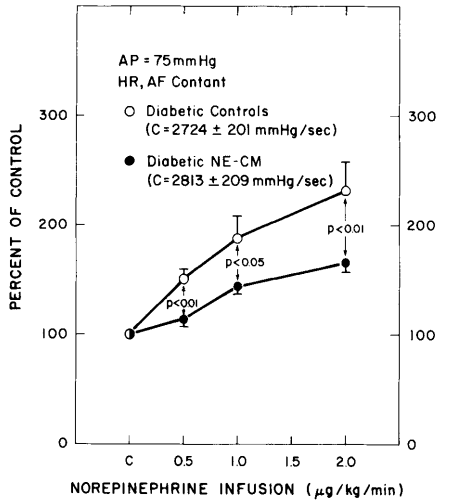

FIG. 2. Norepinephrine dose–response curves relating dose to maximal rate of rise of left ventricular pressure (*dP/dt* max) expressed as percentage of initial values. Vertical brackets indicate SE. Responses in cardiomyopathic diabetic rabbits were significantly less compared with diabetics without prior NE injury.

tricular *dP/dt* measurements were recorded simultaneously on a multichannel oscillograph (Hewlett–Packard 7758 B system).

Left ventricular function curves were constructed from step-wise increments of venous return while maintaining constant arterial blood pressure and heart rate. Stroke volume was calculated for each step and plotted in relation to simultaneous left ventricular end-diastolic pressure (LVEDP). Subsequently each curve was analyzed by determining the stroke volume (SV) ejected by the ventricle at the end-diastolic pressure of 10 cm H_2O. This value (SV_{10}) permits a quantitative comparison of relative "pump function" capabilities of the ventricle. The choice of SV_{10} as a measure of ventricular performance has been discussed previously (15).

Responses to adrenergic stimulation were assessed during continuous norepinephrine infusions at rates of 0.5, 1.0, and 2.0 μg/min/kg. A ventricular function curve was obtained during the maximum infusion rate (2 μg/min/kg). The effects of hypercapnia on ventricular performance were determined by gradually adding CO_2 to the inspired gas mixture using a Simet gas mixing device to yield arterial PCO_2 values in excess of 60 Torr. A period of 15 min was allowed to achieve a steady-state equilibrium before the ventricular function curve was obtained. Arterial blood samples were drawn before each curve for measurement of blood gas tensions and pH (Instrumentation Laboratories System) and hematocrit values (Microhematocrit tubes, Clay Adams).

Data are expressed as means ± SE. Student's *t* test was used to evaluate the significance between means. Differences were considered significant when the *P* values were less than 5% (16).

Results. *Comparison of left ventricular pump function.* Left ventricular function was compared in six diabetic animals and five diabetics with superimposed NE cardiomyopathy (NE-CM). Baseline metabolic data are shown in Table I. Curves relating stroke volume to left ventricular end-diastolic pressure from representative rabbits of each group are illustrated in Fig. 1A. The curve obtained from the diabetic with superimposed NE-CM rabbit is considerably lower than that from the rabbit with diabetes only. Stroke volume ejected for any given end-diastolic pressure was less. This indicates that left ventricular pump function in NE-CM diabetic rabbits was depressed when compared with the control diabetics.

Stroke volume at a left ventricular end-diastolic pressure of 10 cm H_2O (SV_{10}) for each curve was obtained and the values for each

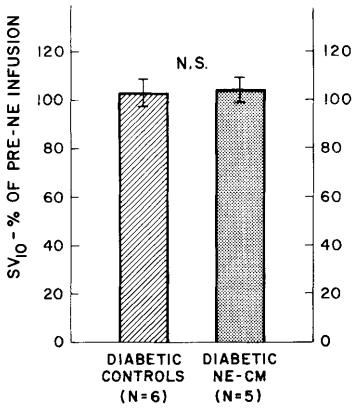

FIG. 3. Left ventricular pump function during norepinephrine (NE) infusion (2 μg/kg/min) in diabetic controls compared with NE-cardiomyopathic (NE-CM) rabbits. Vertical brackets indicate SE. Responses of two groups did not differ.

