Mechanisms of Increased Responses of the Aorta to α -Adrenoceptor Agonists in Streptozotocin-Induced Diabetic Rats

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To investigate the influence of diabetes on the responsiveness of the cardiovascular system, we have examined the effects of various agents on the reactivity of the vascular smooth muscle of aortic strips obtained from age-matched control and diabetic rats. Norepinephrine (NE) contracted the aortic strips obtained from age-matched control and diabetic rats in a concentration-dependent manner, but maximal contraction of aortic strips in response to NE was enhanced in diabetic rats. The EC_{50} value for NE in the diabetic aortic strips was similar to that in the aorta from age-matched controls. Ca-induced contracture of the aortic strips which were depolarized with isotonic K^+ (60 mM) was potentiated in aortic strips from diabetic rats, when compared with those from age-matched controls. Ca-induced contracture of aortic strips, preincubated with 10^{-6} M NE and 10^{-6} M nicardipine in Ca^{2+} -free Krebs-Henseleit solution (KHS), was not significantly different in age-matched control and diabetic rats. Bay K 8644, an activator of calcium channels, produced an increase in the force of contraction of the slightly depolarized aorta from diabetic rats. Phasic contraction induced by phenylephrine (PE) in the presence of 10^{-6} M nicardipine in Ca^{2+} -free KHS was significantly enhanced in aortic strips obtained from diabetic rats. These results demonstrate that NE-induced contraction of the aortic strips obtained from diabetic rats was significantly enhanced, and that this increased contractile response of the aorta to NE may be due to an increased influx of extracellular calcium through the voltage-dependent Ca^{2+} channels, but not through the receptor-operated Ca^{2+} channels. Furthermore, this enhanced response may be responsible for an increased rate of turnover of phosphatidylinositol.

Keywords — diabetes; streptozotocin; aorta; voltage-dependent calcium ion channel; Bay K 8644; phenylephrine; phosphatidylinositol turnover

Introduction

Diabetes mellitus is often accompanied by vascular complications which are responsible for most of the morbidity and mortality associated with the disease. These complications are manifested as retinopathy, nephropathy, coronary heart disease and hypertension.¹⁾ However, the nature of the processes which lead to changes in the peripheral vascular system is not completely understood. An increasing number of reports indicate that abnormal vascular reactivity to various vasoactive agents can occur in animals in which lesions of pancreatic cells have been induced by streptozotocin (STZ) or alloxan.²⁾ Using aortic strips obtained from diabetic animals, it has been found that diabetes is associated with an increased maximal response to norepinephrine (NE) with no change in sensi-

tivity.³⁻⁶⁾ Another research group has also reported an increased sensitivity of the aortic strip to NE in diabetic rats.⁷⁾ An increased sensitivity of the tail artery to NE in diabetic rats has been suggested to reflect a supersensitivity that results from deranged sympathetic neuronal mechanisms.⁸⁾ However, conflicting results have been reported by other researchers. In diabetic rat aortic strips, a decreased maximal response of the aortic strips to NE, with no change in sensitivity has been reported,⁹⁻¹¹⁾ and as well as a decreased maximal response of the aortic strips to NE, a decrease in sensitivity has also been reported.¹²⁾ The discrepancy between the above-mentioned results might be attributable to differences in the species of animal or in the diabetogenic agent used, the duration of hyperglycemia, and the state of hypercholesterolemia in the animals at the time of the various studies. In

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the present study, we investigated the effects of several agents on the reactivity of the aortic strips obtained from age-matched control and STZ-induced diabetic rats. We were especially interested in determining how the activity of voltage-dependent Ca^{2+} channels, that of receptor-operated Ca^{2+} channels, and the turnover of phosphatidylinositol were altered in STZ-induced diabetic rats.

