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Acute hemodynamic effects of insulin-sensitizing agents in isolated perfused rat hearts

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Abstract

Troglitazone has direct effects on the hemodynamics of the heart. We investigated the effects of other insulin-sensitizing agents (rosiglitazone, pioglitazone and JTT-501 (4-[4-[2-(5-methyl-2-phenyl-4-oxazolyl)ethoxy]benzyl]-3,5-isoxazolidinedione)) on the hemodynamics of the heart using isolated perfused rat hearts. Rosiglitazone significantly decreased heart rate and coronary perfusion pressure, and increased peak isovolumic left ventricular pressure, peak rate of rise of left ventricular pressure and peak rate of fall of left ventricular pressure. The effects of rosiglitazone, however, were milder than those of troglitazone. Neither pioglitazone nor JTT-501 had any effect on the heart. D-α-tocopherol, a structural component of troglitazone, did not exert any effect on the heart. The coronary vasorelaxant effect of troglitazone and rosiglitazone was significantly suppressed by indomethacin, but not by N^{ω}-nitro-L-arginine methyl ester. In conclusion, only rosiglitazone, as well as troglitazone, exerted positive inotropic, positive lusitropic, negative chronotropic, and coronary vasorelaxant effects on the heart. The coronary vasorelaxant effect of troglitazone and rosiglitazone was mediated by prostaglandin production.

Keywords: Thiazolidinedione; Hemodynamics; Heart; Coronary vasorelaxant; Prostaglandin

1. Introduction

Insulin resistance is a syndrome associated with a cluster of metabolic disorders including Type II diabetes mellitus, obesity, hypertension, lipid abnormalities, and atherosclerotic cardiovascular disease (Reaven, 1988; Defronzo and Ferrannini, 1991). In addition, hyperinsulinemia and insulin resistances have been shown to be independent risk factors for coronary heart disease (Després et al., 1996). Thus, it is imperative to seek an optimal treatment for patients with hyperinsulinemia and insulin resistance in order to prevent coronary heart disease.

Thiazolidinediones, which act on peripheral tissues as insulin sensitizers, have been developed as appropriate therapies for insulin resistance. Among thiazolidinediones, troglitazone is available for the treatment of Type II diabetes mellitus and potentially of other insulin-resistant states (Nolan et al., 1994). Testing in diabetic animal models (Fujiwara et al., 1988; Ciaraldi et al., 1990; Yoshioka et al., 1993) and human clinical trials (Iwamoto et al., 1991; Suter et al., 1992; Nolan et al., 1994) has shown that troglitazone reduces hyperglycemia, hyperinsulinemia as well as elevated levels of glycosylated hemoglobin A_{1c}, free fatty acids and triglycerides. Other thiazolidinedione insulin-sensitizing agents, such as rosiglitazone and pioglitazone, are now commercially available. JTT-501 (4-[4-[2-(5-methyl-2-phenyl-4-oxazolyl)ethoxy] benzyl]-3,5-isoxazolidinedione), an isoxazolidinedione derivative, is another novel insulin-sensitizing agent (Shibata et al., 1998) and is currently being evaluated for its efficacy in the treatment of Type II diabetes mellitus.

Recently, thiazolidinediones have been reported to exert hemodynamic as well as hypoglycemic effects. Troglitazone and pioglitazone have been shown to lower elevated arterial pressure in rat models of hypertension (Yoshioka et al., 1993; Kaufman et al., 1995) and in diabetic hypertensives (Ogihara et al., 1995). The mechanism of the direct vascular effect of troglitazone or pioglitazone may be in part due to inhibition of inward Ca^{2+} currents through L-type channels in vascular smooth muscle cells (Zhang et al., 1994). On the other hand, the vasodilator effect of troglitazone was abolished by indomethacin, but

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not by N^{ω}-nitro-L-arginine methyl ester (L-NAME), in human small arteries (Walker et al., 1998) and Wistar rat arteries (Song et al., 1997). Troglitazone increased prostaglandin I_2 production in isolated aortic rings, and increased prostaglandin I_2 and prostaglandin E_2 production in 3T6 fibroblasts (Fujiwara et al., 1998).

