A lack of synergistic interaction between insulin and pioglitazone on reactivity of rat aorta from chronically high dose insulin-treated diabetic rats

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Abstract. Our goal was to determine whether hyperinsulinaemic and diabetic state can affect vasodepressor effects of insulin and pioglitazone (PIO), an insulin-sensitizing thiazolidinedione drug. For this purpose, we established an experimental type 2 diabetic model (streptozotocin-nicotinamide model) in adult male rats (DIA group) and some of them were treated with chronically high-dose insulin for 14 weeks (INS-T DIA group). Blood pressure, glucose, HbA_{1C}, triglyceride, cholesterol, plasma insulin levels and body weight were measured. Endothelium-denuded aortic rings were suspended in tissue baths for reactivity studies. Cumulative concentration-response curves of serotonin (5-hydroxytryptamine; 5-HT) were evaluated before and after 1 h incubations with insulin (10^{-7} or 10^{-4} U/l), or PIO (10 μmol/l) or insulin plus PIO. PIO or higher concentration of insulin (10^{-4} U/l), each alone, attenuated 5-HT induced contractions in both groups of aortae. Vasodepressor effect of insulin was diminished by 12% ± 4% in aortae from INS-T DIA group. The presence of PIO in the bath did not affect impaired vasodepressor response of insulin. Contractions induced by KCl, or Bay K 8644 were partly inhibited after PIO incubations, with similar E_{max} and pD_2 values in both groups of aortae. The results indicate that PIO does not modulate directly vasodepressor effect of insulin in hyperinsulinaemic/diabetic state. But, the direct vasodepressor effect of PIO, partly by Ca^{2+} channel inhibition, may be beneficial by improving insulin utilization due to increasing blood flow to the insulin-sensitive tissues in hyperinsulinaemic/diabetic state.

Key words: Insulin — Pioglitazone — Rat aorta — STZ-nicotinamide diabetes model

Introduction

The thiazolidindiones (TZDs), peroxisome proliferator activated receptors gamma agonists, represent a new class of oral agents for treatment of type 2 diabetes that improve glycaemic control by increasing insulin sensitivity in target tissues (Bailey and Day 2001; Vasudevan and Balasubramanyam 2004). TZDs have some direct vascular effects independent from their insulin-sensitizing effects. For example, pioglitazone (PIO) causes acute dilation of peripheral blood vessels by blocking Ca^{2+} channels, and this effect may contribute in part to their antihypertensive actions in type 2 diabetes. (Zhang et al. 1994; Buchanan et al. 1995; Kotchen et al. 1996; Majithiya et al. 2005). On the other hand, besides its key role in the regulation of carbohydrate metabolism, insulin has important cardiovascular effects including vasodilator action (Baron 1994; McNally et al. 1995; Ozturk et al. 1996). Insulin depresses pressor responses of vasoconstrictor agents *in vivo* and *in vitro* (Alexander and Oake 1987; Yagi et al. 1988). It has been shown that systemic hyperinsulinemia induces vasodilation (Anderson and Mark 1993; Tack et al. 1996) and this further enhances the delivery of glucose to insulin sensitive tissues, thus supporting glucose uptake (Baron 1996). In states of insulin resistance, insulin-mediated vasodilation is blunted (Laakso et al. 1990, 1992; Verma et al. 1997; Walker et al. 1997). Insulin resistance at the vascular level has proposed as an attractive hypothesis that hypertension in insulin-resistant states may be partly the result of an inability of the direct vasodilator effect of insulin (Baron 1993; Passa

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1993; Bhanot and McNeill 1996). However, little is known on the effect of chronically high-dose insulin treatment on vascular smooth muscle reactivity. So far, most studies are focused on the mechanisms of attenuated endothelium-dependent relaxations in diabetic state (Pieper 1999). In the present study, we evaluated whether the direct vasodepressor effect of insulin, independent from nitric oxide production, is decreased in hyperinsulinaemic/diabetic state and we tested the hypothesis that PIO may acutly augment the vasodepressor effect of insulin, hence it may increase insulin utilization directly. Previously we have observed that there was no synergic interaction between troglitazone and insulin in aortae from nondiabetic rats (Sahilli et al. 1999).

