Effect of Diabetes on Fast Response to Norepinephrine in Rat Aorta

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The fast and slow components of the mechanical response to 1 μM norepinephrine (NE) were measured in aortic rings isolated from eight spontaneously diabetic rats, six streptozocin-induced diabetic (STZ-D) rats, six STZ-D rats treated with 2.5 U insulin/day during the 4 days before being killed, and six age- and sex-matched control rats. The total contraction to NE (i.e., the sum of fast and slow components) was similar in the four groups: spontaneously diabetic, 16.53 ± 1.72 mN; STZ-D, 15.68 ± 1.41 mN; insulin-treated, 16.17 ± 2.05 mN; and control, 15.27 ± 0.96 mN (NS). The fast component, measured graphically in a total contraction in 1.35 mM Ca, was greater in spontaneously diabetic (12.61 ± 1.07 mN, $P < 0.05$) and STZ-D (12.25 ± 0.89 mN, $P < 0.05$) rats compared with control (9.14 ± 0.74 mN) or insulin-treated (8.58 ± 1.23 mN) rats. The same increase of the fast component was detectable after 3 min of incubation in Ca-free medium + 2 mM EGTA (control 6.54 ± 0.47 mN, spontaneously diabetic 9.07 ± 0.76 mN, $P < 0.05$; STZ-D 8.82 ± 0.72 mN, $P < 0.05$), and it was also abolished by insulin treatment (insulin-treated 6.29 ± 0.36 mN). We conclude that the diabetic state increases the fast component of NE-induced contraction either in the absence or presence of Ca in the medium. This suggests that such an increase depends on a larger release of Ca from intracellular stores. ***Diabetes*** **41:30–34, 1992**

Peripheral vascular disease is a common occurrence in diabetes. Alterations include restrictions of blood flow to the limbs, which frequently causes tissue necrosis (1). The underlying reason for this phenomenon could be exaggerated vasoconstriction and/or deficient vascular relaxation, which might be caused by an abnormally intense response of diabetic vascular smooth muscle to endogenous agonists. Enhanced vasoconstriction has been demonstrated in response to prostanoids (2) and catecholamines in numerous vascular preparations as isolated rat aorta (3,4), rat cremaster (5,6), rat tail artery (7), isolated rat hindlimb (8,9), and perfused femoral bed of the dog (10). Deficient vascular relaxation has also been reported in rat aorta (11) and rat (12) and dog (13–15) coronary vessels, and it was associated in some cases with deficient endothelial activity (16,17).

Vascular response to catecholamines can be divided from the mechanical point of view into fast and slow components (18). These components are caused by mobilization of Ca from different sources (intracellular Ca for the fast, extracellular Ca for the slow), and both processes are mediated by well-identified second messengers (19). Ca entry blockers affect only the slow component because it needs extracellular Ca, whereas, the fast component is not altered due to its intracellular origin.

Although the enhanced vascular response to catecholamines in diabetes has been described, the reports about the influence of the disease on the different components of contraction are scarce (20). This could have important pathological implications, however, because the selective modification of any of the phases could point to a particular site and/or mechanism of action of the disease.

This study investigated the effects of diabetes (spontaneous and streptozocin [STZ]-induced) and of insulin treatment on the fast and slow components of the response to norepinephrine (NE) in rat aorta.

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RESEARCH DESIGN AND METHODS

Norepinephrine, acetylcholine, and EGTA were purchased from Sigma (St. Louis, MO). Drugs for isotonic salt solution (ISS) preparation were of analytical grade and were obtained from local suppliers.

The experiments were carried out in 38 male rats weighing 300–350 g divided into four groups. Eight rats belonged to the eSS strain, which spontaneously develops a non-insulin-dependent diabetic syndrome (21). These rats had basal glycemia of 4.31 ± 0.45 mM but attained abnormally high values (10.98 ± 1.62 mM) after an oral glucose load (2.5 g/kg body wt). Twelve additional rats with basal glycemia of 4.48 ± 0.17 mM were rendered diabetic by one injection of 40 mg/kg body wt i.v. STZ. Seven days after the injection, six of these rats were used as STZ-induced diabetic (STZ-D) rats, and the basal glycemia under these conditions was 16.91 ± 0.34 mM. The remaining six rats were treated with insulin for 4 days (2.5 U/day). Glycemia was 18.26 ± 0.39 mM after STZ administration and was lowered to 11.93 ± 0.34 mM after insulin treatment. The last group was composed of six rats with basal glycemia of 4.98 ± 0.22 mM who received no treatment at all and served as control rats. Because the STZ-D rats were used 7 days after STZ administration and the insulin-treated group was used 11 days after the injection, an additional group of six rats were injected with STZ and used 11 days after the injection to match the treatment length of insulin-treated rats.

