Vascular complications claim most of the mortality and morbidity of human diabetes mellitus. Depending on the specific vascular beds affected, vascular complications can be manifested as atherosclerosis, retinopathy, nephropathy, hypertension, and distal vasculopathy, etc. Notwithstanding the general acknowledgment of the involvement of abnormal Ca\(^{2+}\) handling in these vascular complications, the altered properties of various Ca\(^{2+}\)-mobilizing mechanisms in diabetes are still obscure. Although numerous studies have shown alterations in the KCl depolarization-induced vascular contractions and changes in the intracellular Ca\(^{2+}\) concentrations ([Ca\(^{2+}\)], very little information is available on the properties of voltage-dependent Ca\(^{2+}\) channels (VDCC) in diabetic vascular smooth muscle cells (SMCs). Our previous study has revealed that the KCl-induced increase in [Ca\(^{2+}\)] in tail artery SMCs from streptozotocin-induced diabetic rats were examined. The densities, but not the voltage dependence, of L-type VDCC currents were reduced as diabetes progressed from 1 wk to 3 mo. The inhibitory effect of dibutyryl-cAMP on L-type VDCC currents was greater in diabetic SMCs than in age-matched control cells (P < 0.01). Both the stimulatory effect of BAY K 8644 and the inhibitory effect of nifedipine on L-type VDCC currents were significantly enhanced in diabetic cells. The diabetes-related abnormalities in L-type VDCC currents were mimicked by culturing SMCs with a high concentration of glucose. Our results suggest that the properties of L-type VDCC in diabetic vascular SMCs were significantly altered, partially related to the increased L-type VDCC sensitivity to cAMP and hyperglycemia.

MATERIALS AND METHODS

Animal model of diabetes. Male Sprague-Dawley adult rats weighing 150–180 g were maintained on standard rat chow and tap water ad libitum with 12:12-h light-dark cycles in a quiet environment. Diabetes was induced by a single injection of streptozotocin (STZ, 60 mg/kg body wt) dissolved in sodium citrate buffer (pH 4.5; see Ref. 22) via the lateral tail vein or penis vein after the rats were anesthetized by intraperitoneal injection of pentobarbital sodium (60 mg/kg body wt). Age-matched control rats were treated with an injection of an equal volume of vehicle (sodium citrate buffer). Glycosuria was determined using Chemstrip (Boehringer Mannheim). Mean blood pressure of the anesthetized rats (pentobarbital sodium, 60 mg/kg ip) was determined through the right femoral artery by a pressure transducer connected to a dihydropyridines; patch-clamp; hyperglycemia; calcium channels; artery

Wang, Rui, Yuejin Wu, Guanghua Tang, Lingyun Wu, and Salma Toma Hanna. Altered L-type Ca\(^{2+}\) channel currents in vascular smooth muscle cells from experimental diabetic rats. Am. J. Physiol. Heart Circ. Physiol. 278: H714–H722, 2000.—Vascular complications of diabetes are associated with abnormal Ca\(^{2+}\) handling by vascular smooth muscle cells (SMCs) in which the alteration in L-type voltage-dependent Ca\(^{2+}\) channel (VDCC) currents may play an important role. In the present study, the characteristics of L-type VDCC currents in tail artery SMCs from streptozotocin-induced diabetic rats were examined. The densities, but not the voltage dependence, of L-type VDCC currents were reduced as diabetes progressed from 1 wk to 3 mo. The inhibitory effect of dibutyryl-cAMP on L-type VDCC currents was greater in diabetic SMCs than in age-matched control cells (P < 0.01). Both the stimulatory effect of BAY K 8644 and the inhibitory effect of nifedipine on L-type VDCC currents were significantly enhanced in diabetic cells. The diabetes-related abnormalities in L-type VDCC currents were mimicked by culturing SMCs with a high concentration of glucose. Our results suggest that the properties of L-type VDCC in diabetic vascular SMCs were significantly altered, partially related to the increased L-type VDCC sensitivity to cAMP and hyperglycemia.
Biopac system (Biopac System) and was recorded on a Macintosh computer. At the time of tail artery removal, 1.5-mL blood samples were collected from the rats in the fasted state. The measurement of blood levels of glucose and glycated hemoglobin was performed by a Chemistry Laboratory at the Royal University Hospital of the University of Saskatchewan. To be succinct, 1-mo diabetic SMCs referred to the cells obtained from the rats 1 mo after receiving STZ injection, and so on. Likewise, 1-mo control SMCs referred to the cells obtained from the rats 1 mo after receiving vehicle injection, and so on. Normal SMCs referred to the cells obtained from the rats without receiving STZ or vehicle injection.