Materials and Methods

1) Animals and the Induction of Diabetes

— Male Wistar rats, 8 weeks old and 200–220 g in weight, received a single injection, in the tail vein, of streptozotocin (STZ) of 60 mg/kg dissolved in a citrate buffer solution. Age-matched control rats were injected with the buffer solution alone. Food and water were given *ad libitum*. The concentration of glucose in the plasma was determined by the *o*-toluidine method.¹³⁾

2) Isolation of Aortic Strip — After treatment with STZ or buffer solution, rats were allowed a period of 8-weeks respite, and then the aorta was excised from the age-matched control and diabetic rats. A section of the thoracic aorta between the aortic arch and the diaphragm was removed and placed in oxygenated, modified Krebs-Henseleit solution (KHS). The solution consisted of (mM) NaCl, 118.0; KCl, 4.7; NaHCO_3 , 25.0; CaCl_2 , 1.8; NaH_2PO_4 , 1.2; MgSO_4 , 1.2; dextrose, 11.0. In all experiments, to avoid the possible influence of endothelium-derived relaxing factor, the endothelium of each strip was removed by gentle rubbing of the endothelial surface with a disposable cotton applicator. The aorta was cleaned of loosely adhering fat and connective tissue and cut into helical strips 3 mm in width and 20 mm in length. The tissues were placed in a well-oxygenated (95% O_2 , 5% CO_2) bath of 10 ml KHS at 37 °C, with one end connected to a tissue holder and the other to a force-displacement transducer (Nihon Kohden TB611T). The tissue was equilibrated for 60 min under a resting tension of 1.0 g. During this time the KHS in the tissue bath was replaced 20 min.

3) Vascular Responses of NE and Ca^{2+} —

After equilibration, the aortic strip was contracted by treatment with 10^{-7} M norepinephrine (NE) to ensure stabilization of the smooth muscles and then acetylcholine (ACh) at a final concentration of 10^{-5} M was added to the bath. The removal of endothelial cells by rubbing was confirmed by the fact that tonic contraction of the aorta by NE was not affected by treatment with ACh. The effects of drugs were then tested. The tissue was allowed to relax and equilibrate for 40 min before the next application of drugs. During this interval, the tissue was washed with fresh KHS. NE (10^{-10} – 10^{-6} M) was added cumulatively until a maximal response was achieved. After the addition of each dose, a plateau response was obtained before the addition of a subsequent dose. When Ca-induced contracture in the depolarized muscle was examined, the aortic strips were exposed to Ca^{2+} -free KHS which contains 0.01 mM ethyleneglycol-bis-(β -aminoethyl ether) *N,N,N',N'*-tetraacetic acid (EGTA) and were depolarized with isotonic K^+ (60 mM). Ca^{2+} (0.05–5.0 mM) was cumulatively applied to the depolarized aorta in Ca^{2+} -free KHS. When Ca-induced contracture in the presence of an agonist was examined, the aortic strips were exposed to Ca^{2+} -free KHS that contains 10^{-6} M nicardipine and 0.01 mM EGTA, and then NE was added to the bath to a final concentration of 10^{-6} M. In this solution, NE produced a phasic contraction followed by decreases in tone to the resting levels; Ca^{2+} (0.05–5.0 mM) was then added cumulatively to the bath.

4) Effects of Calcium Channel Agonist — The effects of 10^{-6} M Bay K 8644, a voltage-dependent Ca-channel agonist,^{14, 15)} on the aorta obtained from age-matched control and diabetic rats, were studied in the slightly depolarized muscle with isotonic K^+ (15 mM).

5) Phasic Contraction of Aorta to PE and NE — For the measurement of phasic contraction, the aortic strips were preincubated with 10^{-6} M nicardipine and 0.01 mM EGTA in Ca^{2+} -free KHS. Phenylephrine (PE) and NE were then added to the bath, both at a final concentration of 10^{-6} M.

6) Agents Used — (–)-Norepinephrine hydrochloride, streptozotocin, L-phenylephrine

hydrochloride, nicardipine hydrochloride and EGTA were purchased from Sigma Chemical Co. (St. Louis, MO). Acetylcholine chloride was purchased from Daiichi Pharmaceuticals. Bay K 8644 was generously supplied by the Bayer. Bay K 8644 and nicardipine were first dissolved in dimethyl sulfoxide (DMSO), then diluted with saline. The final concentration of DMSO in the bath was less than 0.01%, and did not affect contraction or relaxation.