The day of the experiments, the animals were anesthetized with 80 mg/kg body wt i.p. pentobarbital sodium, and the thoracic aorta was quickly excised and placed in a petri dish filled with ISS with the following composition: 118 mM NaCl, 5.32 mM KCl, 1.54 mM NaH_2PO_4, 119 mM $MgSO_4$, 24.9 mM $NaHCO_3$, 1.35 mM $CaCl_2$, 0.01 mM EDTA, and 5.6 mM glucose. Rings 2 mm in length were cut and mounted isometrically in thermostatted (37°C) organ baths filled with ISS continuously bubbled with a mixture of 5% CO_2/95% O_2. Force was recorded with a Grass FT.03C force transducer coupled to a Sanborn polygraph. Special care was taken to not damage the endothelium during the mounting procedure.

At the end of the experiments, the rings were contracted with 1 μM NE in ISS. After the contraction stabilized, the presence of functional endothelium was confirmed by the addition of 1 μM acetylcholine, which produced a relaxation of 30–50%. When needed, the endothelium was removed by inserting the points of small forceps and rolling the ring gently over filter paper for 15 s (22). These rings did not relax when exposed to acetylcholine as described previously.

After 1 h of stabilization under a passive force of 2 *g*, the rings were exposed to NE until the contraction reached a plateau (usually 15–20 min). The fast component of the response was measured from the baseline to the point in which the rate of force development decreased abruptly. The slow component was measured from that point to the top of the contraction. The total response was the sum of both components (Fig. 1, *A* and *B*). In some experiments, the fast component was measured after 3 min of exposure to Ca-free ISS + 2 mM EGTA, which prevents the appearance of the slow component (Fig. 1, *C*). In six control and six STZ-D rats, used 11 days after STZ administration, concentration-contraction curves to NE were performed in Ca-free ISS + 2 mM EGTA, obtaining the fast component only. The same experiments were repeated in rings of the same group in which the endothelial lining had been removed.

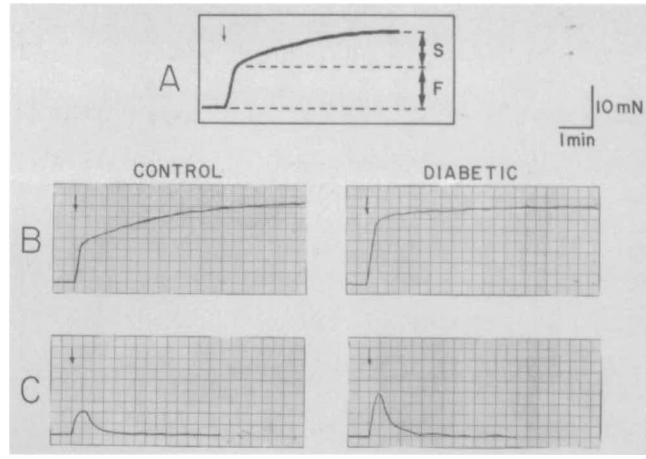

FIG. 1. *A*: graphic method used to measure fast (F) and slow (S) components of response to norepinephrine (NE) in 1.35 mM Ca. *B* and *C*: actual records of 2 NE responses in aortic rings of spontaneously diabetic and control rats, showing that, in the diabetic preparation, F predominated over S. *Arrows*, addition of 1 μM NE to bath.

The results are expressed as means ± SE. Groups were compared by Student's *t* test for unpaired samples or analysis of variance (>2 groups compared at the same time). $P < 0.05$ was significant.

RESULTS

The total response to NE was not significantly different in the four groups of rats (Fig. 2). The fast components of these total responses were measured graphically (Fig. 1), and the results are presented in Fig. 3. The fast component was significantly greater in spontaneously diabetic and STZ-D rats than control or insulin-treated rats.