Cell preparation. Single SMCs were dispersed enzymatically following our established procedure (19) with modifications. Rats were anesthetized by intraperitoneal injection of pentobarbital sodium (60 mg/kg body wt). Tail arteries were isolated, and connective tissue was removed under a dissecting microscope. The arteries were cut open longitudinally and were immersed in a Ca\(^{2+}\)- and Mg\(^{2+}\)-free Hank’s buffered saline solution (HBSS; Gibco) at 4°C. The arterial strips were then processed in the following solutions at 37°C: 1) low-Ca\(^{2+}\) (0.2 mM) HBSS containing collagenase/dispase (1.5 mg/mL; Boehringer Mannheim), elastase (0.5 mg/mL, type II; Sigma, St. Louis, MO), trypsin inhibitor (1 mL/mg; Sigma), and BSA (2 mg/mL; Sigma) for 50 min; 2) Ca\(^{2+}\)-free HBSS in which the tissue was rinsed two times; and 3) Ca\(^{2+}\)-free HBSS with 1 mg/mL collagenase (type II; Sigma) and 2 mg/mL BSA for 20 min. Next, arterial pieces were transferred to a Ca\(^{2+}\)-free HBSS at 4°C and were triturated for 5 min. Ca\(^{2+}\) concentration of the incubating solution was gradually increased to 1.7 mM. The dispersed cells were plated on 35-mm petri dishes in DMEM (Gibco) containing penicillin (100 U/mL; Sigma) and streptomycin (0.1 mg/mL; Sigma) and were maintained at 4°C for at least 4 h. Finally, cells were cultured in DMEM containing 10% FCS (Gibco) at 37°C. The glucose concentration of DMEM was 5 or 25 mM for culturing control SMCs or diabetic SMCs, respectively, unless otherwise specified. The primarily cultured cells were used in electrophysiological recording within 18–30 h of isolation, and some freshly isolated SMCs were used in the experiments within 8 h after isolation.

The principles of laboratory animal care (National Institutes of Health publication no. 85–23, Revised 1985) were followed, and animal experimental protocols were approved by the University Committee on Animal Care and Supply of the University of Saskatchewan.

Ca\(^{2+}\) channel current recording. The whole cell configuration of the patch-clamp technique was used to measure L-type VDCC currents (20). The petri dish with attached cells was mounted on the stage of an inverted phase-contrast microscope. The patch pipette with tip resistance of 2–4 M\(\Omega\) was pulled from microhematocrit capillary tubes (Fisher, Nepean, ON). Ca\(^{2+}\) channel currents were recorded using an Axopatch-200A patch-clamp amplifier, controlled by a Digidata 1200 interface and pClamp software (version 6.02; Axon Instruments). At the beginning of each experiment, junctional potential between pipette solution and bath solution was electronically adjusted to zero (24, 28, 29). Ca\(^{2+}\) channel currents were filtered with a four-pole low-pass Bessel filter at a cutoff frequency of 2 kHz, digitized at a sampling frequency of 5 kHz, and stored in a personal computer for off-line analysis. Leakage and capacitative currents were subtracted using the P/4 protocol with opposite polarity to the depolarization waveform. Cell capacitance and series resistance were electronically compensated. Cell capacitance was estimated by compensating the series resistance using the dial setting of the capacitive cancellation circuits in an Axopatch-200A patch-clamp amplifier. The cells were held at −40 mV, at which the majority of T-type VDCC currents had been inactivated. The test potentials were applied by a run of command pulses with a duration of 300 ms at increasing voltage steps of 10 mV every 8 s. The current recording started when VDCC currents were stabilized (~5 min after achieving the whole cell configuration). The pipette solution contained (in mM): 70 cesium aspartate, 10 EGTA, 2 ATP, 5 MgCl\(_{2}\), 1 CaCl\(_{2}\), 5 potassium pyruvate, 5 potassium succinate, 25 glucose, 10 HEPEs, 5 creatine phosphate-sodium, and 50 U/mL creatine kinase (pH 7.35 with CsOH). The bath solution was of the following composition (in mM): 20 BaCl\(_{2}\), 5 glucose, 140 Trizma base, 20 HEPEs, 5 KCl, and 5 CsCl (pH 7.4 with HCl). When Ca\(^{2+}\) was used as the charge carrier, 20 mM BaCl\(_{2}\) in the bath solution was replaced with 1.8 mM CaCl\(_{2}\) and 27 mM tetraethylammonium chloride. The osmolality of all solutions was adjusted to 300–310 mosmol/kgH\(_{2}\)O. All experiments were carried out at room temperature (20–22°C).