7) Data Analysis — The contractile force of aortic strips from control and diabetic rats is expressed in mg tension mg^{-1} tissue. EC_{50} values are defined as the concentration of the agonist which produces 50% of the maximal response and were calculated by the method of Flemming *et al.*¹⁶⁾ Each set of data is expressed as a mean $\pm$ S.E. Statistical differences between the control and diabetic groups were compared by using Student's t-test for unpaired observations.

Results

Eight to ten weeks after treatment with STZ, body weight was significantly decreased in diabetic rats compared with that of age-matched control rats. The concentration of glucose in plasma was significantly elevated in STZ-diabetic

TABLE I. Body Weight and Plasma Glucose Levels for Age-matched Control and Diabetic Rats

	Body weight (g)	Plasma glucose (mg/dl)
Control	485.3 ± 12.5	128.2 ± 4.0
Diabetic	324.9 ± 13.9 <i>a)</i>	516.3 ± 27.5 <i>a)</i>

Value $\pm$ S.E. Significant difference from age-matched control. *a)* $p < 0.01$.

rats compared with that in age-matched controls (Table I).

1) Concentration-Response Curves of NE and Ca^{2+}

Figure 1 shows the concentration-response curves for the contractile response of aortic strips to NE. NE contracted aortic strips obtained from diabetic and age-matched control rats in a concentration-dependent manner, but maximal contraction of aortic strips in response to NE was significantly enhanced in diabetic rats. EC_{50} values for NE were $5.00 \pm 1.68 \times 10^{-8}$ M and $2.23 \pm 0.48 \times 10^{-8}$ M in age-matched control and diabetic rats, respectively ($n = 6$). The EC_{50} value for NE in diabetic aortic strips was not significantly different from that in control preparations. Figure 2 shows concentration-response curves for Ca-induced contracture of

Fig. 1. Cumulative Concentration-Response Curves for Norepinephrine (NE) in Aortic Strips without Endothelial Cells Obtained from Age-matched Control (—EC) (●) and Diabetic (—EC) (○) Rats

Values represent the mean from 6 experiments, respectively, with the mean $\pm$ S.E. indicated by vertical lines. *a)* $p < 0.01$, *b)* $p < 0.05$.

Fig. 2. Cumulative Concentration-Response Curves for Calcium in Aortic Strips without Endothelial Cells Obtained from Age-matched Control (●) and Diabetic (○) Rats

Aortic strips were previously depolarized with Ca^{2+} -free isotonic K^+ (60 mM). Values represent the mean of 6 experiments respectively with the mean $\pm$ S.E. indicated by vertical lines. *a)* $p < 0.01$, *b)* $p < 0.05$.

Fig. 3. Cumulative Concentration-Response Curves for Calcium in Aortic Strips without Endothelial Cells Obtained from Age-matched Control (●) and Diabetic (○) Rats

Aortic strips were preincubated with 10^{-6} M nicardipine, 0.01 mM EGTA and 10^{-6} M norepinephrine in Ca^{2+} -free Krebs-Henseleit solution. Values represent the mean from 6 experiments, respectively, with the mean $\pm$ S.E. indicated by vertical lines.

aorta which had been depolarized with isotonic K^+ (60 mM). Concentration-response curves for Ca were shifted to the left and maximal contraction was also enhanced in the diabetic aortic strip, as compared with that of age-matched controls. EC_{50} values for Ca^{2+} were $5.51 \pm 0.61 \times 10^{-4}$ M and $2.78 \pm 0.38 \times 10^{-4}$ M in aortic strips from age-matched control and diabetic rats, respectively ($n=6$, $p < 0.01$). However, Ca-induced contracture of aortic strips, preincubated with 10^{-6} M NE in the presence of 10^{-6} M

Fig. 5. Contractile Response to Bay K 8644 10^{-6} M in Rat Aortic Strips Obtained from Age-matched Control (□) and Diabetic (▨) Rats