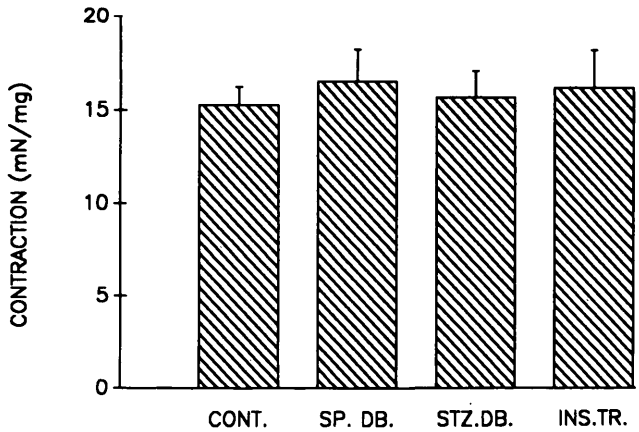

FIG. 2. Total response to 1 μM norepinephrine in 1.35 mM Ca showing similar contraction in all experimental groups. CONT, control, *n* = 6; SP DB, spontaneously diabetic, *n* = 8; STZ DB, streptozocin-induced diabetic, *n* = 6; and INS TR, insulin-treated STZ-DB rats, *n* = 6.

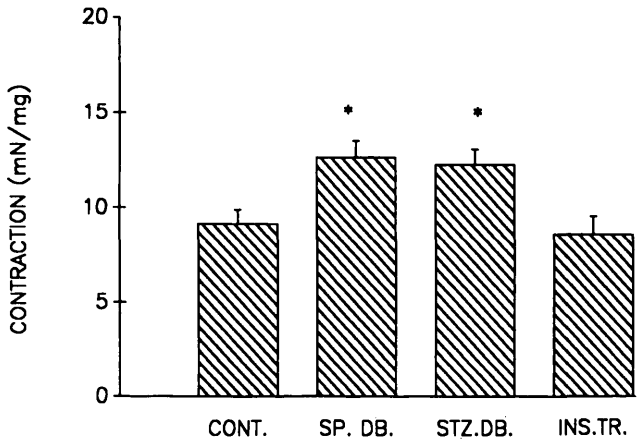

FIG. 3. Fast component to 1 μM norepinephrine in 1.35 mM Ca measured graphically (see METHODS). Fast component is greater in spontaneously diabetic (SP DB, n = 8) and streptozocin-induced diabetic (STZ DB, n = 6) rats than in control (CONT, n = 6) or insulin-treated (INS TR, n = 6) rats. *P < 0.05 vs. CONT or INS TR.

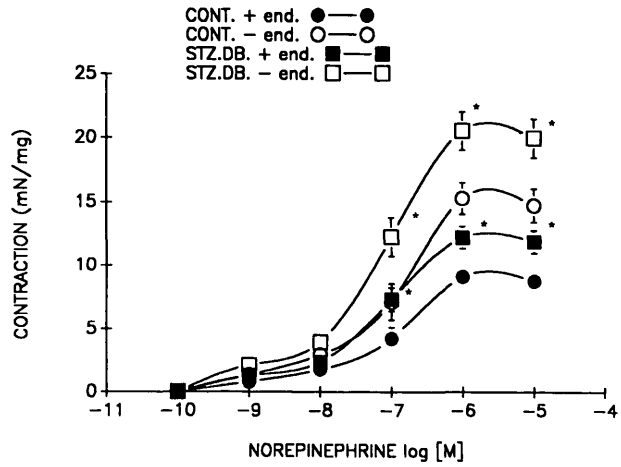

FIG. 5. Fast response to norepinephrine in control (CONT, n = 6) and streptozocin-induced diabetic (STZ DB, n = 6) rat aortic rings with and without endothelium (end.). Greater fast response in STZ DB rats was evident over a wide range of norepinephrine concentrations. Endothelium removal increased contraction in both groups, but the difference between STZ DB and CONT rats persisted. *P < 0.05 STZ DB vs. CONT.

The fast component was further measured under conditions in which the slow component could no longer be present, i.e., incubation in Ca-free ISS for 3 min (Fig. 4). Although the contractile response was attenuated with respect to the response obtained in presence of extracellular Ca (Fig. 3), the fast component was significantly greater in spontaneously diabetic and STZ-D rats compared with control and insulin-treated rats.

This increased fast response to NE did not seem to be correlated with the degree of the metabolic disorder, at least as evaluated by the blood glucose levels. The increase of the fast response to NE was similar in STZ-D and spontaneously diabetic rat aortas despite much higher glycemia in the former. Moreover, insulin treatment reverted the abnormal mechanical response in the insulin-treated rats despite blood glucose levels that were still considerably higher than in control conditions.