Data analysis and chemicals. The current density of L-type VDCC was calculated by normalizing the peak amplitude of current from cell capacitance (pA/pF). Conductance (G) was obtained according to the equation: G = I \(_{\text{peak}}\)/(V \(_{\text{test}}\) − V \(_{\text{rev}}\)), where I \(_{\text{peak}}\) is the peak amplitude of the current, V is the test potential, and V \(_{\text{rev}}\) is the reversal potential. The potential at the zero current level was tentatively used as the reversal potential, which was estimated in the current-voltage (I-V) curve or in some cases by extrapolating the I-V relationship until the inward current became outwardly directed.

For estimating the steady-state activation, L-type VDCC conductances were plotted against test potentials. The derived activation curve was fitted to data with a Boltzmann equation: G/G \(_{\text{max}}\) = 1 + exp[(V \(_{1/2}\) − V)/k] \(^{-1}\), where G/G \(_{\text{max}}\) is the relative conductance normalized by the maximal conductance, V \(_{1/2}\) is the potential required for half-activation of the current, and k is the Boltzmann coefficient.

The steady-state inactivation of L-type VDCC was examined using a double-pulse protocol. After employing conditioning holding potentials (HP) at different voltage levels for 2 s, the test pulse of 20 mV with a duration of 300 ms was applied to evoke L-type VDCC currents. The normalized current amplitudes (I/I \(_{\text{max}}\)) were plotted against the conditioning HP. The steady-state inactivation curve was drawn by fitting the data to the Boltzmann distribution: I/I \(_{\text{max}}\) = 1 + exp[(V − V \(_{i/2}\))/k] \(^{-1}\), where I/I \(_{\text{max}}\) is the relative current amplitude compared with the maximum current amplitude, V is the conditioning HP, and V \(_{i/2}\) is the potential required for half-inactivation of the current (20).

The data were expressed as means ± SE. Statistical significance was determined with the unpaired Student’s t-test in conjunction with the Newman-Keuls test where applicable. A significant difference level was set at P < 0.05.

Dibutyryl-cAMP (DBcAMP), nifedipine, STZ, and other chemicals were purchased from Sigma Chemical. BAY K 8644 was from Calbiochem (San Diego, CA).

RESULTS

Development of experimental diabetes. Changes in body weight of the rats after receiving either STZ or vehicle injection are shown in Fig. 1A. The body weight increase of diabetic rats, but not the control rats, was retarded after STZ injection. There was no difference in mean blood pressure among 1-, 2-, and 3-mo control rats or diabetic rats (Fig. 1B). Glycosuria was detected 36–48 h after STZ injection and continued for the
ensuing 1–3 mo. The fasting glucose concentration of plasma was elevated from 7.6 ± 0.4 mM (n = 21) in control rats to 25–35 mM in 1- to 3-mo diabetic rats (Fig. 1C). The blood level of glycated hemoglobin of 1- to 3-mo diabetic rats was also significantly higher than that of control rats (Fig. 1D).