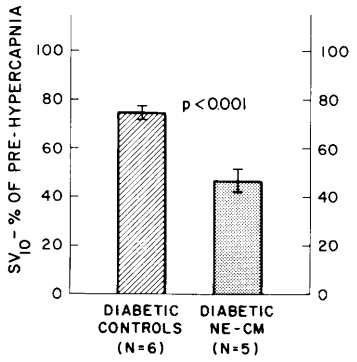

FIG. 4. Effects of hypercapnia on left ventricular performance in diabetic rabbits without and with NE injury. Greater depression of left ventricular function appeared in the cardiomyopathic group. Vertical brackets indicate SE.

group are summarized in Fig. 1B. SV_{10} in the control diabetic group averaged 1.22 (±0.08) ml; in contrast, the mean SV_{10} in diabetic animals with superimposed NE-CM was 0.95 (±0.01 ml; $P < 0.05$).

Inotropic responses to norepinephrine. In all animals, norepinephrine infusion (0.5–2.0 μg/kg/min) produced dose-dependent increases in left ventricular *dP/dt* max while aortic pressure and heart rate were kept constant. These data are summarized in Fig. 2. The changes in LV *dP/dt* max were plotted as percentage increase over control values obtained immediately prior to giving NE. Control values for LV *dP/dt* max in controls and NE-CM diabetic rabbits did not differ significantly. However, the inotropic responses to NE infusion were less at all dose levels in the NE-CM diabetic rabbits compared to the control diabetics.

The effect of NE infusion on ventricular pump function was assessed during the highest dose (2 μg/kg/min) by obtaining ventricular function curves relating SV to LVEDP. The SV_{10} during NE infusion averaged 1.23 (±0.09) and 0.98 (±11) ml in the control and NE-CM diabetic groups, respectively. These values were not significantly different from their respective preinfusion values (1.22 ± 0.08 and 0.95 ± 0.08). When these data were expressed as percentages of preinfusion SV_{10}, the mean values for both groups were almost identical (Fig. 3).

Effects of hypercapnia. Mean values for arterial pH, PO_2, PCO_2, and hematocrit in control and NE-CM diabetic rabbits before and during hypercapnia are listed in Table I. It can be seen that the degree of respiratory acidosis induced by the addition of CO_2 to the inspired gas mixture was almost identical in the two groups as reflected by comparable levels of arterial pH and PCO_2.

Induction of severe hypercapnic acidosis caused a depression in left ventricular function (downward shift of the ventricular function curve) in all animals. As shown in Fig. 4, the mean reduction of SV_{10} in control diabetic rabbits was about 26%. However, in those with superimposed cardiomyopathy the reduction of SV_{10} during hypercapnia was greater than 50%. The difference between the groups was highly significant ($P < 0.001$). Reduction in ventricular function was substantially less when NE was given during hypercapnic stress in both groups (Fig. 5). However, the cardiomyopathic hearts continued to manifest greater intolerance to hypercapnic acidosis.

Discussion. The study was undertaken primarily to examine the effect of experimental NE cardiomyopathy on left ventricular performance in diabetic rabbits. The left ventricular performance was assessed by ventricular function curves and measurements of ventricular *dP/dt* max while aortic pressure and heart rate were held constant. The mean values of both SV_{10} (stroke volume ejected by the ventricle at end-diastolic pressure of 10 cm H_2O) and *dP/dt* max were slightly higher in diabetics

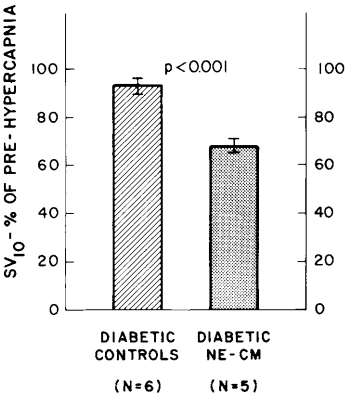

FIG. 5. Effects of hypercapnia on left ventricular function during inotropic stimulation with norepinephrine (NE; 2 μg/kg/min). LV performance was well-maintained in diabetics, but fell substantially in those with coexisting NE cardiomyopathy. Vertical brackets indicate SE.

as compared with those of our previously reported nondiabetic group of animals (12).