Materials and Methods

Materials

PIO was kindly supplied from Takeda Chem. Ind., Ltd. (Osaka, Japan), streptozotocin (STZ), nicotinamide, phenylephrine, acetylcholine, serotonin (5-hydroxytryptamine; 5-HT), Bay K 8644, sodium nitroprusside and dimethyl sulphoxide (DMSO) were obtained from Sigma Chemical Comp., Inc. (St. Louis, MO, U.S.A), KCl was obtained from Baker (U.S.A.) and insulin (Humulin N) was obtained from Eli Lilly (U.S.A). PIO was dissolved in DMSO.

Animals and treatment

Adult male Wistar rats (200–230 g) were assigned randomly to be made diabetic (DIA group), insulin-treated (hyperinsulinaemic) diabetic group (INS-T DIA group) and age-matched controls. Diabetes was established according to the method described by Masiello et al. (1998) that appears to be closer to type 2 diabetes mellitus than other available animal models. It was induced by a single i.p. injections of STZ (65 mg/kg body weight) and nicotinamide (230 mg/kg body weight). Nicotinamide was given 20 min before STZ injection. STZ was dissolved in 0.1 mmol/l citrate buffer (pH 4.5) and nicotinamide was dissolved in saline immediately before injections. Age-matched control rats were given vehicles. 10 days after injections, animals with nonfasting blood glucose levels >160 mg/dl (Accucheck-go, Roche Diagnostics) were considered diabetic and were included in the study. Blood was sampled from the tail vein. Because the rats were not hyperinsulinaemic in this model and since hyperinsulinaemia is an important feature of clinical type 2 diabetes, some of them were treated with high-dose insulin (gradually increasing dose: 10–20 IU/kg/day, s.c.) for 14 weeks starting at 2nd week of diabetes. The initial dose of insulin was 10 IU/kg/day. It was given for the remainder of the study with adjustments in dosing as needed to keep glucose levels within the critical range. The rats gradually required high insulin doses than initial dosing. Food and water were given *ad libitum*. The study was approved by local Ethic Comitee.

Measurement of blood parameters

16 weeks after the STZ-nicotinamide or vehicle injections, rats were anesthetized with pentobarbital sodium (60 mg/kg body weight, i.p.). Prior to sacrifice, blood glucose (AccuCheck-go, Roche Diagnostics), cholesterol, triglyceride (Accutrend GCT meter, Roche Diagnostics), and HbA_{1C} levels (DCA 100+, Bayer Corp.) were measured. Insulin levels were measured in plasma samples (stored at –70°C) by immunoradioassay.

Measurement of blood pressure

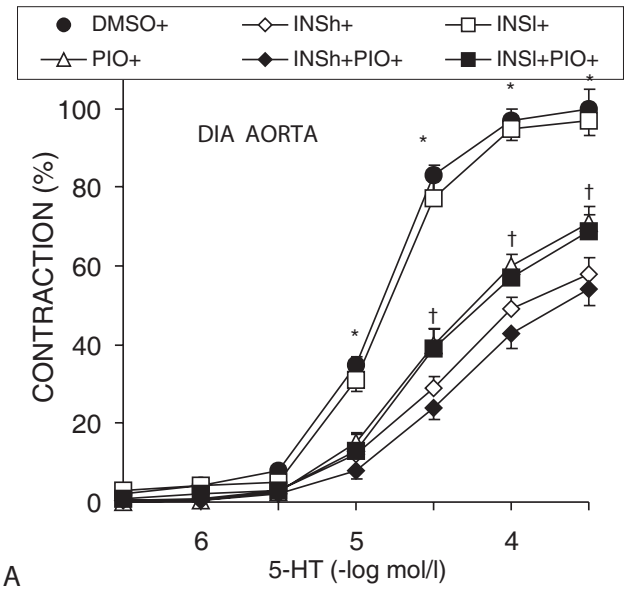
Systolic blood pressure was measured non invasively, by tail-cuff method, using pressure meter (Commat Ltd., Turkey). The tail of rats were pre-warmed to 40°C for 5 min. The tail-cuff and piezoelectric pulse sensor were placed at the base of the tail and were connected to a fully automatic blood pressure analyzer for each rat, 10 individual readings were recorded, and the average of the remaining 8 readings was taken as an individual systolic blood pressure.