Cell capacitance and current densities of L-type VDCC. There was no significant difference in the capacitance of tail artery SMCs from normal rats (12.7 ± 0.6 pF, n = 29), control rats at 1 mo (13.1 ± 0.4 pF, n = 11) and 3 mo (13.5 ± 0.5 pF, n = 10), or among the rats at different diabetic stages. The values of cell capacitance of 1-wk, 1-mo, 2-mo, or 3-mo diabetic SMCs were 13.7 ± 0.5 pF (n = 19), 14.1 ± 0.6 pF (n = 20), 13.6 ± 0.6 pF (n = 20), or 14.4 ± 0.7 pF (n = 19), respectively.

As demonstrated in our previous study (19), L-type VDCC currents in rat tail artery SMCs became stable within 5 min of the formation of the whole cell recording configuration and were maintained at the stable level for >20 min. Correspondingly, L-type VDCC current densities, measured after the current activity became stable, of tail artery SMCs from different groups of rats were compared. The current densities of L-type VDCC remained unchanged between normal rats and 1-wk to 3-mo control rats (Fig. 2). The current densities of L-type VDCC in 1-wk diabetic SMCs (n = 18) were smaller than that of control SMCs, although the difference did not reach a statistically significant level (Fig. 2A). As diabetes of the rats progressed, the decrease in the current densities of L-type VDCC became significant. Figure 2B shows that the current densities of L-type VDCC of 1-mo diabetic SMCs (n = 30) or 2-mo diabetic SMCs (n = 37) were significantly smaller than those of normal SMCs (n = 59) or 2-mo control SMCs (n = 16) between test potentials of 0 and +50 mV. The current densities of L-type VDCC of 3-mo diabetic SMCs (n = 32) were also significantly smaller than that of 3-mo control SMCs (n = 27) between test potentials of −20 and +60 mV (Fig. 2C). Although the current densities of L-type VDCC were similar between 1- and 2-mo diabetic SMCs (Fig. 2B), it further decreased in 3-mo diabetic SMCs. The maximum current densities of L-type VDCC (at +20 mV) of 3- and 2-mo diabetic SMCs were −2.2 ± 0.2 and −3.0 ± 0.2 pA/pF (P < 0.05), respectively. The maximum current densities of L-type VDCC of normal SMCs and 3-mo control SMCs were not different. It appeared that the I-V relationships of L-type VDCC were not affected by the diabetes status of the rats (Fig. 2).

As demonstrated previously (19), the density of L-type VDCC currents with 1.8 mM Ca^{2+} as the charge carrier (Ca^{2+} currents) in tail artery SMCs was too...
small to be readily detected. In a total of 30 cells from 1-mo control rats, 8 cells exhibited detectable Ca\textsuperscript{2+} currents with a mean peak density at +20 mV of -0.7 ± 0.2 pA/pF. However, only 1 out of 21 diabetic SMCs from 1-mo diabetic rats showed a visible Ca\textsuperscript{2+} current with a peak density at +20 mV of -0.3 pA/pF. These results demonstrated the same changes of L-type VDCC current density in diabetic SMCs whether barium or Ca\textsuperscript{2+} was used as the charge carrier. Because the tiny density of Ca\textsuperscript{2+} currents in these vascular SMCs rendered further analysis difficult, we used barium as the charge carrier in all following studies.

In another group of experiments, SMCs were isolated from 1-mo control rats or 1-mo diabetic rats and were used within 8 h without primary culture. The maximum current densities of L-type VDCC (at +20 mV) of control SMCs (n = 4) or diabetic SMCs (n = 5) were -4.2 ± 0.2 and -2.9 ± 0.1 pA/pF (P < 0.05), respectively. The similar results obtained in freshly isolated cells (within 8 h after isolation) and primarily cultured cells (18–30 h after isolation) indicated that the difference in the current densities of L-type VDCC of normal SMCs and diabetic SMCs did not result from different responses of these cells to in vitro primary culture conditions.