In a study on hemodynamics, a significant increase in stroke volume and cardiac output was observed in patients treated with troglitazone; its effects were considered to be a result of decreased peripheral resistance (Ghazzi et al., 1997). Recently, we used an isolated perfused heart model to demonstrate that troglitazone exerted direct positive inotropic, positive lusitropic, negative chronotropic and coronary vasodilator effects on the heart. The direct positive inotropic, positive lusitropic and negative chronotropic effects of troglitazone were not mediated by α- or β-adrenoceptors or by Ca^{2+} channels (Shimoyama et al., 1999). Based on these findings, it is possible that the improvement of hyperglycemia or hyperinsulinemia induced by thiazolidinediones is mediated via their direct cardiovascular effects, and such hemodynamic effects may be beneficial for Type II diabetic patients with coronary artery disease and heart failure.

The direct cardiovascular effects of other insulin-sensitizing agents remain unclear. Since there is growing interest in the potential use of insulin-sensitizers to prevent coronary heart diseases in patients with Type II diabetes mellitus and insulin-resistance, and coronary artery disease and heart failure are major causes of mortality in diabetic patients, it is clinically important to evaluate the direct effects of other insulin-sensitizing agents on cardiovascular hemodynamics and to clarify whether the cardiovascular effects of thiazolidinediones are involved in the improvement of insulin resistance. In the present study, we investigated the direct impact of various insulin-sensitizing agents (troglitazone, rosiglitazone, pioglitazone and JTT-501) on cardiac hemodynamics using isolated perfused rat hearts.

2. Methods

2.1. Isolated heart preparation

Male Wistar rats (300 to 350 g, 12 weeks old) were used for standard isolated isovolumic heart preparations. All procedures were carried out in strict accordance with Tottori University Guide for the Care and Use of Laboratory Animals. The procedure for creating isolated perfused rat hearts was similar to that previously described (Ogino et al., 1995). Briefly, the rat was heavily anesthetized with an i.p. injection of ketamine. HCl (40 mg) and xylazine (2.0 mg) were dissolved in heparin (1000 IU). After bilateral sternotomy, the heart was rapidly excised, and the aorta was cannulated for retrograde perfusion with a 14-gauge needle connected to a modified Langendorff perfusion system. The system consisted of a warmed storage vat for perfusate solutions, an adjustable-speed rotary pump (model 7518-10, 7520-35, Masterflex, Cole-Parmer Instrument, IL, USA), and a condenser. The vat and condenser were warmed to 37°C using a Constant Temperature Circulator (model T-80, Tokyo Rikakikai, Tokyo, Japan). After rapid cannulation of the aorta, the coronary perfusion pressure was held at 80 mmHg by maintaining a constant flow of modified Tyrode's solution (144 mmol/l Na^+, 5 mmol/l K^+, 1.5 mmol/l Ca^{2+}, 6 mmol/l HEPES, 0.9 mmol/l Mg^{2+}, 152 mmol/l Cl^+, and 5 mmol/l glucose). Lidocaine (5 μg/ml) was added to suppress ventricular ectopy. The pH was adjusted to 7.40. The solution was equilibrated with 100% oxygen before use and then oxygen was continuously bubbled through the perfusate during the experiment. The perfusate was not recirculated.

Ventricular function was assessed by measuring left ventricular pressure with a fluid-filled latex balloon inserted into the left ventricle through the mitral valve and held in place by a suture tied around the left atrium. The other end of the catheter was connected to a pressure transducer (model MP 5100, Baxter, Tokyo, Japan) for continuous measurement of left ventricular pressure. A second transducer connected to the perfusion line just before the heart was used to measure coronary perfusion pressure. Both transducers were connected to a Mac Lab (model 200, AD Instruments, NSW, Australia) to monitor and record heart rate, left ventricular pressure and coronary perfusion pressure. After the heart was attached to the Langendorff perfusion system, it was allowed to stabilize for at least 30 min, during which left ventricular pressure and coronary perfusion pressure were monitored. The intraventricular balloon was inflated to give an end-diastolic pressure of 5 mmHg, and the balloon volume was held constant thereafter. After the equilibration period, each drug (1.0 μmol) was administered as a bolus injection directly into the condenser of the perfusion system (which contained 20 ml of perfusate), to avoid changes in perfusion pressure, temperature and pH due to the injection.

