British Journal of Pharmacology (1998) 123, 675–682

Inhibitory action of insulin-sensitizing agents on calcium channels in smooth muscle cells from resistance arteries of guinea-pig

Yoshito Nakamura, [1]Yusuke Ohya, Uran Onaka, Koji Fujii, Isao Abe & Masatoshi Fujishima

Second Department of Internal Medicine, Faculty of Medicine, Kyushu University, Fukuoka, Japan

1 The actions of troglitazone, pioglitazone, metformin and bezafibrate, agents that improve insulin-resistance, on voltage-dependent Ca^{2+} channels in arterial smooth muscle cells were examined by use of the conventional and nystatin-perforated whole-cell clamp methods. Single cells were freshly isolated from resistance mesenteric arteries of guinea-pigs. The actions of these agents on 77 mM K^+-induced contraction of the isolated arteries were also examined with the use of isometric tension recording.

2 The thiazolidinedione derivatives, troglitazone and pioglitazone, inhibited whole-cell Ca^{2+} currents in a dose-dependent manner with dissociation constants of 3.0 μM and 44.9 μM and Hill coefficients of 0.61 and 0.68, respectively. These two agents inhibited the 77 mM K^+-induced contraction with similar potencies as those inhibiting the Ca^{2+} currents. Metformin and bezafibrate had no apparent effects on the Ca^{2+} current or high K^+-induced contraction.

3 The inhibitory action of troglitazone on Ca^{2+} currents was not affected by the command potential, the holding potential, or the stimulation frequency, suggesting that its mode of the action differs from that of known organic Ca^{2+} channel antagonists.

4 The inhibitory action of troglitazone on Ca^{2+} currents was not affected by the addition of insulin to, or the removal of glucose from, the solutions.

5 In conclusion, the thiazolidinedione derivatives directly inhibited the voltage-dependent Ca^{2+} channels in a different manner from that of organic Ca^{2+} channel antagonists. This inhibitory action on Ca^{2+} channels was not a common feature of insulin-sensitizing agents.

Keywords: Insulin resistance; calcium channel; vascular smooth muscle; electrophysiology; troglitazone; pioglitazone; metformin; bezafibrate

Introduction

Thiazolidinedione derivatives, such as troglitazone and pioglitazone, are new agents that improve the metabolism of glucose by increasing insulin sensitivity in insulin-sensitive tissues (Ciaraldi *et al*., 1990; Hoffman *et al*., 1991). Metformin and clofibrate also possess this insulin-sensitizing action (Kotchen, 1996). These agents have been shown to lower blood pressure in animals and man, especially in the presence of obesity or insulin-resistance (Yoshioka *et al*., 1993; Kemnitz *et al*., 1994; Lee *et al*., 1994; Buchanan *et al*., 1995; Kaufman *et al*., 1995; Ogihara *et al*., 1995). However, some conflicting results exist (Giugliano *et al*., 1993; Landin *et al*., 1994). These observations may be related to the hypothesis that insulin resistance and hyperinsulinaemia raise arterial pressure (Ferrannini *et al*., 1985; Kotchen, 1996; Landsberg, 1996). However, recent studies suggest that some insulin-sensitizing agents may have a direct vascular effect. For example, pioglitazone inhibits the contractile response of rat aorta both in the presence and absence of insulin (Buchanan *et al*., 1995; Kotchen, 1996). This action may result from the inhibition of Ca^{2+} entry, since pioglitazone inhibits the agonist-induced increase in cytosolic Ca^{2+} in cultured aortic cells, as measured by Ca^{2+}-sensitive dye (Buchanan *et al*., 1995). Ciglitazone, another thiazolidinedione derivative, also inhibits the increase in cytosolic Ca^{2+} in human glioblastoma cells (Pershadsingh *et al*., 1993). In addition, a recent study with the patch-clamp method shows that pioglitazone inhibits L-type Ca^{2+} currents in vascular smooth muscle cells (Zhang *et al*., 1994). However, it is not known (1) whether the ability to inhibit Ca^{2+} channels is a common property of all insulin-sensitizing agents, (2) whether the mode of inhibitory action of insulin-sensitizing agents on Ca^{2+} channels is the same as that for organic Ca^{2+} channel antagonists, and (3) whether the inhibitory action on Ca^{2+} channels is modified in the presence of insulin. To address these questions, we examined the actions of the insulin-sensitizing agents, troglitazone, pioglitazone, metformin, and bezafibrate on voltage-dependent Ca^{2+} channels in arterial smooth muscle cells by use of the conventional and perforated whole-cell patch clamp methods.

[1]Author for correspondence at: Second Department of Internal Medicine, Faculty of Medicine, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-82, Japan.

Methods

Female guinea-pigs (body weight 200–250 g) were anaesthetized with ether and then decapitated. Single smooth muscle cells were obtained by collagenase treatment (Wako Chemical Co., Tokyo, Japan) from mesenteric arterial branch (diameter <300 μm), as described previously (Ohya *et al*., 1993; Setoguchi *et al*., 1995).

Voltage clamp recording

Conventional and nystatin-perforated whole-cell patch clamp methods were performed with a patch pipette through a voltage clamp amplifier (Axopatch 1-D, Axon Instruments Inc., Foster City, CA, U.S.A.). The conditions and procedures for the conventional and perforated methods were basically the same as those previously described (Horn & Marty, 1988; Ohya *et al*., 1993; Setoguchi *et al*., 1995). Recording electrodes were made from Pyrex glass capillary tubing. Current recording was carried out at room temperature (22 to 24°C). The arterial cells

were held at −80 mV and command potentials were applied every 10 s or 20 s. Data were obtained after the current amplitude had been stabilized (usually 3 to 4 min after the whole-cell configuration was obtained). The Ca^{2+} current did not run-down over the next 20 min under these conditions (Ohya & Sperelakis, 1989; Setoguchi *et al*., 1995). Membrane currents were low-pass-filtered at 2 kHz, digitized with a sampling frequency of 5 to 10 kHz, and stored in a personal computer system for subsequent analysis. The liquid junction potential of 10 mV was corrected. The leak and residual capacitive currents were subtracted by use of the P/4 protocol. The traces finally shown were low-pass-filtered at 1 kHz.