Steady-state inactivation and activation of L-type VDCC. The steady-state activation of L-type VDCC of 1- to 2-mo diabetic SMCs was not different from those of normal SMCs (not shown). In 3-mo diabetic SMCs (V<sub>1/2</sub> = 8.5 mV, k = -10.2 mV, n = 6), the steady-state activation curve of L-type VDCC appeared to shift to the right in the depolarizing direction compared with normal SMCs (V<sub>1/2</sub> = 4.1 mV, k = -8.5 mV, n = 5; Fig. 3A). However, the difference was not significant (P > 0.05).

The steady-state inactivation curves of L-type VDCC were nearly overlapped among three groups of SMCs.
Altered responses of L-type VDCC currents to cAMP and dihydropyridines. The inhibition of L-type VDCC currents by cAMP was significantly enhanced in diabetic SMCs. DBcAMP (1 mM) decreased the maximum current densities of L-type VDCC (120 mV) by 27.4 ± 2.1% (n = 6) or 25.1 ± 3.4% (n = 5) in normal or 1-mo control SMCs, respectively. A 43 ± 3.4% inhibition of the maximum current densities of L-type VDCC (120 mV) by DBcAMP (1 mM) was observed in 1-mo diabetic SMCs (P < 0.01 vs. 1-mo control SMCs).

The sensitivities of L-type VDCC in diabetic SMCs to dihydropyridines were further examined. BAY K 8644 at 5 µM increased the amplitude of L-type VDCC currents by nearly threefold in 1-mo control SMCs (Fig. 4, A and C). An approximately fourfold increase in L-type VDCC currents by BAY K 8644 was seen in all diabetic SMCs (P < 0.05 vs. control SMCs; Fig. 4, B and C). As we reported previously, BAY K 8644 induced a leftward peak shift of the I-V relationship of L-type VDCC currents (20). This effect of BAY K 8644 on the I-V relationship of L-type VDCC was not altered in diabetic SMCs (Fig. 4, A and B). Figure 4D shows that the concentration-response curve of L-type VDCC to BAY K 8644 was significantly shifted to the left in 1-mo diabetic SMCs compared with that of 1-mo control SMCs. The sensitivity of L-type VDCC to the L-type VDCC antagonist nifedipine was also significantly increased in diabetic SMCs. The IC50 of nifedipine was 90 ± 30 nM for L-type VDCC of 1-mo control SMCs (n = 8). The IC50 of nifedipine was reduced to 12 ± 10 nM in 1-mo diabetic SMCs (n = 8, P < 0.01 vs. control; Fig. 5).

Effects of glucose concentrations of culture media on the properties of L-type VDCC currents. To examine the influence of hyperglycemia on the altered properties of L-type VDCC in diabetic SMCs, the isolated rat tail artery SMCs were cultured in media having different concentrations of glucose. When SMCs were cultured for >24 h, the cells started to proliferate, and the recording of detectable L-type VDCC currents became difficult (not shown). Therefore, in the present study, SMCs were cultured only for 24 h. The current density of L-type VDCC of 3-mo diabetic SMCs was significantly smaller than that of 2-mo diabetic SMCs when both groups of cells were cultured in the modified...
DMEM containing 25 mM glucose for 24 h, as described earlier (Figs. 2B, 2C, and 6A). Interestingly, after culturing 3-mo diabetic SMCs for 24 h in normal DMEM containing 5 mM glucose, the current density of L-type VDCC was not distinguishable from that of 2-mo diabetic SMCs cultured with 25 mM glucose (Figs. 2B and 6A). More importantly, the current densities of L-type VDCC of 3-mo diabetic SMCs were significantly increased when cultured for 24 h with 5 mM glucose compared with the 25 mM glucose culture (P < 0.05; Fig. 6A). The current densities of L-type VDCC of 3-mo diabetic SMCs were not significantly different when cultured for 24 h with the normal DMEM (5 mM glucose) or with the modified DMEM containing 5 mM glucose and 20 mM mannitol (Fig. 6C). These results support the contention that hyperglycemia, but not hyperosmolality, may be responsible for the altered properties of L-type VDCC in diabetes. The diabetes-related abnormalities of L-type VDCC currents were also observed in control SMCs by increasing the glucose concentration of culture medium. As shown in Fig. 6B, after culturing 1-mo control SMCs for 24 h in the modified DMEM containing 25 mM glucose, the current density of L-type VDCC was decreased and became indistinguishable from that of 1-mo diabetic SMCs cultured in the modified DMEM containing 25 mM glucose. However, replacement of 25 mM glucose with 5 mM glucose plus 20 mM mannitol in the modified DMEM failed to replicate the inhibitory effect of hyperglycemia on the current density of L-type VDCC of control SMCs (Fig. 6C).