Aortic strips were previously depolarized with isotonic K^+ 15 mM. Values represent the mean of 6 experiments, respectively, with the mean $\pm$ S.E. indicated by vertical lines. a) $p < 0.01$.

nicardipine and 0.01 mM EGTA in Ca^{2+} -free KHS, was not significantly different between diabetic and age-matched control rats (Fig. 3). EC_{50} values for Ca^{2+} were $2.62 \pm 0.26 \times 10^{-4}$ M and $2.91 \pm 0.66 \times 10^{-4}$ M in age-matched control and diabetic rats, respectively ($n=6$).

2) Effect of Ca Channel Agonist

Figure 4 shows an example of Bay K 8644-induced contractile responses of aortic strips obtained from age-matched control and diabetic

Fig. 4. Typical Recordings of Contractile Responses of Aorta Obtained from Age-matched Control and Diabetic Rats Induced by Bay K 8644 10^{-6} M in the Presence of Isotonic K^+ 15 mM

Fig. 6. Typical Recordings of Contractile Responses of Aortic Strips Obtained from Age-matched Control and Diabetic Rats Induced by 10^{-6} M Phenylephrine in the Presence of 10^{-6} M Nicardipine and 0.01 mM EGTA in Ca^{2+} -free Krebs-Henseleit Solution

Fig. 7. Contractile Response to 10^{-6} M Phenylephrine and 10^{-6} M Norepinephrine in Aortic Strips Obtained from Age-matched Control (□) and Diabetic (▨) Rats

Aortic strips were preincubated with 10^{-6} M nicardipine and 0.01 mM EGTA in Ca^{2+} -free Krebs-Henseleit solution. Values represent the mean from 6 experiments, respectively, with the mean $\pm$ S.E. indicated by vertical lines. ^{a)} $p < 0.01$.

rats using the slightly depolarized muscle in isotonic 15 mM K^+ . Bay K 8644, an activator of calcium channels, produced an increase in the force of contraction in the aortic strips from diabetic rats greater than that in the aortic strips from age-matched control rats (Fig. 5). The maximal contractile responses to Bay K 8644 10^{-6} M were 36.4 ± 2.5 mg mg⁻¹ tissue and 53.8 ± 2.1 mg mg⁻¹ tissue in aortic strips from age-matched control and diabetic rats, respectively ($n = 6$, $p < 0.01$).

3) Phasic Contractions of Aorta to PE and NE

Figure 6 shows typical recordings of phasic contraction responses to 10^{-6} M PE an α_1 -adrenoceptor agonist, in the presence of 10^{-6} M nicardipine in Ca^{2+} -free KHS. Phasic contraction in response to 10^{-6} M PE was potentiated in aortic strips from diabetic rats when compared with those from age-matched controls (Fig. 7). The maximal contractile forces in response to 10^{-6} M PE were 29.9 ± 1.4 mg mg⁻¹ tissue and 45.9 ± 2.1 mg mg⁻¹ tissue in aortic strips from age-matched control and diabetic rats, respectively ($n = 6$, $p < 0.01$). Phasic contractions in response to 10^{-6} M NE in the presence of 10^{-6} M nicardipine in Ca^{2+} -free KHS were also significantly enhanced in aortic strips obtained from di-

abetic rats (Fig. 7). Maximal contractile responses to 10^{-6} M NE were 24.5 ± 2.2 mg mg⁻¹ tissue and 36.9 ± 1.9 mg mg⁻¹ tissue in aortic strips from age-matched control and diabetic rats, respectively ($n = 6$, $p < 0.01$).