This increased fast-component response in STZ-D rats was detected over a wide range of concentrations (Fig. 5). Endothelium removal increased the fast-component responses in both control and STZ-D rings, but the difference between both groups persisted. The concentration to reach half-maximal contraction was not significantly different between groups (control rats 1.58 × 10^{-7} M, STZ-D rats 7.49 × 10^{-8} M, NS).

Because the total response to NE was similar in all groups, and the fast component was increased in diabetic rats, the slow component was, as anticipated, significantly depressed in the spontaneously diabetic rats (data not shown).

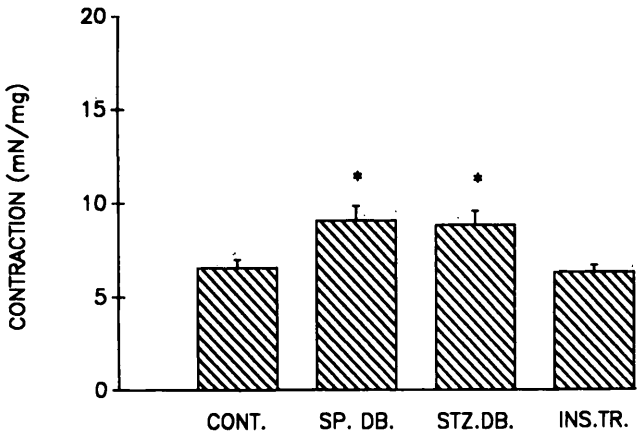

FIG. 4. Fast component to 1 μM norepinephrine measured in Ca-free isotonic saline solution + 2 mM EGTA. Although forces are smaller than those obtained in 1.35 mM Ca, fast component is still greater in spontaneously diabetic (SP DB, n = 8) and streptozocin-induced diabetic (STZ DB, n = 6) rats than control (CONT, n = 6) or insulin-treated (INS TR, n = 6) rats. *P < 0.05 vs. CONT or INST TR rats.

DISCUSSION

Diabetes is frequently associated with peripheral vascular disease (1). Although various structural vascular lesions can be encountered that produce a deficient blood supply to the tissues involved, there is also an exaggerated vasoconstrictor response to sympathetic stimulation, which has been demonstrated in several vascular preparations (3–10). Less attention has been focused on which of the two components of the response to NE is altered by the disease, which was the main goal of this study.

Contrary to the description of several investigators (3–10), we did not find an enhanced total response to NE in our vascular preparations of diabetic rats. However, when the response was separated into fast and slow components, the fast component was augmented in diabetic rats. Furthermore, the appearance of the same alteration in two different models of diabetes seems to indicate that the augmented fast component is a consequence of elevated glucose levels and/or a decrease of insulin availability rather than an inherited trait. In contrast, Carrier and White (22) have shown increased dependency of the adrenergic response on extracellular Ca. This could be ascribed to their use of Sprague-

Dawley rats, which differ in several aspects of cytoplasmic Ca handling from Wistar rats (23).

The reason for the discrepancy between our results and those of others, in which most found an increased response to NE and other vasoconstrictors, is not evident. The difference could be partly due to the different species and/or etiology of diabetes. Although most studies were conducted in rats (3–9), only two studies tested the aorta (3,4), but the STZ dose was greater than in our study (65 mg/kg body wt). Two of the other studies used alloxan-induced diabetes (6,8), but neither used aorta (5–9). The remaining study used the femoral bed of alloxan-induced diabetic dogs (10). In neither case did the investigators differentiate between fast and slow components of response. In addition, experiments carried out in vessels in which the fast component predominates could lead to overestimation of the response to NE. Similarly, if the fast component is measured too early during an NE contraction, part of the slow component can be overlooked.

Both phases of the response to NE involve different sources of activator Ca (18). Although the slow component reflects Ca influx through receptor-operated channels, the fast component is caused by release of NE-sensitive intracellular Ca stores (19). Therefore, an increase of the fast component in diabetes could reflect an accumulation of intracellular Ca caused by this disease and/or an augmented Ca release due to increased formation of inositol trisphosphate and other intracellular mediators (20). The augmented release could stabilize the membrane and diminish Ca entry through Ca channels, and in this way, the fast would predominate over the slow component (24–26). Another possibility is that an augmented intracellular Ca release would open more Ca-sensitive K^+ channels, leading to repolarization and decreased slow component of the contraction (26).

The analysis of the concentration-contraction curves to NE in control and STZ-D rats revealed an increased maximal response of the fast component in the latter, with no differences in the NE concentration needed to reach half-maximal contraction. This indicates that the sensitivity to NE is similar in both groups, but much more Ca is released from the intracellular stores in diabetic compared with control rats. Note that the STZ-D rats in this group had been used 11 days after STZ administration (as was the case with the insulin-treated rats), showing no differences with respect to STZ-D rats used 7 days after injection. This rules out any effect attributable to the different duration of treatment in both cases.