In contrast to the chronic effect of hyperglycemia (24 h culture), acute exposure (15 min) of the patch-clamped normal SMCs to 25 mM glucose (n = 5), or the patch-clamped 1-mo diabetic SMCs to 5 mM glucose (n = 5), did not alter the properties of L-type VDCC in the respective cells (data not shown).

The influence of hyperglycemia on the sensitivities of L-type VDCC to cAMP and dihydropyridines was further examined. A 36 ± 4.1% inhibition of L-type VDCC currents by DBcAMP (1 mM) was observed (n = 5) in 1-mo control SMCs cultured for 24 h with 25 mM glucose, which was significantly greater than the effect of DBcAMP on 1-mo control SMCs incubated with 5 mM glucose (25.1 ± 3.4%; P < 0.05). On the other hand, BAY K 8644 (5 µM) increased the current amplitude of L-type VDCC by 314 ± 53% in 1-mo control SMCs (n = 5) that were cultured for 24 h with 25 mM glucose. This effect of BAY K 8644 was not significantly different from a 268 ± 37% increase in L-type VDCC currents in 1-mo control SMCs cultured for 24 h with 5 mM glucose (P > 0.05; Fig. 4C).

DISCUSSION

Altered properties of VDCC in diabetes may significantly affect the Ca²⁺ homeostasis of vascular SMCs and the vascular functions as well. The contractile response evoked by K⁺ depolarization has been found to be diminished (4, 8), increased (26), or not influenced (1, 14) in diabetic blood vessels, depending on the experimental models, vascular tissue types, and the duration of diabetes. For instance, the contraction and ⁴⁵Ca uptake of aortic tissues induced by high K⁺ were both suppressed in STZ-induced 2- to 3-mo diabetic rats (7). These results suggested the abnormalities of VDCC in diabetic vascular SMCs. However, the characteris-

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**Fig. 6.** Effects of glucose concentrations of culture media on the current densities of L-type VDCC in control or diabetic SMCs. A: diabetes-related decrease in the current densities of L-type VDCC was partially reversed by culturing diabetic SMCs with 5 mM glucose for 24 h (Ọ; n = 27). Some SMCs from 3-mo diabetic rats were cultured with 25 mM glucose for 24 h (●; n = 32). Other SMCs from 3-mo diabetic rats were cultured with 5 mM glucose for 24 h (Ọ; n = 17). *P < 0.05 between two groups of 3-mo diabetic SMCs. B: diabetes-related decrease in current densities of L-type VDCC was partially mimicked by culturing control SMCs with 25 mM glucose for 24 h. SMCs from 1-mo diabetic rats were cultured with 25 mM glucose for 24 h (●; n = 30). Some SMCs from 1-mo control rats were cultured with 5 mM glucose for 24 h (Ọ; n = 10). Other SMCs from 1-mo control rats were cultured with 25 mM glucose for 24 h (●; n = 11). *P < 0.05 vs. 1-mo control SMCs. C: effects of hyperglycemia and hyperosmolality on current densities of L-type VDCC in 3-mo normal SMCs or 3-mo diabetic SMCs. Concentrations of glucose or mannitol in the culture medium are shown. HP, -80 mV; test potential, +20 mV; n = 5–27 cells for each group. *P < 0.05 vs. results from cells exposed to 5 mM glucose culture.
tics of VDCC in diabetic vascular SMCs had not been directly studied using the patch-clamp technique. Our study showed that the current density of L-type VDCC in tail artery SMCs from 1- to 3-mo diabetic rats was significantly reduced. A similar decrease in L-type VDCC current density has been reported in ventricular myocytes isolated from 4- to 5-mo diabetic rats (18). In contrast, Magyar et al. (11) reported that L-type VDCC current density of ventricular myocytes isolated from rats with a diabetes history of several days to weeks was not different from that of age-matched control rats. In fact, our result from 1-wk diabetic vascular SMCs did not show a significant decrease in L-type VDCC current density. It appears that the abnormalities of L-type VDCC in diabetes depend on the stage of diabetes.