Preparation of aortic tissues

Thoracic aortae were removed and placed in standart bicarbonate-buffered physiological salt solution with the composition (in mmol/l): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4{\cdot}7H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11.1 (pH 7.4), and they were cleaned of adherent connective tissues. The endothelium was denuded by a gently rubbing the intimal surface of the vessel with curved forceps, cut into rings 3–4 mm in length with following suspension in 20-ml isolated tissue baths maintained at 37°C and aerated with 5% CO_2 in O_2. Resting tension was maintained at 2 g. As a routine, the rings were allowed to equilibrate for 1 h before the start of the experiments. During this period, they were washed in every 15 min. Isometric tensions were recorded on a computer assisted data acquisition system with force-displacement transducers (Commat Ltd., Turkey).

Experimental protocol in aortae

After equilibration period, each vessel was contracted twice with KCl (60 mmol/l) to assess their viability. After 20 min, the vessels were stimulated by the sub-maximal concentration of phenylephrine (3×10^{-6} mol/l) and checked for the absence of endothelium by confirming no more than 10% relaxation to acetylcholine (10^{-5} mol/l). Then the tissues were



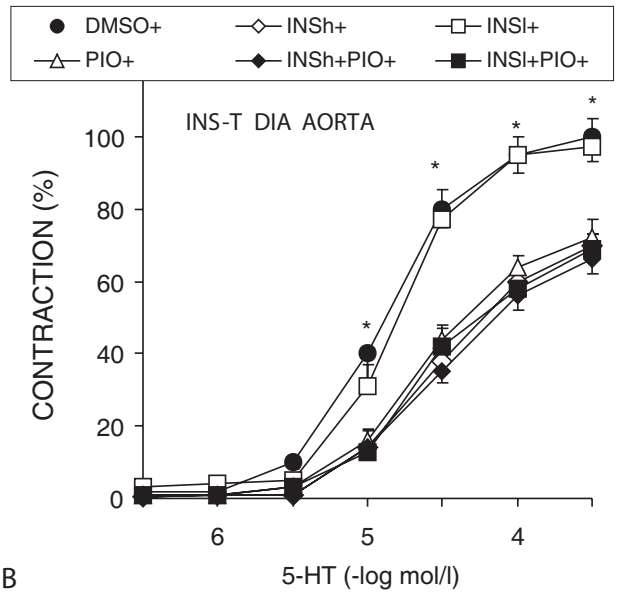


Figure 1. 5-HT concentration-response curves, after 1-h incubations, in diabetic (DIA) (A) and insulin-treated (hyperinsulinaemic) diabetic (INS-T DIA) aortae (B). * significance with others except 10^{-7} U/1 insulin incubation; † significance to INS (10^{-4} U/1) + PIO incubation, $p < 0.05$, $n = 5–7$. DMSO, vehicle; INSh (high)+, 10^{-4} U/1 insulin incubation; INSl (low)+, 10^{-7} U/l insulin incubation; PIO, 10 μmol/l pioglitazone incubation.

serially washed and were rested for 30 min. The following protocol for incubations was applied in separate rings: i) a cumulative concentration-response curve to 5-HT in the absence or presence of insulin (physiologic concentration 10^{-7} U/l or suprapyhsiologic concentrations 10^{-4} U/l); ii) a cumulative concentrations-response curve to 5-HT in the absence or presence of PIO (10 μmol/l); iii) a cumulative concentration-response curve to 5-HT in the absence or presence of insulin (10^{-7} or 10^{-4} U/l) plus PIO (10 μmol/l); iv) a cumulative concentration-response curves to KCl or Bay K 8644 in the absence or presence of PIO (10 μmol/l). Incubation periods with insulin or PIO were 1 h in each protocol. Incubations without drugs were evaluated with DMSO as a vehicle. Maximum concentration of DMSO were ≤0.1% (v/v) in the tissue bath and had no significant effect on the reactivity of the aortae during 1-h incubation periods.