To isolate inward Ca^{2+} currents, the pipette was filled with a high Cs^+ solution. The pipette solution for the conventional method contained (mM): Cs aspartate 130, CsCl 20, ATP-Na_2 3, EGTA 10, HEPES 10, and pH 7.3 titrated with CsOH. For the perforated method, the pipette solution contained (mM): Cs-aspartate 110, Na-aspartate 25, NaCl 20, $MgCl_2$ 2, HEPES 10, and pH 7.3 titrated by CsOH, and nystatin (200–300 μg ml^{-1}, Sigma, St. Louis, MO, U.S.A.). Nystatin was dissolved in 100% dimethyl sulphoxide (30 mg ml^{-1}), frozen in aliquots, and diluted within 1 h before use. As a carrier of Ca^{2+} currents, either Ba^{2+} or Ca^{2+} ion was used. The Ba^{2+}-containing bath solution consisted of (mM): $BaCl_2$ 10, NaCl 150, glucose 5.4, HEPES 5, and pH 7.3 titrated with NaOH. For the Ca^{2+}-containing bath solution, $BaCl_2$ was replaced with 5 or 10 mM $CaCl_2$. To make glucose-free solution, glucose was replaced with (+)-mannitol.

Isometric tension recording

Isometric tension was recorded from circularly cut ring prepared from the second branch of mesenteric artery (diameter around 300–400 μm). The endothelium was removed by gentle rubbing of internal surface with tips of forceps. Isometric tension changes were monitored with a transducer (UM-203, Medico Kishimoto, Kyoto, Japan) connected to a carrier amplifier (AP-5, Ufer, Co., Ltd. Kyoto, Japan). The arterial ring was mounted in an organ chamber with a capacity of 5 ml filled with Krebs solution (mM: Na^+ 137.4, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, HCO_3^- 15.5, $H_2PO_4^-$ 1.2, Cl^- 134 and glucose 11.5; bubbled with O_2 95% and CO_2 5%). A contraction was evoked by replacing the bath solution with 77 mM K^+ solution (mM: Na^+ 66.2, K^+ 77.1, Ca^{2+} 2.5, Mg^{2+} 1.2, HCO_3^- 15.5, $H_2PO_4^-$ 1.2, Cl^- 134 and glucose 11.5; bubbled with O_2 95% and CO_2 5%). The resting tension was adjusted to obtain maximum contraction (about 0.4 to 0.6 g). For each ring, 77 mM K^+ solution was at first applied to obtain a control contraction. Then the test contraction was obtained 15 min after incubation with either troglitazone, pioglitazone, metformin or bezafibrate.

Chemicals

The drugs used were the thiazolidinedione derivatives, troglitazone ((±)-5-[4-(6-hydroxy-2,5,7,8-tetramethylchroman-2-ylmethoxy) benzyl]-2,4-thiazolidinedione, a gift from Sankyo Co., Ltd., Tokyo, Japan) and pioglitazone ((±)-5-[p-[5-ethyl-2-pyridyl-2-ethoxy] benzyl]-2,4, thiazolidinedione, a gift from Takeda Chemical Industries, Ltd., Osaka, Japan), as well as a biguanide derivative, metformin (N,N-dimethylimidodicarbonimidic diamide; 1,1-dimethylbiguanide, Sigma), and a clofibrate derivative, bezafibrate (2-[p-[2-(p-chlorobezamido) ethyl]phenoxy]-2-methylpropionic acid, Sigma) (Figure 1). Troglitazone, pioglitazone and bezafibrate were dissolved in 100% dimethyl sulphoxide (DMSO) as 100 mM stock

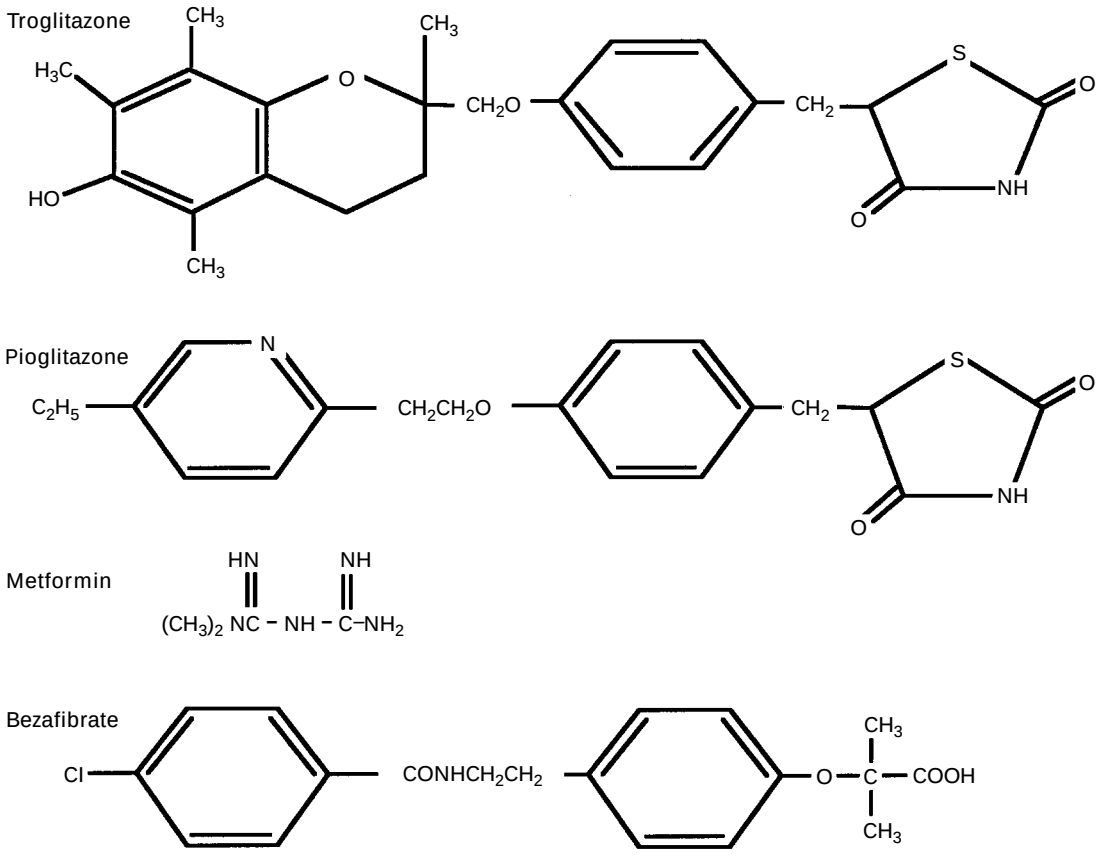

Figure 1 Chemical structures of troglitazone, pioglitazone, metformin, and bezafibrate.

solutions. Metformin was dissolved in a distilled water to 100 mM. The stock solution was diluted at least 1000 times when used. The final concentration of each drug is stated in the text. DMSO at 0.1% had no effect on membrane currents or contraction.