2.2. Drugs

Troglitazone and rosiglitazone were kindly donated by Sankyo (Tokyo, Japan). Pioglitazone and JTT-501 were kindly donated by Takeda Chemical Industries (Osaka, Japan) and Japan Tabacco (Tokyo, Japan), respectively. Each drug was dissolved in 0.1 ml of distilled water containing 5% dimethyl sulfoxide, and 19% bovine serum albumin. As the control, 0.1 ml of the same medium but without a drug was used. The other drugs were purchased from Sigma (St. Louis, MO, USA).

2.3. Experimental protocol

The purpose of the present study was to elucidate whether other insulin-sensitizing agents (rosiglitazone, pioglitazone and JTT-501) exerted direct hemodynamic effects on the heart. We first investigated the acute effects of

Table 1
Baseline values of heart rate, coronary flow, coronary perfusion pressure, LVP_{max}, dP/dt_{max}, and dP/dt_{min}

	Vehicle ($n=4$)	Troglitazone ($n=4$)	Rosiglitazone ($n=4$)	Pioglitazone ($n=4$)	JTT-501 ($n=4$)	D-α-tocopherol ($n=4$)
Heart rate (bpm)	177.5 ± 4.6	190.7 ± 2.8	172.8 ± 4.8	181.0 ± 8.5	190.5 ± 6.9	168.8 ± 1.3
CF (ml/min)	12.6 ± 0.2	10.8 ± 0.4	11.8 ± 0.6	10.8 ± 0.8	11.4 ± 1.0	10.0 ± 0.6
CPP (mmHg)	80.3 ± 0.9	81.3 ± 0.8	79.3 ± 0.6	81.8 ± 0.7	81.0 ± 0.8	80.2 ± 0.4
LVP_{max} (mmHg)	113.6 ± 6.1	116.2 ± 11.3	111.4 ± 2.7	116.6 ± 11.9	128.1 ± 10.8	120.9 ± 6.2
dP/dt_{max} (mmHg)	2982 ± 93	2808 ± 254	2821 ± 114	3097 ± 424	3324 ± 199	2649 ± 122
dP/dt_{min} (mmHg)	−1778 ± 57	−1547 ± 110	−1582 ± 44	−1721 ± 199	−1777 ± 144	−1605 ± 79

CF: coronary perfusion flow, CPP: coronary perfusion pressure, LVP: left ventricular pressure.

each of these insulin-sensitizing agents on isolated perfused rat hearts ($n=4$, each). We administered 1.0 μmol of each drug and measured the impact on heart rate, coronary perfusion pressure, peak isovolumic left ventricular pressure (max left ventricular pressure), peak rate of rise of left ventricular pressure (dP/dt_{max}), and peak rate of fall of left ventricular pressure (dP/dt_{min}) after each injection. Next, to examine whether the chemical structure of vitamin E was involved in the hemodynamic effects of troglitazone, we assessed the effects of D-α-tocopherol (1.0 μmol) on isolated perfused rat hearts ($n=4$). Finally, to assess whether nitric oxide or prostaglandin was involved in the mechanism of coronary vasodilatation induced by these insulin-sensitizing agents, we examined the effect of L-NAME (300 μmol/l) and indomethacin (10 μmol/l). Each was added to the perfusate 20 min before each drug was administered and was present throughout the experiments ($n=4$, each drug). In every experiment, the impact of each agent on hemodynamic parameters was recorded at 1, 2, 3, 5, 10, 20 and 30 min after each injection. Changes in all parameters are expressed as percent changes from the baseline values.