In some aortae, concentration-response curves of sodium nitroprusside, an endothelium-independent relaxant agent, were evaluated in rings pre-contracted with phenylephrine (3×10^{-6} mol/l).

Data analyses and statistics

Contractile responses to agonists before and after incubations were expressed as a percentage of maximum contractions of agonists (% E_{max}). Agonist pD_2 values (apparent agonist affinity constant: –log EC_{50} – the negative log of the molar concentration of the drug giving 50% of the maximal response) were calculated by linear regression analysis of the concentration-response curves and taken as a measure of the sensitivity of the aortae to the agonists. Results are expressed as means ± SE; *n* represents the number of rings. Differences between pre- and post-incubation data were analysed by the paired Student's *t*-test. Group differences were tested by ANOVA followed by Newman–Keul's test. A level of probability less than 0.05 was regarded as significant.

Results

Characteristics of animals

Diabetic rats showed moderate hyperglycaemia. Insulin levels were not significantly different from their age-matched controls. Exogenous insulin treatment caused a dramatic rise in the insulin levels with critical low levels of glucose. At the end of the treatment, systolic blood pressure values increased significantly in INS-T DIA group.

Table 1 summarizes the characteristics of the animals at the end of the study.

In vitro experiments

Incubations with physiologic concentration of insulin (10^{-7} U/l) alone did not exert any inhibitory effect on the contractility to 5-HT, but supraphysiologic concentration (10^{-4} U/l) depressed the response to 5-HT significantly

in aortae from DIA and INS-T DIA groups. E_{max} of 5-HT was significantly diminished by 42% ± 4% in aortae from DIA group and 30% ± 3% in aortae from INS-T DIA group (n = 5–7, each) (Fig. 1 and Table 2). Vasodepressor effect of insulin was attenuated significantly by 12% ± 4% in aortae from INS-T DIA group when compared to DIA group, $p < 0.05$ (Fig. 2, Table 2). PIO (10 μmol/l) alone, depressed the responses to 5-HT significantly in both groups of aortae. After PIO incubations, E_{max} of 5-HT was depressed by 33% ± 4% in aortae from DIA group and 43% ± 6% in aortae from INS-T DIA group. There was no significant difference between the groups regarding the vasodepressive effect of PIO (Fig. 1 and Table 2).Vasodepressor effect of insulin did not change significantly when used in combination with PIO in both groups of aortae (Fig. 1 and Table 2). PIO (10 μmol/l) alone, also depressed the contractile responses to either KCl or Bay K 8644 (Ca^{2+} channel opener) after incubations. The drug depressed E_{max} of KCl and Bay K 8644 by 20% ± 7 % and 69% ± 10% respectively in aortae from DIA group and there were no significant differences on the vasodepressor effect of PIO on the contractile agents when compared to aortae from INS-T DIA group. (Fig. 3, Table 3). SNP responses were similar in both groups of aortae (Fig. 4).