These findings are consistent with our earlier reports on the effect of alloxan diabetes on newborn lambs (17). Using isolated cardiac papillary muscle preparations, Fein *et al.* (18) were unable to identify significant alteration in rats with chronic streptozotocin diabetes. Similar results were also found in isolated perfused heart preparations with normal ranges of ventricular filling pressure (4, 19). But the ability of the ventricle to respond to the higher ventricular filling pressure (15–20 cm H_2O) was decreased in diabetes. At high ventricular filling pressures there is greater tension development and increased myocardial oxygen demand. Thus decreased ventricular performance at higher filling pressures in isolated perfused heart preparations could be the result of imbalance of oxygen supply and demand, coupled with intrinsic metabolic and structural derangements. An exquisite sensitivity of diabetic hearts to reduced O_2 delivery in isolated heart preparations and compromised coronary circulation in diabetics (17, 20) has been demonstrated.

Intrinsic abnormalities of diabetic myocardium have been uncovered by various stresses (2, 3, 21). These include a number of biochemical parameters such as depressed SR calcium transport (22, 23), reduced CA^{2+}-activated ATPase (8, 24), and altered sodium pump activity (25). These defects are likely associated with alterations in the composition of cardiac membranes (23, 26). For example, Lopaschuk *et al.* (26) reported that cardiac SR calcium transport is depressed and this is associated with the accumulation of LC acylcarnitines in the SR fraction. This can be prevented by carnitine treatment, but the appearance of cardiac dysfunction is unaffected. Functional depression in diabetes is not associated with concomitant hypothyroidism (18). Thus, other abnormalities must be considered.

The hypercapnia studies are consistent with important underlying abnormalities in the diabetic hearts. Depression of ventricular function was significantly greater in diabetics than in normal hearts. Severe hypercapnia reduced ventricular pump function 10% in controls and 25% in NE-CM hearts (12). In contrast, the mean reduction of ventricular pump function was 50% in diabetics with superimposed NE-CM (Fig. 4). The depressive effect of hypercapnia can be countered in part by the administration of NE (Fig. 5). The importance of adrenergic activity in supporting cardiac function during acute hypercapnia and acidosis has been amply demonstrated in normal animals (27, 28). Nevertheless, this study shows that 8 weeks of diabetes produced no significant alterations in ventricular responses to NE infusion. More importantly, we observed that the intrinsic exquisite sensitivity of diabetic hearts to hypercapnia was not related to the depressed myocardial adrenergic system. This concept is consistent with earlier reports. Vadlamudi and McNeill (29) found no changes either in cardiac cAMP responses, or in the sensitivity or maximum inotropic response to diabetic rat hearts to isoproterenol. Miller *et al.* (30) reported similar results with epinephrine. In contrast Ingebretsen *et al.* (31) demonstrated that acute alloxan-induced diabetes in the rat produced no change in basal cardiac cAMP or GMP content, or in cardiac protein kinase or phosphorylase activities. However, a marked reduction in isoproterenol-induced changes in cAMP and protein kinase activity was identified. The discrepancies between these studies may relate to differences in duration and severity of the diabetic state. Clarification of this issue awaits further investigation.

The excellent technical assistance of Ronald Gordon and Victor Hardaswick is gratefully acknowledged.

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Received July 22, 1985. P.S.E.B.M. 1986, Vol. 181.
Accepted November 1, 1985.