2.4. Statistical analysis

All data are expressed as means ± S.E.M. Multiple comparisons among three or more groups were carried out

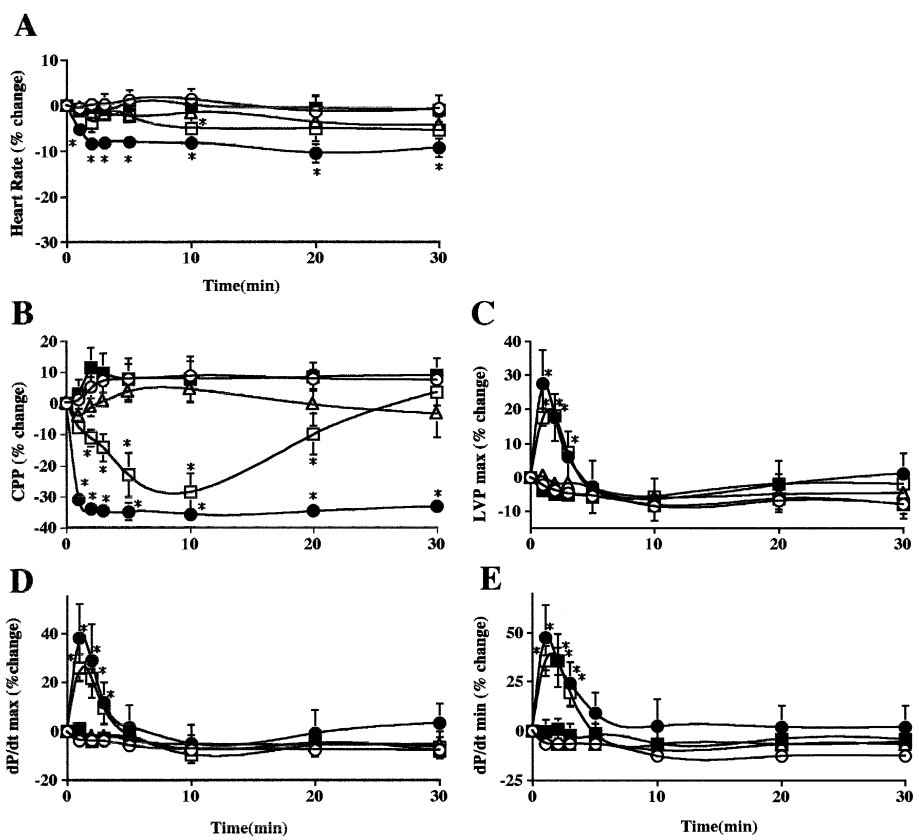

Fig. 1. Effects of troglitazone (●), rosiglitazone (□), pioglitazone (■) and JTT-501 (△) on (A) heart rate, (B) coronary perfusion pressure, (C) max left ventricular pressure, (D) dP/dt_{max} and (E) dP/dt_{min} under constant coronary flow ($n=4$ each). Results are expressed as percent changes from baseline values. $^{*}P<0.05$ vs. the vehicle (○) at each determination point.

by two-way analysis of variance and by Fisher's exact test for post hoc analysis. Differences with a P value < 0.05 were considered statistically significant.

3. Results

We examined the impact of each insulin-sensitizing agent on the heart. We found no statistical difference in the baseline values of heart rate, coronary flow, max left ventricular pressure, dP/dt_{max} or dP/dt_{min} among the groups (Table 1). Rosiglitazone had a significant impact on heart rate, coronary perfusion pressure, max left ventricular pressure, dP/dt_{max} or dP/dt_{min} (Fig. 1). Rosiglitazone decreased heart rate significantly, and a minimum was reached at 10 min ($-5.0 \pm 2.9\%$ from the baseline value) (Fig. 1A). Similarly, rosiglitazone decreased coronary perfusion pressure within 1 min, and minimum values were reached at 10 min after injection ($-28.4 \pm 5.8\%$ from the baseline value) (Fig. 1B). In contrast to the effect of troglitazone, coronary perfusion pressure gradually decreased and returned to baseline 30 min after injection. The coronary vasodilator effect (decrease in coronary perfusion pressure) of rosiglitazone was weaker than that of troglitazone ($P < 0.05$). Rosiglitazone increased the max left ventricular pressure and dP/dt_{max} within 1 min with a corresponding decrease in dP/dt_{min}. The maximum effects were seen at 1–2 min ($18.2 \pm 1.7\%$, $25.9 \pm 5.5\%$, and $-35.4 \pm 7.6\%$, respectively) and then values returned to baseline at 5 min (Fig. 1C,D,E). These actions were rapid and transient, similar to those of troglitazone. The inotropic (max left ventricular pressure and dP/dt_{max}) and lusitropic effects (dP/dt_{min}) of rosiglitazone were weaker than those of troglitazone ($P < 0.05$). Neither pioglitazone nor JTT-501 had any significant impact on heart rate, coronary perfusion pressure, max left ventricular pressure, dP/dt_{max} or dP/dt_{min} (Fig. 1).