To exclude some artifact that could have been caused by the graphic estimation of the fast and slow components, measurements were carried out with previous exposure to Ca-free ISS. In these conditions, only the fast component occurs (18). The results were in agreement with the protocol carried out in presence of 1.35 mM Ca, with the exception that all forces obtained were smaller in Ca-free ISS. This phenomenon could be explained if part of the fast component is caused by rapid entry of Ca tightly bound to the membrane through NE-operated channels. In any case, the persistence of an increased fast component in absence of any extracellular Ca contribution points to an abnormality of intracellular origin.

Removal of endothelium increased the fast-component response to NE in both control and STZ-D rats. This increase was expected because of the elimination of the relaxant influence that the endothelium normally exerts on the vascular smooth muscle (22). However, the fast-component response was still greater in STZ-D than control rats, indicating that the mechanism of production of this phenomenon was not mediated by a decreased relaxant function of the endothelium in the diabetic rat.

Several mechanisms could have been involved in the presence of an augmented fast-component response in diabetes. First, it is possible that inositol trisphosphate formation is augmented by the disease (20). Second, increased adrenergic stimulation, which has been demonstrated to occur in diabetes, leads to intracellular Ca accumulation (27). This Ca, in turn, would provide the basis for an augmented fast component on exposure to NE. Finally, insulin stimulates Na^+-K^+-dependent ATPase activity (28) at physiological concentrations of insulin in, e.g., heart (29) and kidney (30). If insulin is absent, Na^+-K^+-ATPase could be less active, leading to intracellular Na and Ca accumulation (by depression of the Na-Ca exchanger). This could also provide the basis for an augmented fast-component response on exposure to NE and also for the beneficial effect of insulin treatment that was evident in the insulin-treated rats.

The pathological importance of an increased fast-component response in diabetic aortic rings cannot be firmly established. However, we can speculate about the contractile and therapeutic implications in vascular smooth muscle function. An increased vasoconstriction due to augmented Ca stores would be difficult to relieve with Ca entry blockers, which act mainly on Ca influx from the extracellular space. Furthermore, an increased Ca store would attenuate the buffer function of these stores (31), thus enhancing even the vascular responses that are not receptor mediated, such as those due to activation of voltage-operated channels.

In summary, our experiments suggest that diabetes promotes an augmented Ca release on NE stimulation of isolated rat aorta. This phenomenon indicates that an increase of the Ca releasing capacity of NE-sensitive intracellular stores can be caused by diabetes.

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REFERENCES

1. Williams RH, Porte D Jr: The pancreas. In *Textbook of Endocrinology*. Williams RH, Ed. Philadelphia, PA, 1974, p. 572–74
2. Kimura I, Pancho LR, Isoi Y, Kimura M: Diabetes-induced enhancement of prostanoid-stimulated contraction in mesenteric veins of mice. *Jpn J Pharmacol* 51:403–10, 1989