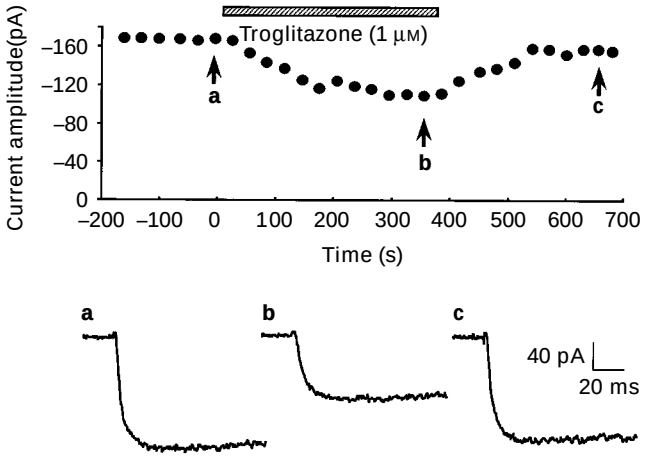

Figure 2 Effects of the application and washout of troglitazone on voltage-dependent Ca^{2+} channels in arterial smooth muscle cells. The current was evoked with a command potential of 10 mV from a holding potential of −80 mV, a stimulation frequency of 0.1 Hz, by use of the conventional whole-cell patch clamp method. The Ba^{2+}-containing bath solution and the high Cs^{+}-pipette solution were used. Changes in the amplitudes of Ca^{2+} currents during the application (bar) and removal of 1 μM troglitazone are shown. Current traces were obtained before (a) and after (b) the application, and the removal (c) of troglitazone.

To evaluate the effects of insulin, the cells were preincubated with 10 μM insulin (human; Sigma) for 10 min before recording of Ca^{2+} currents. This concentration has been shown to induce a maximal response of the various effects of insulin (Fleig *et al*., 1984; Fujiwara *et al*., 1988; Ciaraldi *et al*., 1990; Zhu *et al*., 1993).

Curve fitting and statistics

Fitting of the data to each equation was performed by the nonlinear least-squares method. Data are expressed as mean ± s.e. Statistical significance was determined by Student's *t* test (unpaired) or one-way analysis of variance. A level of $P<0.05$ was considered statistically significant.

Results

In guinea-pig mesenteric artery cells, whole-cell Ca^{2+} currents were evoked by the depolarizing command potentials. The major component of inward current under this condition was of L-type Ca^{2+} channels (Ohya *et al*., 1993; Setoguchi *et al*., 1995). Figure 2 shows the effects of the application and removal of troglitazone on the Ca^{2+} currents. Addition of troglitazone inhibited the Ca^{2+} current and the current recovered after the removal of troglitazone (with 1 μM troglitazone, the amplitude decreased to 65 ± 1%

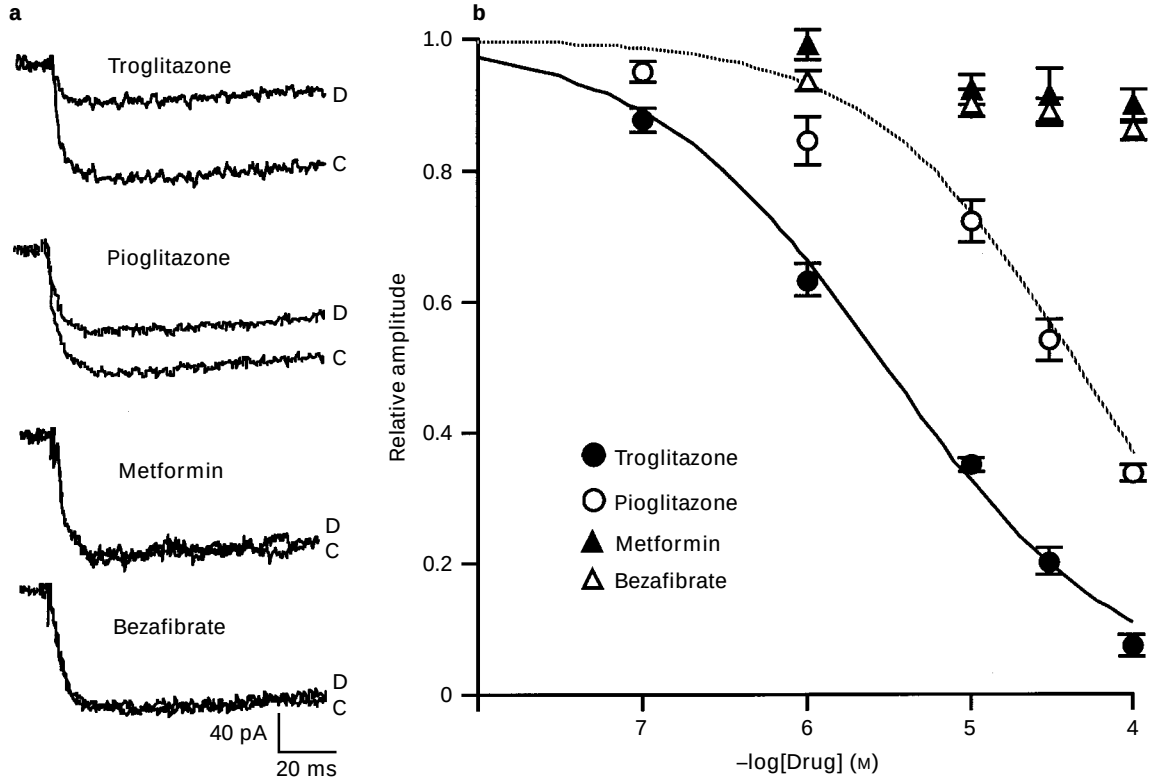

Figure 3 Effects of troglitazone, pioglitazone, metformin, and bezafibrate on the Ca^{2+} currents. (a) Current traces before (C) and after (D) application of 10 μM of the drug. Conditions for the current recording were the same as in Figure 2. (b) Dose- response relationship of action of the insulin-sensitizing agents on the current amplitudes. Relative amplitudes of the currents (mean with vertical lines showing s.e.) with various drug-concentrations are plotted; troglitazone ($n=5$ to 19), pioglitazone ($n=5$ to 9), metformin ($n=4$ to 6) and bezafibrate ($n=4$ to 6). A control amplitude (before application of drugs) was normalized to 1.0. Continuous curves for troglitazone and pioglitazone were obtained by fitting data to the following equation: $I_{drug}/I_{control}=1-1/\{1+(K_d/[drug])^{n_H}\}$, where I_{drug} is the current amplitude recorded with a certain concentration of drug. $I_{control}$ is the current amplitude recorded before application of the drug, K_d is a dissociation constant (troglitazone, 3.0 μM; pioglitazone, 44.9 μM), and n_H is Hill coefficient (troglitazone, 0.61; pioglitazone, 0.68).

of the control; after wash-out, 93±2% of the control; $n=5$ cells).