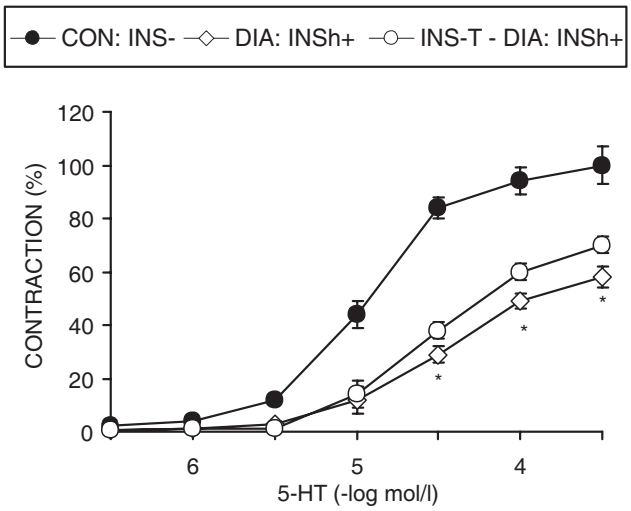


Figure 2. Vasodepressor effect of insulin (high: 10^{-4} U/1), after 1-h incubations, on 5-HT –induced contractions in aortic groups. * significance with other groups, $p < 0.05$, n = 5–7; CON: INS–, control age-matched group and no insulin incubation; DIA: INSh+, diabetic group with insulin incubation; INS-T DIA: INSh+, insulin-treated (hyperinsulinaemic) diabetic group with insulin incubation.

Discussion

In this study, we established an experimental type 2 diabetic model as described by Masiello et al. (1998). It has been reported that this experimental model is closer to human type 2 diabetes than other commonly used animal models. In this model, nicotinamide partly protects the pancreatic cells from cytotoxic effect of STZ, and reduced pancreatic insulin stores could maintain to some extent responsiveness to glucose and sulphonylurea, but there is no hyperinsulinaemia. We treated diabetic animals chronically with high dose exogenous insulin to produce hyperinsulinaemic state. We observed that vasodepressor effect of insulin is blunted by 12% ± 4% in aortae from insulin-treated rats. Kobayashi

Table 1. Some characteristics of animals

	CON	DIA	INS-TDIA
	n = 10	n = 10	n = 6
Body weight (g)	280 ± 5	274 ± 6	320 ± 9*
Blood glucose (mg/dl)	109 ± 7	202 ± 9*	75 ± 5*
Plasma insulin (μU/ml)	35.1 ± 5.3	30.3 ± 5.1	125 ± 11*
HbA1C (%)	4.1 ± 0.05	6.0 ± 0.2*	4.9 ± 0.4
Blood triglyceride (mg/dl)	88 ± 6	92 ± 6	90 ± 5
Blood cholesterol (mg/dl)	69 ± 6	80 ± 7	72 ± 5
Syst. blood pressure (mm Hg)	125 ± 8	128 ± 8	159 ± 10*

CON, age-matched control group; DIA, diabetic group; INS-T DIA, insulin-treated (hyperinsulinaemic) diabetic group; * significance to other groups, $p < 0.05$.

Table 2. pD2 and % E_{max} values for 5-hydroxytryptamine (5-HT) after INS or PIO incubations

	Incubation	pD_2	% E_{max}
DIA	DMSO (con)	4.94 ± 0.05*	103 ± 5*
	INS (10^{-7} U/l)	4.95 ± 0.06	97 ± 8
	INS (10^{-4} U/l)	4.45 ± 0.06	58 ± 3†
	PIO (10 μmol/l)	4.50 ± 0.04	70 ± 4
	INS (10^{-7} U/l) + PIO	4.48 ± 0.07	69 ± 4
	INS (10^{-4} U/l) + PIO	4.35 ± 0.05	54 ± 4
INS-T DIA	DMSO (con)	4.84 ± 0.05*	105 ± 7*
	INS (10^{-7} U/l)	4.87 ± 0.06	96 ± 5
	INS (10^{-4} U/1)	4.40 ± 0.05	70 ± 4
	PIO (10 μmol/l)	4.48 ± 0.05	62 ± 8
	INS (10^{-7} U/l) + PIO	4.42 ± 0.07	69 ± 4
	INS (10^{-4} U/1) + PIO	4.39 ± 0.06	65 ± 4

pD_2, –log EC_{50} (mol/l); % E_{max}, percentage of maximum contraction of agonist; DIA, diabetic group; DMSO, dimethyl sulphoxide (vehicle); con, incubation with vehicle; INS, insulin; PIO, pioglitazone; INS-T DIA, INS-treated diabetic group; * significance to other incubations except to INS (10^{-7} U/l) incubation; † significance to INS (10^{-4} U/1) + PIO incubations, $p < 0.05$, n = 5–7.