Discussion

In the present study, increased maximal contractile responses to NE were observed in aortic strips obtained from diabetic rats (Fig. 1). However, there was no significant difference in the EC_{50} values of NE that produces contraction between aortic strips from age-matched control and diabetic rats. Diabetes mellitus is known to produce alterations in the reactivity of blood vessels to neurotransmitters and various other agents. In diabetic blood vessels, similar results to ours have previously been observed.^{3-6, 17-20} In contrast to our observation, conflicting results have been reported by other research groups. In diabetic rat aortic strips, decreased maximal response has been observed, with no change in sensitivity.⁹⁻¹¹ This discrepancy may be attributable to differences in the species of animal or diabetogenic agent used, and duration of hyperglycemia and state of hypercholesterolemia in the animals at the times of the respective studies. In the present study, we found that the responses of vascular smooth muscle to NE were increased without any change in the EC_{50} value in diabetic rats. These results suggest that while the affinity or number of α -adrenoceptors may not be altered, changes in transmembrane responses might occur, including rate of turnover of phosphatidylinositol or availability of Ca^{2+} in the aorta.

It has been reported recently that there are two types of calcium channel: voltage-dependent calcium channels (VDC) and receptor-operated calcium channels (ROC).²¹ To investigate the mechanism of the increased response of the aortic strip to NE in diabetic rats, we examined which type of Ca-channel was altered in diabetes. Concentration-response curves for Ca-induced contracture of the depolarized aortic strip were shifted to the left and the EC_{50} value for Ca^{2+} was significantly decreased in diabetic aortic strip as compared to that in aortic strips

from age-matched controls (Fig. 2). However, Ca-induced contracture of aortic strips, preincubated with 10^{-6} M NE in the presence of 10^{-6} M nifedipine and 0.01 mM EGTA in Ca^{2+} -free KHS, was not significantly different in aortic strips from diabetic and age-matched control rats (Fig. 3). These results suggest that an increased contractile response of the aortic strip in diabetic rats may be, at least in part, due to an increased influx of extracellular Ca^{2+} through the VDC but not through the ROC. The EC_{50} value obtained from concentration-response curves for Ca-induced contracture was reduced, indicating that the open-time or open-probability or density of the VDC may be increased in the diabetic aortic strip. This increase may be responsible for the increased contractile response of the aortic strip to NE in diabetic rats. Our observations are in agreement with those of Agrawal and McNeill.¹⁹⁾

Recently, a novel derivative of dihydropyridine, Bay K 8644, has been reported to have a calcium-agonistic action.^{14,15)} Bay K 8644 has both vasoconstrictor and positive inotropic effects. More recent studies have confirmed the agonistic actions of Bay K 8644 on voltage-dependent Ca^{2+} channels in various preparations of vascular smooth muscle.²²⁻²⁵⁾ Bay K 8644 (10^{-6} M) caused contraction of the aortic strips obtained from age-matched control and diabetic rats in isotonic K^+ 15 mM KHS, but contraction of aortic strips in response to Bay K 8644 was significantly enhanced in diabetic rats (Figs. 4 and 5). These results are consistent with the Ca-induced contracture of the aortic strip which had been depolarized with isotonic 60 mM K^+ . These results further demonstrate that the increased contractile response of the aortic strip in response to NE may be due to an increased influx of extracellular Ca^{2+} through the VDC. Our observations are supported by a recent study of isolated mesenteric arteries obtained from spontaneously diabetic and STZ-induced diabetic rats.²⁰⁾ It is well known that contraction of vascular smooth muscle may be mediated either by an influx of extracellular calcium ions or *via* mobilization of intracellular calcium from store sites.²⁶⁾ To investigate the intracellular mobilization of Ca^{2+} , we examined the phasic