Figure 3 shows the effect of troglitazone, pioglitazone, metformin and bezafibrate on the amplitude of Ca^{2+} currents. Figure 3a shows the changes in current traces in the presence of 10 μM troglitazone, pioglitazone, metformin and bezafibrate. Troglitazone and pioglitazone inhibited Ca^{2+} channel currents. However, pioglitazone exerted a smaller inhibitory effect than troglitazone (10 μM troglitazone, by 65±5%, $n=19$; 10 μM pioglitazone, by 28±4%, $n=8$). Metformin and bezafibrate did not have any apparent effects. The dose-response relationships of these agents on the current amplitudes were obtained (Figure 3b). Only troglitazone and pioglitazone exerted a significant effect within the concentrations examined. The dissociation constants for troglitazone and pioglitazone were 3.0 μM and 44.9 μM, and Hill coefficients for these drugs were 0.61 and 0.68, respectively.

To evaluate whether this inhibitory action causes the vasodilatation, we examined effects of these insulin-sensitizing agents on the isometric tension of isolated arteries (Figure 4). Troglitazone (1 μM and higher) and pioglitazone (10 μM and higher) inhibited the 77 mM K^+-induced contraction (10 μM: by 48±10%, $n=8$; and 11±2%, $n=6$, respectively). On the other hand, metformin and bezafibrate, at concentrations up to 100 μM, had no apparent effects on the 77 mM K^+-contraction (Figure 4).

The mode of troglitazone action on Ca^{2+} channels was then evaluated. Figure 5 shows the action of troglitazone on the current-voltage relationship of Ca^{2+} currents. Troglitazone inhibited the currents evoked by command potentials of −30, −10 and 10 mV (Figure 5a). The full current-voltage relationships before and after the application of 1 μM troglitazone are shown in Figure 5b ($n=19$ cells). The same degree of inhibition was observed at all command potential levels. In Figure 5c, the average inhibitions by 1 μM troglitazone at the given command potentials are shown. This graph clearly shows that the inhibition was not affected by the strength of the command potential.

The effect of troglitazone on voltage-dependent inactivation of Ca^{2+} channels was then examined (Figure 6a). The steady-state inactivation curve was obtained by use of a double-pulse protocol before and after the application of 1 μM troglitazone. Troglitazone did not alter the steady-state inactivation curve. Figure 6b shows the effect of repetitive depolarization on the current inhibition induced by troglitazone. The amplitudes of the current evoked with the 18th pulse in the repetitive stimulation with various frequencies (0.2, 1 and 3 Hz) are shown. The train pulses caused only a slight accumulation of the inhibition in the absence and presence of troglitazone. These results suggest that the current inhibition by troglitazone was not holding potential-dependent or frequency-dependent.

The inhibitory action of troglitazone on the Ca^{2+} channels was examined in the presence and absence of insulin (Figure 7). Cells were preincubated with 10 μM insulin for 10 min before the current was recorded. Experiments were first performed with the Ba^{2+}-containing bath solution and the conventional patch clamp method (Figure 7a). The current inhibition by troglitazone was not affected in the presence of insulin. We also examined the action of troglitazone with or without glucose in the solution. Removal of glucose from the bath solution did not affect the action of troglitazone (Figure 7a).

We also examined the effect of troglitazone action by the perforated patch clamp method with the Ca^{2+}-containing bath solution (Figure 7b). In the perforated patch clamp method, the wash-out of the second-messenger system is minimal (Horn & Marty, 1988). The troglitazone actions on the Ba^{2+} currents recorded with the conventional method and those on the Ca^{2+} currents recorded with the perforated patch clamp method