3. Owen MP, Carrier GO: Calcium dependence of norepinephrine-induced vascular contraction in experimental diabetes. *J Pharmacol Exp Ther* 212:253–58, 1980
4. Mulhern M, Docherty JR: Effects of experimental diabetes on the responsiveness of rat aorta. *Br J Pharmacol* 97:1007–12, 1989
5. Morff RJ: Microvascular reactivity to norepinephrine at different arteriolar levels and durations of streptozocin-induced diabetes. *Diabetes* 39:354–60, 1990
6. Fortes ZB, Scivoletto R, García-Leme J: Functional changes in the microcirculation of alloxan-induced diabetic rats. *Gen Pharmacol* 20:615–20, 1989
7. Storozhevykh TP, Zadkova GF, Markov KHM: Sosudistye reaktsii spontanno gipertensivnykh i normotensivnykh krys pri sakharnom diabete. *Probl Endokrinol* 35:68–72, 1989
8. Brody MJ, Dixon RL: Vascular reactivity in experimental diabetes mellitus. *Circ Res* 14:494–501, 1964
9. Friedman JJ: Vascular sensitivity and reactivity to norepinephrine in diabetes mellitus. *Am J Physiol* 256:H1134–38, 1989
10. Pogatsa G, Koltai MZ, Hadnazy P, Koszeghy A: Insulin-induced reversibility of altered responsiveness in femoral arterial bed of diabetic dogs. *Diabetes Res* 9:41–45, 1988
11. Kamata K, Miyata N, Kasuya Y: Functional changes in potassium channels in aortas from rats with streptozocin-induced diabetes. *Eur J Pharmacol* 166:319–23, 1989
12. Sjogren A, Edvinsson L: Vasomotor changes in isolated coronary arteries from diabetic rats. *Acta Physiol Scand* 134:429–36, 1988
13. Gebremedhin D, Koltai MZ, Pogatsas A, Magvar K, Hadhazy P: Influence of experimental diabetes on the mechanical responses of canine coronary arteries: role of endothelium. *Cardiovasc Res* 22:537–44, 1988
14. Koltai MZ, Rosen P, Hadhazy P, Ballagi-Pordany G, Koszeghy A, Pogatsa G: Relationship between vascular adrenergic receptors and prostaglandin biosynthesis in canine diabetic coronary arteries. *Diabetologia* 31:681–86, 1988
15. Koltai MZ, Rosen P, Pogatsa G: Diminished vasodilation: imbalance of synthesized cyclooxygenase products by adrenergic mediation in diabetic coronaries of the dog. *Prog Clin Biol Res* 301:449–53, 1989
16. Kamata K, Miyata N, Kasuya Y: Impairment of endothelium-dependent relaxation and changes in levels of cyclic GMP in aorta from streptozocin-induced diabetic rats. *Br J Pharmacol* 97:614–18, 1989
17. Kappagoda T, Jayakody L, Rajotte R, Thomson AB, Senaratne MP: Endothelium-dependent relaxation to acetylcholine in the aorta of streptozocin-induced diabetic rat and the BB-diabetic rat. *Clin Invest Med* 12:187–93, 1989
18. Bohr DF: Vascular smooth muscle: dual effect of calcium. *Science* 139:597–99, 1963
19. Berridge MJ, Irvine RF: Inositol triphosphate, a novel second messenger in cellular signal transduction. *Nature* (Lond) 312:315–21, 1984
20. Sakai Y, Aihara K, Honda H, Inazu M: Calcium mobilization and phosphatidylinositol turnover in vas deferens smooth muscle of diabetic rats. *Eur J Pharmacol* 162:475–81, 1989
21. Martínez SM, Tarres MC, Montenegro S, Milo R, Picena JC, Figueroa N, Rabasa SL: Spontaneous diabetes in eSS rats. *Acta Diabetologica Lat* 25:303–13, 1988
22. Carrier GO, White RE: Enhancement of alpha-1 and alpha-2 adrenergic agonist-induced vasoconstriction by removal of endothelium in rat aorta. *J Pharmacol Exp Ther* 232:682–87, 1985
23. Rinaldi GJ, Bohr DF: Endothelium-mediated spontaneous response in aortic rings of deoxycorticosterone acetate-hypertensive rats. *Hypertension* 13:256–61, 1989
24. Rinaldi GJ, Bohr DF: Plasma membrane and its abnormalities in hypertension. *Am J Med Sci* 295:389–95, 1988
25. Rinaldi GJ, Bohr DF: Potassium-induced relaxation of arteries in hypertension: modulation by extracellular calcium. *Am J Physiol* 256:H707–12, 1989
26. Furspan PB, Rinaldi GJ, Hoffman K, Bohr DF: Dietary calcium and cell membrane abnormality in genetic hypertension. *Hypertension* 13:727–30, 1988
27. Ganguly PK, Beamish RE, Innes IR, Dhalla NS: Abnormalities of adrenergic mechanisms in diabetic cardiomyopathy. In *Pathophysiology and Pharmacology of Heart Disease*. Anand IS, Wahi PL, Dhalla NS, Eds. Boston, MA, Kluwer, 1989, p. 197–204
28. Clausen T: Regulation of active Na^+-K^+-transport in skeletal muscle. *Physiol Rev* 66:542–80, 1986
29. Rinaldi GJ, Gelpi RJ, Mosca SM, Cingolani HE: Acción de la insulina sobre el miocardio. *Medicina (B Aires)* 40:295–301, 1980
30. Finch D, Davis G, Bower J, Kirchner K: Effect of insulin on renal sodium handling in hypertensive rats. *Hypertension* 15:514–18, 1990
31. Saida K, Van Breemen C: Characteristics of the norepinephrine-sensitive Ca^{2+} store in vascular smooth muscle. *Pfluegers Arch Eur J Physiol* 397:166–67, 1983