Multiple etiological factors, such as the altered hemodynamics due to hypertension or diabetic nephropathy, may contribute to the altered properties of L-type VDCC of vascular SMCs in diabetes. Blood pressure change in STZ-diabetic animals has been controversial. Not only hypertension (5) but also hypotension (10) in STZ-Injected diabetic rats have been reported. Myers and Messina (15) did not find any change in blood pressure in STZ-diabetic rats. We cannot explain this discrepancy in the reported blood pressure change, even when taking into account the differences in the rat strains or the methods for administration of STZ, etc. In any case, our results demonstrated that 1–3 mo after STZ injection, mean blood pressure of diabetic rats was not altered. Therefore, the possibility is remote that the altered L-type VDCC functionality observed in our study might be secondary to the altered hemodynamics in diabetes. On the other hand, a strong correlation between the altered properties of L-type VDCC in diabetic SMCs to hyperglycemia was indicated from our study. We have previously studied the hyperglycemia-related abnormal Ca^{2+} handling of vascular SMCs (22). When the glucose concentration of extracellular solutions was kept at 5.5 mM, KCl induced a fast increase in [Ca^{2+}], that then slowly declined (type 1 response) in 83% of tail artery SMCs from nondiabetic rats. In 9% of nondiabetic SMCs, KCl induced a slow increase in [Ca^{2+}] (type 2 response). Interestingly, under the same culture conditions, KCl induced type 1 and type 2 responses in 47% and 35% of diabetic SMCs, respectively. When SMCs from nondiabetic or diabetic rats were cultured in 36 mM glucose, KCl induced a fast increase in [Ca^{2+}], that, however, was maintained at a high level (type 3 response; see Ref. 22). These results provide indirect evidence that VDCC may undergo significant changes under hyperglycemic conditions. Furthermore, exposure of nondiabetic vascular tissues to the elevated glucose level can imitate the abnormal vascular contractilities seen in diabetic rats. We have previously found that the KCl-induced maximum tension development of rat tail artery tissues was significantly reduced by hyperglycemia (21). Kam et al. (9) reported that the contractile responses to KCl of aortae from nondiabetic rats were significantly reduced after exposure to elevated concentrations of glucose in vitro, suggesting that "the elevated glucose level leads to an impaired Ca^{2+} influx via the (slow) L-type Ca^{2+} channels." In the present study, we showed that the reduced current density of L-type VDCC in diabetic SMCs was replicated by culturing nondiabetic control SMCs for 24 h, but not 15 min, with hyperglycemic medium. A 15-min incubation of 1-mo diabetic SMCs with 5 mM glucose failed, but a 24-h incubation was able, to reverse the diabetic-related decrease in the current density of L-type VDCC. These observations link the altered properties of L-type VDCC in diabetic SMCs to hyperglycemia. In keeping with the idea that hyperglycemia could affect protein expression, Williams et al. (27) have reported that culturing human vascular SMCs in 20 mM glucose increased vascular permeability factor (VPF) mRNA expression within 3 h (3-fold vs. 5 mM glucose) and significantly increased VPF peptide production within 24 h (1.5-fold). This time- and glucose concentration-dependent increase in VPF mRNA expression was rapidly reversed after normalizing the extracellular glucose concentration.