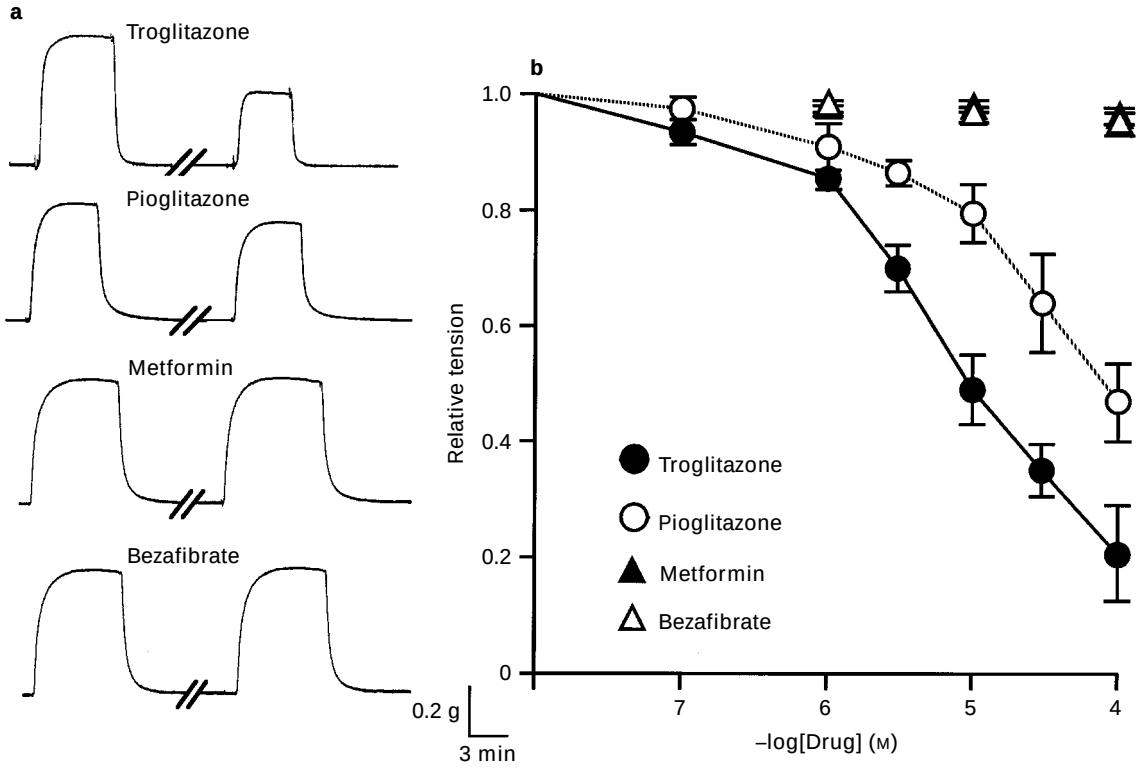

Figure 4 The effects of insulin sensitizing-agents on the 77 mM K^+-induced contraction of mesenteric arteries with the isometric tension recording. (a) Representative traces of 77 mM K^+-induced contraction before (left trace) and after application of 10 μM of the drug (right trace). (b) Relative tensions after the application of drugs are shown. Control tension (before application of drugs) was normalized to 1.0. Data are shown as mean and vertical lines indicate s.e. from 5 to 7 mesenteric arteries.

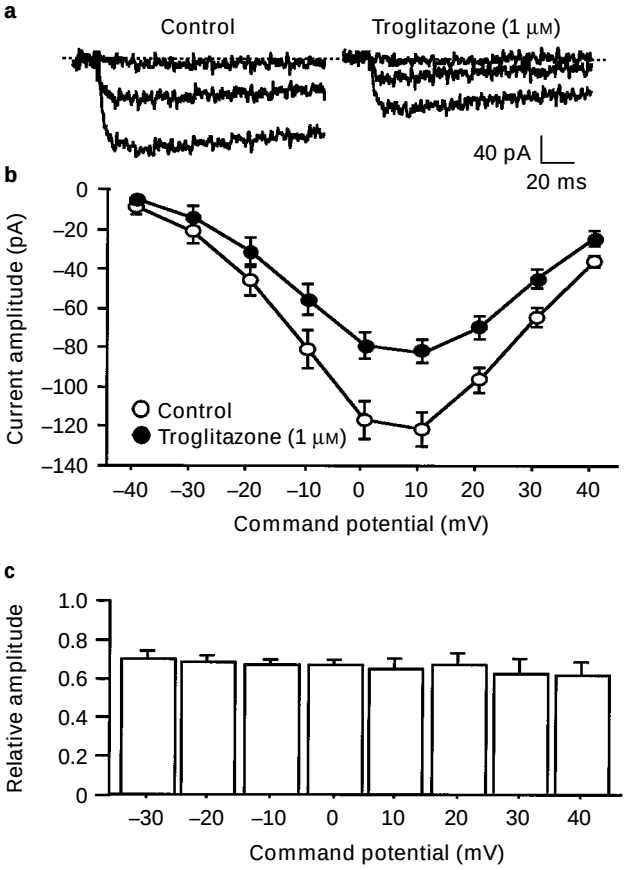

Figure 5 Action of troglitazone on the current-voltage relationship of Ca^{2+} currents. (a) Traces of the current obtained at command potentials of −30, −10 and 10 mV. Other conditions for the current recording were same as in Figure 2. Dotted line is zero current level. (b) Peak amplitudes of the currents, recorded in the absence (control) and presence of 1 μM troglitazone, are plotted against the command potentials. Each value represents the mean with vertical lines showing s.e., from 19 cells. (c) Relationship between the command potential and the current inhibition. The amplitudes at any given command potential in the absence of troglitazone are normalized to 1.0 and those observed at the same potential in the presence of 1 μM troglitazone are expressed as a relative value. Data are the same as in (b).

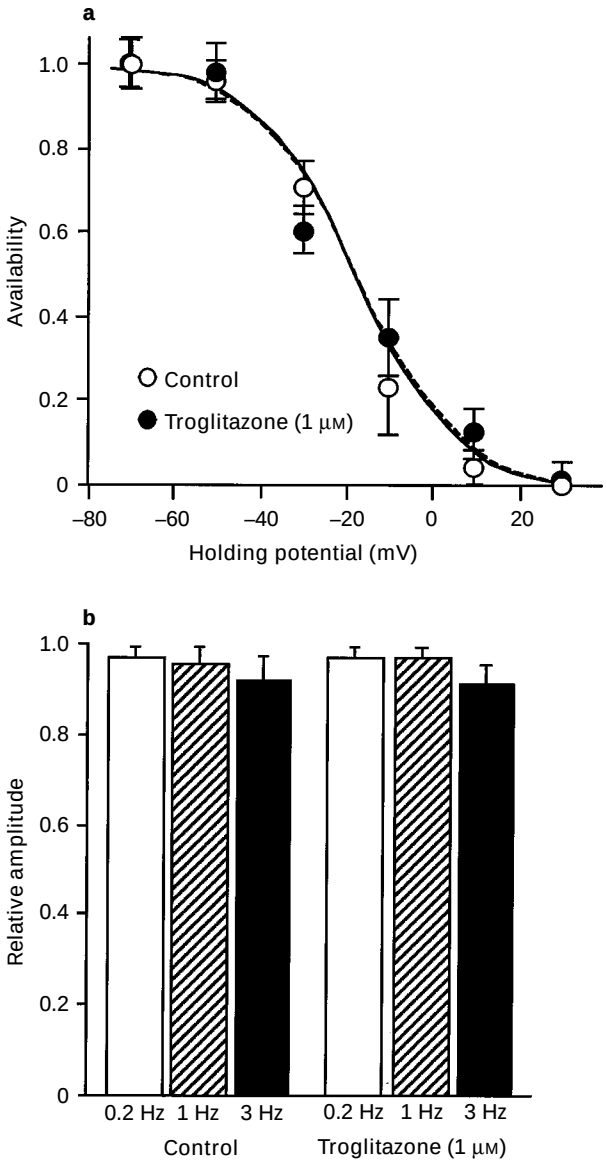

Figure 6 (a) Steady-state inactivation curves obtained in the absence ($n=5$) and presence of 10 μM troglitazone ($n=5$). Conditioning pulse was applied for 10 s at various potentials before a test pulse to 10 mV. Amplitude of the current evoked by the test pulse with various holding (conditioning) potentials was normalized to that evoked with a holding potential of −80 mV, and plotted against the holding potential. Each point represents the mean, and vertical lines s.e., of 4 to 8 values. Two curves were obtained by fitting data to the Boltzman distribution: $P=1/[1+\exp\{(V-V_h)/k\}]$, where P is the availability, V is the holding (conditioning) potential, V_h is the potential required for half-inhibition of the current, and k is the Boltzman coefficient. The V_h values for control and troglitazone were 10.6 mV and 11.4 mV, respectively, and k values were 11.6 mV and 12.1 mV, respectively. (b) Repetitive depolarizations did not potentiate the inhibition of Ca^{2+} current by troglitazone. Columns represent the relative amplitude of the current evoked by the 18th pulse in repetitive depolarization at 0.2 Hz, 1 Hz, and 3 Hz (mean ± s.e. of 4 to 7 cells). The amplitude at the first pulse was normalized to 1.0. The current was evoked by a command potential of 10 mV (duration 100 ms) from a holding potential of −80 mV. The Ba^{2+}-containing bath solution and the conventional patch clamp method were used for (a) and (b).

were comparable (compare a and b in Figure 7). Moreover, the addition of insulin to or removal of glucose from the solutions did not affect the effect of troglitazone in the perforated patch clamp method either (Figure 7b).