To confirm whether higher doses of pioglitazone and JTT-501 had hemodynamic effects, we examined the effects of 5.0 μmol of pioglitazone and JTT-501. While both pioglitazone and JTT-501 slightly decreased coronary perfusion pressure ($-6.7 \pm 2.3\%$ and $-11.1 \pm 0.6\%$, respectively, $n = 3$), neither pioglitazone nor JTT-501 showed an inotropic effect.

To clarify whether vitamin E was involved in the effects of troglitazone, we perfused isolated rat hearts with D-α-tocopherol. D-α-tocopherol did not exert direct effect on heart rate, coronary perfusion pressure, max left ventricular pressure, dP/dt_{max} or dP/dt_{min} in isolated perfused hearts (Fig. 2).

To evaluate the involvement of nitric oxide or prostaglandin production on the coronary vasodilator effects of

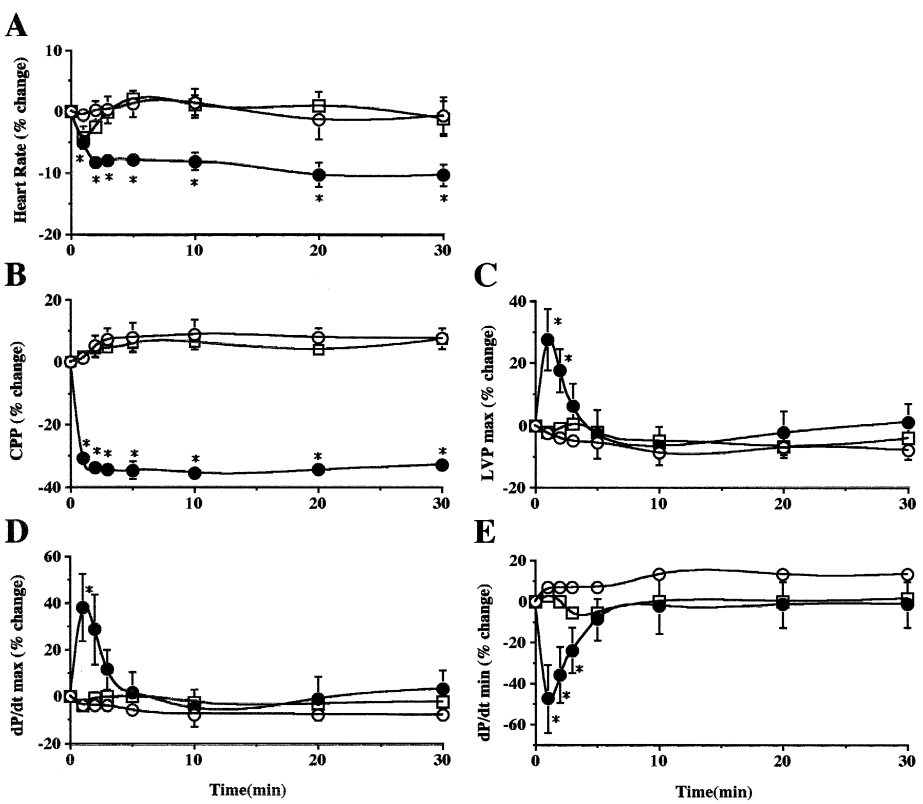

Fig. 2. Effects of D-α-tocopherol on (A) heart rate, (B) coronary perfusion pressure, (C) max left ventricular pressure, (D) dP/dt_{max}, and (E) dP/dt_{min} ($n = 4$ each). Results are expressed as percent changes from baseline values. vehicle (○), troglitazone (●), vitamin E (□) $^{*}P < 0.05$ vs the vehicle at each determination point.