The vascular complications of diabetes mellitus are accompanied by a variety of metabolic abnormalities in vascular SMCs. Included are the activation of protein kinase C (27), altered G protein function (25), decreased Na^{+}-K^{+}-ATPase activity (16), and abnormal cAMP metabolism. Miller et al. (12) reported that the activity of adenyl cyclase was significantly increased and the hydrolysis of cAMP reduced in aortic SMCs from STZ-induced diabetic rats. In contrast, Weber et al. (25) did not find any difference in cAMP levels between control and STZ-induced diabetic rat aortae. Because L-type VDCC currents in tail artery SMCs are subjected to the inhibition by cAMP (23), either increased cellular cAMP concentration or enhanced sensitivities of L-type VDCC to cAMP would result in the suppressed current densities of L-type VDCC. To test this hypothesis, we compared the effect of the membrane-permeable analog of cAMP, DBcAMP, on L-type VDCC currents in diabetic SMCs and nondiabetic control SMCs. Our results showed that DBcAMP inhibited L-type VDCC currents more in diabetic SMCs than in nondiabetic control cells. The observed effect of glucose concentration of culture media on the properties of L-type VDCC also indicates that the increased sensitivity to cAMP of L-type VDCC in diabetes may be directly related to hyperglycemia. Thus, no matter whether the cAMP level is altered or not in diabetes, the enhanced sensitivity of L-type VDCC to cAMP may significantly alter the properties of L-type VDCC in diabetic vascular SMCs. Because the sensitivity of L-type VDCC to cAMP is determined by their carboxy-terminal-located phosphorylation sites (13), we speculate that hyperglycemia-related glycosylation of L-type VDCC might increase the affinity of these phosphorylation sites to the cAMP-dependent protein kinase. Alternatively, chronic hyperglycemia may increase the expression of cAMP-dependent protein kinase (6), thus enhancing the effect of cAMP on L-type VDCC.
The relative responsiveness of L-typeVDCC to dihydropyridines was much higher in diabetic SMCs than in normal SMCs. Because the basal activity of L-typeVDCC in normal SMCs was significantly greater than in diabetic SMCs, the absolute current amplitude of L-typeVDCC after the application of BAY K 8644 was still greater in normal SMCs than in diabetic cells. This is consistent with a previous observation that development of contraction force induced by BAY K 8644 was lesser in diabetic aortic tissues than in nondiabetic control tissues (7). In contrast to the influence of hyperglycemia to the current density and the cAMP sensitivity, the mechanisms for the altered dihydropyridine sensitivity of L-typeVDCC in diabetes may be different since hyperglycemic culture did not alter the effect of BAY K 8644 on L-typeVDCC. However, it should be mentioned that the glycosylation of dihydropyridine binding sites of L-typeVDCC may require a time course longer than 24 h. A prolonged hyperglycemic culture period may be more relevant to the chronic diabetic situation. Because of the plausible phenotype change and difficulty in recording L-typeVDCC currents in the SMCs cultured for >24 h, we did not examine further the properties of L-typeVDCC in SMCs in culture beyond 24 h. In any case, the altered dihydropyridine sensitivity of L-typeVDCC in diabetes would impose a great impact on the regulation of blood pressure, the peripheral blood flow rhythm and patterns (2, 3), and the proliferation of vascular SMCs (17). Thus elucidation of the altered dihydropyridine sensitivity of L-typeVDCC in vascular SMCs during diabetes may shed light on the pharmacological intervention of diabetic vascular complications.

In conclusion, the current density of L-typeVDCC in diabetic tail artery SMCs was significantly reduced as diabetes progressed. Although their I-V relationship and steady-state activation and inactivation remained unchanged, L-typeVDCC became more sensitive to dihydropyridines in diabetic vascular SMCs. These abnormalities of L-typeVDCC in diabetic vascular SMCs may result from the increased cAMP sensitivity and may be under the influence of hyperglycemia. The illustration of these diabetes-associated abnormalities of L-typeVDCC in vascular SMCs may help to understand the altered vascular structure and functions encountered in the vascular complications of diabetes. Because the severity and properties of vascular complications of diabetes varies among different subtypes of diabetes mellitus, further investigations on the properties of L-typeVDCC in vascular SMCs in other experimental models of diabetes should also be considered.

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