Discussion

The new findings of the present study were as follows: (1) the thiazolidinedione derivatives, troglitazone and pioglitazone, but not metformin and bezafibrate, inhibited Ca^{2+} current in arterial cells and high-K^+ induced contraction of isolated arteries, (2) troglitazone exhibited a greater potency than pioglitazone at inhibiting currents and contractions, (3) the action of troglitazone on Ca^{2+} channel currents was not affected by the command potential, holding potential, or stimulation frequency, and (4) the inhibitory action of troglitazone on Ca^{2+} currents was not affected by the addition of insulin or the removal of glucose.

The present study demonstrated that the thiazolidinedione derivatives, but not metformin and bezafibrate, inhibited the Ca^{2+} current and high-K^+ induced contraction. Thus, only thiazolidinedione derivatives inhibit voltage-dependent Ca^{2+} channels. These findings suggest that the inhibitory action on Ca^{2+} channels is not a common feature of the insulin-sensitizing agents. Chemical structures of troglitazone,

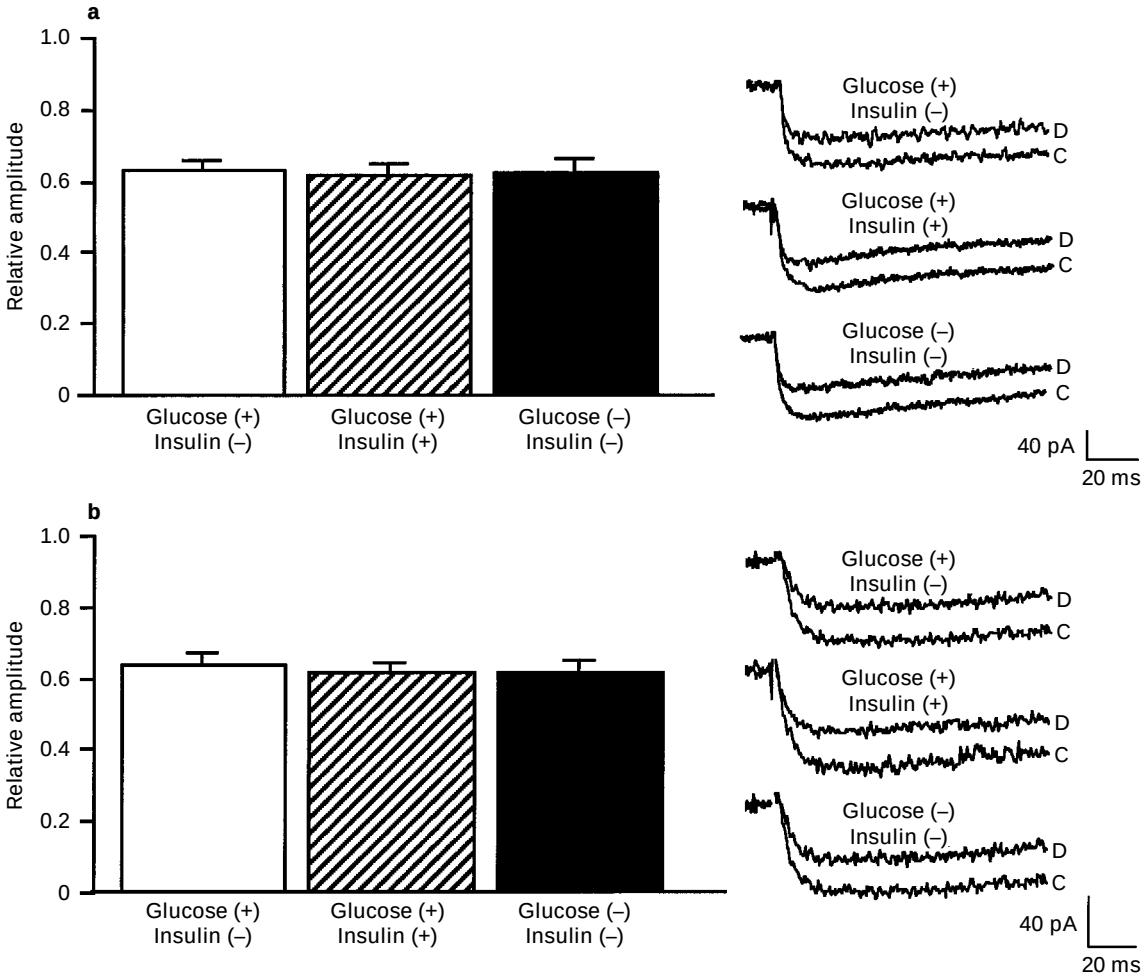

Figure 7 Effect of troglitazone on the Ca^{2+} channels in the presence and absence of insulin and glucose. Experiments were performed on Ba^{2+} currents recorded with the conventional method (a) and on Ca^{2+} currents recorded with the nystatin-perforated method (b). The relative amplitudes of the currents after application of 1 μM troglitazone in the presence of glucose (5.4 mM) and absence of insulin (open columns; a, $n=19$; b, $n=5$), the presence of both glucose and insulin (10 μM) (shaded columns; a, $n=8$; b, $n=5$), and the absence of glucose and insulin (solid columns; a, $n=8$; b, $n=5$). Control amplitude (before application of drug) was normalized to 1.0. Insets: the current traces before (C) and after application of 1 μM troglitazone (D). The current was evoked by a command potential of 10 mV from a holding potential of -80 mV with Ba^{2+}-containing bath solution (a) or Ca^{2+}-containing bath solution (b).

pioglitazone, metformin and bezafibrate are shown in Figure 1. The right half of the structure including benzyl-thiazolidinedione shared by troglitazone and pioglitazone appears to be essential for the inhibition of Ca^{2+} channels. The other half of the structure of these drugs may affect the potency to inhibit Ca^{2+} channels. Even known organic Ca^{2+} channel antagonists do not possess this structure.