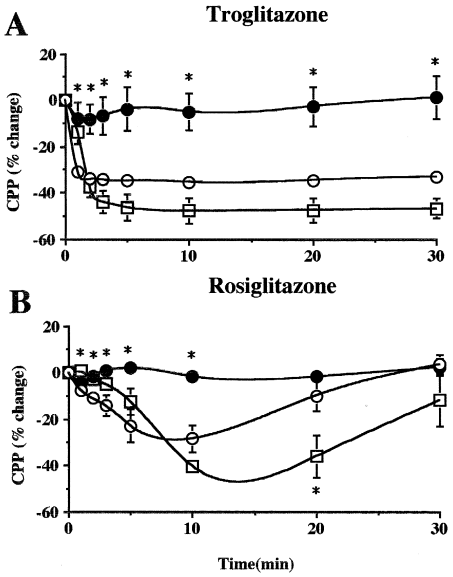

Fig. 3. Effects of (A) troglitazone and (B) rosiglitazone on coronary perfusion pressure ($n = 4$ each) in the presence of indomethacin (●), L-NAME (□) or vehicle (○). Results are expressed as percent changes from baseline values. $P < 0.05$ vs the vehicle at each determination point.

troglitazone and rosiglitazone, we examined the effect of L-NAME and indomethacin on that of troglitazone and rosiglitazone on coronary perfusion pressure. There were significant differences from baseline values of coronary flow in the L-NAME and indomethacin groups. Since L-NAME induced coronary vasoconstriction, the coronary flow of the L-NAME group (5.5 ± 0.7 ml/min) was significantly lower than that in the control group (11.1 ± 0.5 ml/min) and the indomethacin group (13.1 ± 1.0 ml/min). Thus, the rightward shifts of the response curves for coronary perfusion pressure in response to troglitazone and rosiglitazone were due to the low coronary flow. In isolated hearts perfused with indomethacin, the coronary vasodilator effects of troglitazone and rosiglitazone were significantly suppressed (85.6% and 94.0% at 10 min after injection, respectively, Fig. 3). In contrast, L-NAME did not inhibit the coronary vasodilatory effects of troglitazone and rosiglitazone (Fig. 3).

4. Discussion

In this study, we demonstrated that only rosiglitazone, and not pioglitazone or JTT-501, exerted acute effects, similar to those observed with troglitazone, on isolated, perfused hearts. This finding indicates that not all insulin-sensitizing agents, either thiazolidinediones or isoxazolidinediones, exert acute effects on the heart. In addition, we showed that D-α-tocopherol, which is contained in the chemical structure of troglitazone, did not have any impact on the heart. Troglitazone, a thiazolidinedione, contains the vitamin E structure; rosiglitazone and pioglitazone, both thiazolidinediones, do not contain the vitamin E structure. Still, rosiglitazone exerted acute effects on hemodynamic parameters similar to those of troglitazone. These results suggest that the acute effects of troglitazone are not mediated by the vitamin E structure in its molecule.

Branched chains of thiazolidinedione, other than the vitamin E structure, might play a pivotal role in the direct effects of these agents. Thiazolidinediones and an isoxazolidinedione are reported to bind to and activate the peroxisome proliferator-activated receptor-γ (PPAR-γ), the molecular target now widely believed to be central to the insulin-sensitizing effect of thiazolidinediones and isoxazolidinedione. Since not all insulin-sensitizing agents exert acute effects on the heart, the activation of PPAR-γ seems not to be involved in the acute effects of troglitazone and rosiglitazone.

In the present study, pretreatment with indomethacin, but not with L-NAME, abolished the coronary vasodilator effect of troglitazone and rosiglitazone. This finding supports the idea that the coronary vasodilator effects of troglitazone and rosiglitazone are mediated by the prostaglandin pathway, not by nitric oxide. Troglitazone increased prostaglandin I_2 production in isolated aortic rings, as well as that of prostaglandin I_2 and prostaglandin E_2 by 3T6 fibroblasts (Fujiwara et al., 1998). The vasodilator effect of troglitazone on rat tail arteries has been reported to be independent of nitric oxide generation (Song et al., 1997) and is abolished by indomethacin in human small arteries (Walker et al., 1998). We also measured 6-keto prostaglandin $F_{1\alpha}$ (the stable hydrolysis product of prostacyclin) in recirculated perfusate (data not shown). Prostaglandin $F_{1\alpha}$ levels increased gradually after injection of troglitazone and rosiglitazone; however the increase in prostaglandin $F_{1\alpha}$ levels was faster and higher after troglitazone than that after rosiglitazone ($91.2 \pm 14.3\%$ and $39.8 \pm 5.8\%$ at 10 min after injection, respectively, $n = 2$). This may explain the different time course of the change in coronary perfusion pressure after troglitazone and rosiglitazone. Thus, the results of our present study support the hypothesis of prostaglandin involvement in the acute coronary vasorelaxant effect of troglitazone and rosiglitazone.