contraction of the aortic strip in response to PE and NE. Phasic contraction of the aortic strip in response to 10^{-6} M PE, an α_1 -adrenoceptor agonist, and to 10^{-6} M NE, a nonselective α -agonist, in the presence of 10^{-6} M nifedipine and 0.01 mM EGTA in Ca^{2+} -free KHS, was significantly enhanced in aortic strips obtained from diabetic rats compared with that in aortic strips from age-matched control rats (Figs. 6 and 7). Recently, it has been reported that phasic contraction, in response to α -agonists, in rat thoracic aorta may mobilize intracellular calcium by activation of turnover of phosphatidylinositol.²⁷⁾ It has also been reported that inositol 1,4,5-trisphosphate, which is produced by breakdown of phosphatidylinositol, is released from the plasma membrane by Ca^{2+} -mobilizing agonists and can act as a signal for release of Ca^{2+} from the sarcoplasmic reticulum.^{28,29)} In the present study, phasic contraction of the aortic strips in response to PE and NE in diabetic rats was significantly enhanced. This increased contractile response of the aorta may be due to an increased rate of turnover of phosphatidylinositol. Although phasic contraction of the aortic strips in response to α -agonists in Ca^{2+} -free medium may indirectly reflect the rate of turnover of phosphatidylinositol, an increased maximal response of the aortic strip to NE in diabetic rats may also be involved in an increased turnover of phosphatidylinositol.

In conclusion, we have demonstrated that maximal contraction of aortic strips from diabetic rats response to NE was significantly enhanced, and that this increased contractile response of the aorta to NE may be due to an increased influx of extracellular calcium through the voltage-dependent Ca^{2+} channels, but not through receptor-operated Ca^{2+} channels. Furthermore, phasic contractions in response to PE and NE of the aortic strips obtained from diabetic rats were significantly enhanced, suggesting that this increased contractile response of the aorta to NE may be due to an increase in the turnover of phosphatidylinositol.