The mode of action of troglitazone apparently differs from that of the organic Ca^{2+} channel antagonists. First, the action of troglitazone on Ca^{2+} channels was not modified by the holding potential or stimulation frequency. In contrast, organic Ca^{2+} channel antagonists, such as dihydropyridines, benzothiazepines and phenylalkylamines, inhibit Ca^{2+} channels in a holding potential-dependent and stimulation frequency-dependent manner (Lee & Tsien, 1983; Terada *et al*., 1987). Using cells from guinea-pig mesenteric arteries, we showed that nifedipine, diltiazem and gallopamil shifted the steady-state inactivation curve by -24, -19 and -17 mV, respectively. Diltiazem and gallopamil also caused frequency-dependent inhibition; during repetitive stimulation at 2 Hz, the amplitude of 10th pulse became 0.70 and 0.70 of the 1st pulse, respectively, in the presence of these drugs (Setoguchi *et al*., 1995; Ohya *et al*., 1997). Second, the Hill coefficient of the troglitazone action was about 0.6, which suggests that troglitazone exerts its action on Ca^{2+} channels with a negative cooperativity. In contrast, the Hill coefficient of the action of organic Ca^{2+} channel antagonists was about 1.0 (Bean, 1984).

The mechanism of the inhibitory effect of thiazolidinedione derivatives on Ca^{2+} channels is not known. Since the inhibition disappeared after removal of the drug, the troglitazone action on Ca^{2+} channel is not the result of current run-down or irreversible inhibition of the channels. In addition, it is unlikely that the inhibitory mechanism involves insulin- or glucose-dependence, since (1) the addition of insulin or the removal of glucose did not modify the inhibitory action, and (2) troglitazone had a greater inhibitory effect on Ca^{2+} channels than pioglitazone, although both drugs have been shown to possess similar potency in improving sensitivity to insulin *in vivo* (Ciaraldi *et al*., 1990; Hoffman *et al*., 1991).

The perforated patch clamp method minimizes the possible washout of the intracellular component. In the present study, the results obtained by the conventional method and those by the perforated method were comparable. It is thus thought that the troglitazone action is not mediated by diffusable

intracellular factors or that factors which mediate the troglitazone action disappear with the conventional method.

From pharmacokinetic data, we estimated that troglitazone and pioglitazone exert their insulin-sensitizing actions at plasma concentrations of 0.2 to 0.9 μg ml^{-1} (0.6 to 2.7 μM) and 0.3 to 3.0 μg ml^{-1} (0.8 to 8 μM), respectively (Shibata *et al*., 1993; Hiraga, 1997). These concentrations are nearly the same as those required for the inhibition of Ca^{2+} channels. Thus, in clinical use, these drugs may exert a vasodilating action by inhibiting Ca^{2+} channels.

The mechanism of the antihypertensive effect of the thiazolidinedione derivatives has not been fully clarified. Since hyperinsulinaemia is thought to contribute to hypertension by affecting vascular structure, function, or both, as well as stimulating sympathetic activity and renal sodium retention (Ferrannini *et al*., 1990; Landsberg, 1996), the decrease in insulin concentration during treatment with these drugs is considered to be a major mechanism for their antihypertensive effect. Results of the present study suggest that the thiazolidinedione derivatives may contribute in part to their antihypertensive actions by dilating the resistance arteries via inhibition of the voltage-dependent Ca^{2+} channels, regardless of insulin status.

Vasodilators such as α-adrenoceptor antagonists, Ca^{2+} channel antagonists, and angiotensin-convering enzyme inhibitors improve the sensitivity to insulin (Kaplan, 1992; Passa, 1993). One mechanism for this effect is thought to involve vasodilatation, and hence, an increase in blood flow to the insulin-sensitive tissues. It is possible that thiazolidinediones could contribute to the insulin-sensitizing action via its vasodilating action, although their effect on the post-receptor-binding steps of the insulin response in the insulin-sensitive tissue is their major mechanism of action (Ciaraldi *et al*., 1990; Hoffman *et al*., 1991).

In conclusion, we have shown that thiazolidinedione derivatives directly inhibit voltage-dependent Ca^{2+} channels with a different mode of action from that of organic Ca^{2+} channel antagonists. However, their inhibitory effect on the Ca^{2+} channels is not common to the insulin-sensitizing agents. The precise mechanism of the inhibitory action of thiazolidinedione derivatives remains to be clarified.

We thank Sankyo, Co., Ltd., Tokyo, Japan for providing troglitazone, and Takeda Chemical, Industries, Ltd., Tokyo, Japan, for providing pioglitazone. This work was supported by grants from the Ministry of Education, Science, and Culture, Japan (Nos. 07770497 and 09670725), and from Japan Heart Foundation Grant for Research on Vascular Metabolism.

References

BEAN, B.P. (1984). Nitrendipine block of cardiac calcium channels: High-affinity binding to the inactivated state. *Proc. Natl. Acad. Sci. U.S.A.,* **81,** 6388 – 6392.

BUCHANAN, T.A., MEEHAN, W.P., JENG, Y.Y., YANG, D., CHAN, T.M., NADLER, J.L., SCOTT, S., RUDE, R.K. & HSUEH, W.A. (1995). Blood pressure lowering by pioglitazone – evidence for a direct vascular effect. *J. Clin. Invest.,* **96,** 354 – 60.

CIARALDI, T.P., GILMORE, A., OLEFSKY, J.M., GOLDBERG, M. & HEIDENREICH, K.A. (1990). In vitro studies on the action of CS-045, a new antidiabetic agent. *Metabolism,* **39,** 1056 – 1062.

FERRANNINI, E., HAFFINER, S.M. & STERN, M.P. (1990). Hypertension: an insulin-resistance state. *J. Cardiovasc. Pharmacol.,* **15** (Suppl 5), S18 – S25.

FERRANNINI, E., SMITH, J.D., COBELLI, C., TOFFOLO, G., PILO, A. & DEFRONZO, R.A. (1985). Effect of insulin on the distribution and disposition of glucose in man. *J. Clin. Invest.,* **76,** 357 – 364.

FLEIG, W.E., NOETHER-FLEIG, G., FUSSGAENGER, R. & DITSCHNEIT, H. (1984). Modulation by insulin-dependent glycogenesis, but not of insulin binding, in cultured rat hepatocytes – evidence for postreceptor mechanism of action. *Diabetes,* **33,** 285 – 290.