Long-term oral administration of insulin-sensitizing agents, including pioglitazone, lowers blood pressure (Yoshioka et al., 1993; Kemnitz et al., 1994; Nolan et al., 1994). The chronic vasorelaxant effect of these agents is due in part to inhibition of the inward Ca^{2+} current through L-type channels in vascular smooth muscle cells (Zhang et al., 1994; Song et al., 1997; Kawasaki et al., 1998). These findings, including our results, suggest the mediation of the acute vasorelaxant effect of insulin-sensitizing agents via the production of prostaglandin, while the chronic vasorelaxant effect is mediated via the inhibition of inward Ca^{2+} currents. Furthermore, while troglitazone rapidly decreased coronary perfusion pressure and this effect persisted throughout the experiment, coronary perfusion pressure gradually decreased in the presence of

rosiglitazone and returned to baseline values. This chronological discrepancy of the appearance of coronary vasodilator effects between troglitazone and rosiglitazone seems to indicate the existence of two or more mechanisms. To elucidate the exact hemodynamic mechanism of insulin-sensitizing drugs is a key area for future investigations.

The present study showed that not all insulin-sensitizing agents exert acute effects on the heart. The effects of these agents on hemodynamic parameters are probably unrelated to their hypoglycemic effects. However, these insulin-sensitizing agents have been reported to lower blood pressure, and thiazolidinediones have been considered to improve insulin resistance, at least partially, by increasing blood flow (Yoshioka et al., 1993; Kemnitz et al., 1994; Nolan et al., 1994; Buchanan et al., 1995). Vasoconstriction may result in a decreased blood supply in insulin-sensitive skeletal muscle and in a further increase in insulin resistance. The ability of vasodilators to increase insulin sensitivity may be an indirect consequence of drug-induced vasodilatation and hence of an increased blood supply in insulin-sensitive tissues (Laakso et al., 1990; Buchanan et al., 1993). Thus, diverse antihypertensive-vasodilator agents, such as angiotensin-converting enzyme inhibitors (Pollare et al., 1989; Herings et al., 1995) and α-adrenoceptor blockers (Suzuki et al., 1992; Tomiyama et al., 1994), also improve insulin sensitivity. The mechanisms by which thiazolidinediones or isoxazolidinedione improve insulin resistance remain unclear.

We have to comment on the limitations of the study. First, since levels of, and sensitivity to, prostaglandin and nitric oxide are markedly altered in diabetes, it is possible that the effects of these insulin-sensitizing agents are different in the clinical situation. We observed that the coronary vasorelaxant effect of troglitazone was attenuated in streptozotocin-induced diabetic rats (unpublished data). Thus, the coronary endothelial dysfunction in diabetes might be responsible for the attenuation of the effect. It will be necessary to clarify the cardiac effects of these agents in diabetic states. Secondly, the acute effects of drugs seen in this study may not be identical to the effects seen after chronic administration of drugs. Previous studies have shown that blood pressure was decreased after long-term treatment with troglitazone (Ogihara et al., 1995; Saku et al., 1997; Sung et al., 1999). Thus, the vasodilator effects of these agents observed in this study might continue in the chronic situation.

We previously demonstrated that troglitazone exerted a strong coronary vasorelaxant effect and an inotropic effect on the heart without increasing heart rate. In the present study, we showed that rosiglitazone as well as troglitazone, but not pioglitazone or JTT-501, exerted these unique cardiovascular effects and that the coronary vasorelaxant effects were mediated by prostaglandin production. Since diabetes mellitus and insulin resistance are independent major risk factors of coronary heart disease, these unique cardiovascular effects of troglitazone or rosiglitazone would be beneficial for diabetic patients with coronary heart disease or hypertension. Further studies are necessary to clarify the effects of these agents on hemodynamic parameters in the diabetic state.

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