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References

- 1) A. R. Christlieb: Diabetes and hypertensive vascular disease. Mechanism and treatment, *Am. J. Cardiol.*, **32**, 592—606 (1973).
- 2) M. J. Brody and R. L. Dixon: Vascular reactivity in experimental diabetes mellitus, *Circ. Res.*, **14**, 494—501 (1964).
- 3) M. P. Owen and G. O. Carrier: Alteration in vascular smooth muscle sensitivity to vasoconstrictor agents by streptozotocin-induced diabetes, *Proc. West Pharmacol. Soc.*, **22**, 363—366 (1979).
- 4) M. P. Owen and G. O. Carrier: Calcium dependence of norepinephrine-induced vascular contraction in experimental diabetes, *J. Pharmacol. Exp. Ther.*, **212**, 253—258 (1980).
- 5) N. L. Scarborough and G. O. Carrier: Increased α_2 -adrenoceptor mediated vascular contraction in diabetic rats, *J. Auton. Pharmacol.*, **3**, 177—183 (1983).
- 6) N. L. Scarborough and G. O. Carrier: Nifedipine and α adrenoceptors in rat aorta. II: Role of extracellular calcium in enhanced α_2 - adrenoceptor mediated-contraction in diabetes, *J. Pharmacol. Exp. Ther.*, **236**, 603—609 (1984).
- 7) K. M. MacLeod and J. H. McNeill: The influence of chronic experimental diabetes on contractile responses of rat isolated blood vessel, *Can. J. Physiol. Pharmacol.*, **63**, 52—57 (1985).
- 8) S. Ramanadham, W. H. Lyness and T. E. Tenner: Alterations in aortic and tail artery reactivity to agonists after streptozotocin treatment, *Can. J. Physiol. Pharmacol.*, **62**, 418—423 (1984).
- 9) S. Sullivan and H. V. Sparkes: Diminished contractile response of aortas from diabetic rabbits, *Am. J. Physiol.*, **236**, H301—306 (1978).
- 10) M. A. Pfaffman, C. R. Ball, A. Darby and R. Hilman: Insulin reversal of diabetes-induced inhibition of vascular contractility in the rat, *Am. J. Physiol.*, **242**, H490—495 (1982).
- 11) R. J. Head, P. A. Longhurst, R. L. Panek and E. Stizel: A contrasting effect of the diabetic state upon the contractile responses of aortic preparations from the rat and rabbit, *Br. J. Pharmacol.*, **91**, 275—286 (1987).
- 12) P. D. M. V. Turlapaty, G. Lum and B. M. Altura: Vascular responsiveness and serum biochemical parameters in alloxan diabetes mellitus, *Am. J. Physiol.*, **239**, E 412—421 (1980).
- 13) K. M. Dubowski: An *o*-toluidine method for body-fluid glucose determination, *Clin. Chem.*, **8**, 215—235 (1962).
- 14) M. Schramm, G. Thomas, R. Towart and G. Franckowiak: New dihydropyridines with positive inotropic action through activation of Ca^{2+} channels, *Nature* (London), **303**, 536—538 (1983).
- 15) M. Schramm, G. Thomas, R. Towart and G. Franckowiak: Activation of calcium channels by novel 1,4-dihydropyridines. A new mechanism for positive inotropics or smooth muscle stimulants, *Arzneim-Forsch.*, **33**, 1268—1272 (1983).
- 16) W. W. Flemming, D. P. Westfall, I. S. Delande, and J. B. Jeller: Log distribution of equieffective doses of norepinephrine and acetylcholine in several tissues, *J. Pharmacol. Exp. Ther.*, **181**, 339—345 (1972).
- 17) R. Cseuz, T. L. Wenger, G. Kunos and M. Szentivanyi: Changes in adrenergic reaction pattern in experimental diabetes mellitus, *Endocrinology*, **93**, 752—755 (1973).
- 18) C. V. Jackson and G. O. Carrier: Supersensitivity of isolated mesenteric arteries to noradrenaline in the long-term experimental diabetic rat, *J. Auton. Pharmacol.*, **1**, 399—405 (1981).
- 19) D. K. Agrawal, S. Bhimji and J. H. McNeill: Effects of chronic experimental diabetes on vascular smooth muscle function in rabbit carotid artery, *J. Cardiovasc. Pharmacol.*, **9**, 584—593 (1987).
- 20) D. K. Agrawal and J. H. McNeill: Vascular responses to agonists in rat mesenteric artery from diabetic rats, *Can. J. Physiol. Pharmacol.*, **65**, 1484—1490 (1987).
- 21) K. D. Meisner and C. Van Breemen: Evidence for two separate Ca^{2+} pathways in smooth muscle plasmalemma, *J. Membr. Biol.*, **59**, 19—25 (1981).
- 22) H. Yamamoto, O. Hwang and C. Van Breemen: Bay K 8644 differentiates between potential and receptor-operated Ca^{2+} channels, *Eur. J. Pharmacol.*, **102**, 555—557 (1984).
- 23) C. M. Su, V. C. Swamy and D. J. Triggle: Calcium channel activation in vascular smooth muscle by Bay K 8644, *Can. J. Physiol. Pharmacol.*, **62**, 1401—1410 (1984).
- 24) Y. Kanmura, T. Itoh, and H. Kuriyama: Agonist actions of Bay k 8644, a dihydropyridine derivative, on the voltage-dependent calcium influx in smooth muscle cells of the rabbit mesenteric artery, *J. Pharmacol. Exp. Ther.*, **231**, 717—723 (1984).
- 25) G. Gopalakrishnan, L. E. Park and C. R. Triggle: The effects of the calcium channel agonist, Bay k 8644 on human vascular smooth muscle, *Eur. J. Pharmacol.*, **113**, 447—451 (1985).
- 26) P. M. Vanhoutte and T. J. Rimele: Calcium and α -adrenoceptor in activation of smooth muscle, *J. Cardiovasc. Pharmacol.*, **4**, S 280—286 (1982).
- 27) E. Legan, B. Chernow, J. Parrillo and B. L. Roth: Activation of phosphatidylinositol turnover in rat aorta by α_1 -adrenergic receptor stimulation, *Eur. J. Pharmacol.*, **110**, 389—390 (1985).
- 28) M. J. Berridge and R. F. Irvine: Inositol trisphosphate, a second messenger in cellular signal transduction, *Nature* (London), **312**, 315—321 (1984).
- 29) A. V. Somlyo, M. Bond, A. P. Somlyo and A. Scarpa: Inositol trisphosphate-induced calcium release and contraction in vascular smooth muscle, *Proc. Natl. Acad. Sci. U.S.A.*, **82**, 5231—5235 (1985).