FUJIWARA, T., YOSHIOKA, S., YOSHIOKA, T. & HORIOSHI, H. (1988). Characterization of new oral antidiabetic agent CS-045-study in KK and ob/ob mice and Zuker fatty rats. *Diabetes,* **37,** 1549 – 1558.

GIUGLIANO, D., QUATRARO, A., CONSOLI, G., MINEI, A., CERIELLO, A., DEROSA, N. & D'ONOFRIO, F. (1993). Metformin for obese, insulin-treated diabetic patients: improvement in glycaemic control and reduction of metabolic risk factors. *Eur. J. Clin. Pharmacol.,* **44,** 107 – 112.

HIRAGA, K. (1997). The clinical phase I study of AD-4833 – Single-dose study and repeated-dose study – (in Japanese). *Jpn. J. Clin. Exp. Med.,* **74,** 1184 – 1201.

HOFFMAN, C., LORENTZ, K. & COLA, J.R. (1991). Glucose transport deficiency in diabetic animals is corrected by treatment with the oral antihyperglycemic agent pioglitazone. *Endocrinology,* **129,** 1915 – 1925.

HORN, A. & MARTY, A. (1988). Muscarinic activation of ionic currents measured by a new whole-cell recording method. *J. Gen. Physiol.,* **92,** 145 – 159.

KAPLAN, N.M. (1992). Effects of antihypertensive therapy on insulin resistance. *Hypertension,* **19** (Suppl I), I 116 – I 118.

KAUFMAN, L.N., PETERSON, M.M. & DEGRANGE, L.M. (1995). Pioglitazone attenuates diet-induced hypertension in rats. *Metabolism,* **44,** 1105 – 1109.

KEMNITZ, J.W., ELSON, D.F., ROEKER, E.B., BAUM, S.T., BERGMAN, R.N. & MEGLASSON, M.D. (1994). Pioglitazone increases insulin sensitivity, reduces blood glucose, insulin, and lipid levels, and lowers blood pressure, in obsese, insulin-resistant rhesus monkeys. *Diabetes,* **43,** 204 – 211.

KOTCHEN, T.A. (1996). Attenuation of hypertension by insulin-sensitizing agents. *Hypertension,* **28,** 219 – 222.

LANDIN, K., TENGBORN, L. & SMITH, U. (1994). Metformin and metoprolol CR treatment in non-obese men. *J. Intern. Med.,* **235,** 335 – 341.

LANDSBERG, L. (1996). Insulin sensitivity in the pathogenesis of hypertension and hypertensive complication. *Clin. Exp. Hypertens.,* **18,** 337 – 146.

LEE, K.S. & TSIEN, R.W. (1983). Mechanism of calcium channel blockade by verapamil, D600, diltiazem and nitrendipine in single dialysed heart cells. *Nature,* **302,** 790 – 794.

LEE, M.K., MILES, P.D., KHOURSHEED, M., GAO, K.M., MOOSSA, A.R. & OLEFSKY, J.M. (1994). Metabolic effects of troglitazone on fructose-induced insulin resistance in the rat. *Diabetes,* **43,** 1435 – 1439.

OGIHARA, T., RAKUGI, H., IKEGAMI, H., MIKAMI, H. & MASUNO, K. (1995). Enhancement of insulin sensitivity by troglitazone lowers blood pressure in diabetic hypertensives. *Am. J. Hypertens.,* **8,** 316 – 320.

OHYA, Y., ABE, I., FUJII, K., TAKATA, Y. & FUJISHIMA, M. (1993). Voltage-dependent Ca^{2+} channels in resistance arteries from spontaneously hypertensive rats. *Circ. Res.,* **73,** 1090 – 1099.

OHYA, Y., ADACHI, N., ABE, I. & FUJISHIMA, M. (1997). Effects of CP-060S on membrane channels in vascular smooth muscle cells from guinea-pig. *Eur. J. Pharmacol,* (in press).

OHYA, Y. & SPERELAKIS, N. (1989). ATP regulation of the slow calcium channels in vascular smooth muscle cells of guinea pig mesenteric artery. *Circ. Res.,* **64,** 145 – 154.

PASSA, P. (1993). Insulin resistance and hypertension. *Clin. Exp. Hypertens.,* **15,** 1047 – 1059.

PERSHADSINGH, H.A., SZOLLOSI, J., BENSON, S., HYUN, W.C., FEUERSTEIN, B.G. & KURTZ, T.W. (1993). Effects of ciglitazone on blood pressure and intracellular calcium metabolism. *Hypertension,* **21,** 1020 – 1023.

SETOGUCHI, M., OHYA, Y., ABE, I. & FUJISHIMA, M. (1995). Inhibitory action of betaxolol, α β_1-selective adrenoceptor antagonist, on voltage-dependent calcium channels in guinea-pig artery and vein. *Br. J. Pharmacol.,* **115,** 198 – 202.

SHIBATA, H., NII, S., KOBAYASHI, M., IZUMI, T., SASAHARA, K. & YAMAGUCHI, K. (1993). Phase I study of a new hypoglycemic agent CS-045 in healthy volunteers — safety and pharmacokinetics in reported administration — (in Japanese). *Rinsho Iyaku,* **9,** 1519–1537.

TERADA, K., KITAMURA, K. & KURIYAMA, H. (1987). Blocking actions of Ca^{2+} antagonists on the Ca^{2+} channels in the smooth muscle cell membrane of rabbit small intestine. *Pflügers Arch.,* **408,** 552–557.

YOSHIOKA, S., NISHINO, H., SHIRAKI, T., IKEDA, K., KOIKE, H., OKUNO, A., WADA, M., FUJIWARA, T. & HORIKOSHI, H. (1993). Antihypertensive effects of CS-045 treatment in obese Zucker rats. *Metabolism,* **42,** 75–80.

ZHANG, F., SOWERS, J.R., RAM, J.L., STANDLEY, P.R. & PEULER, J.D. (1994). Effects of pioglitazone on calcium channels in vascular smooth muscle. *Hypertension,* **24,** 170–175.

ZHU, Z., TEPEL, M., NEUSSER, M., MHERING, N. & ZIDEK, W. (1993). Concentration-dependent effects of insulin on Ca^{2+} influx in vascular smooth muscle cells of normotensive and spontaneous hypertensive rats. *Clin. Sci.,* **85,** 425–429.

(Received August 11, 1997
Accepted November 11, 1997)