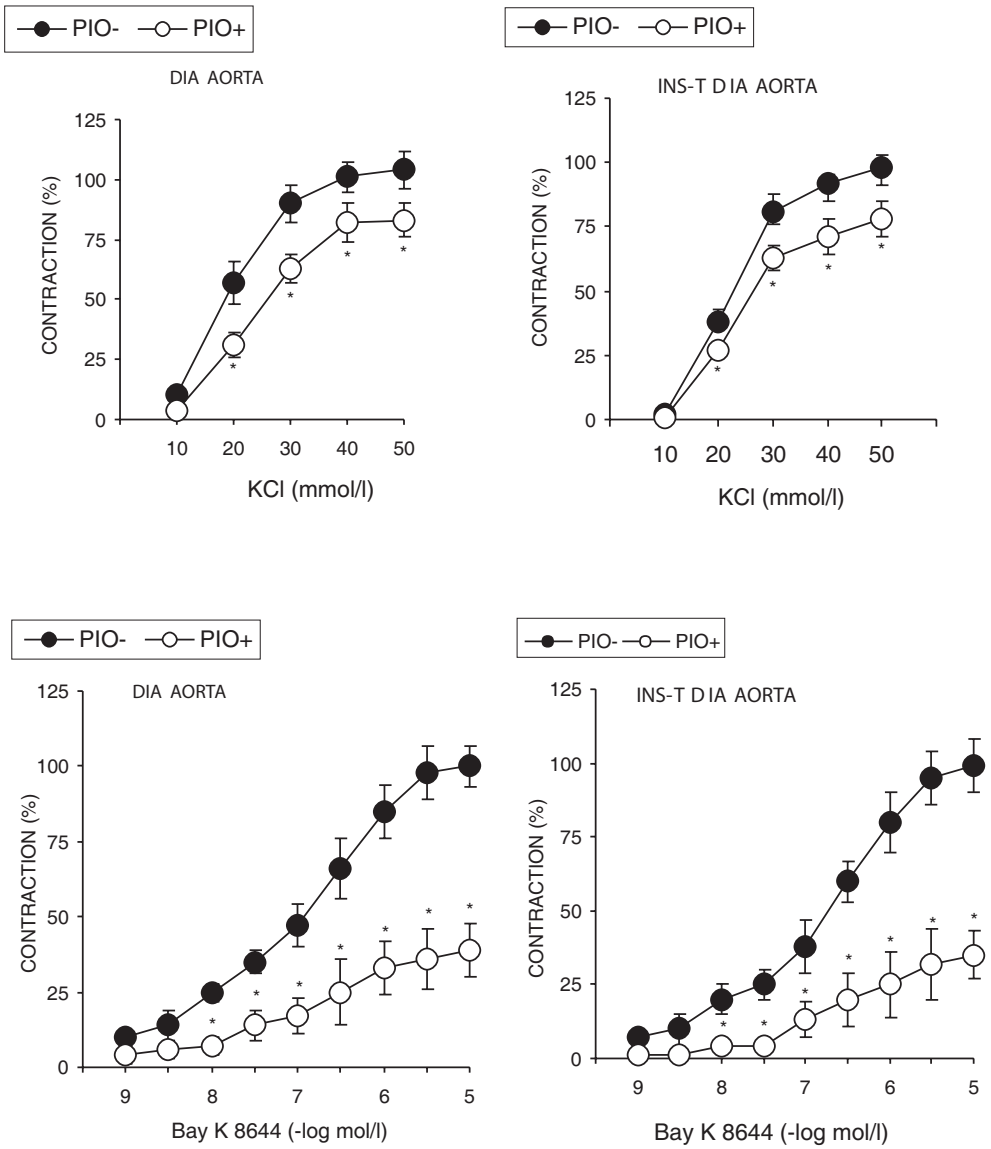


Figure 3. Vasodepressor effect of pioglitazone (PIO – 10 μmol/l), after 1-h incubations, on KCl- and Bay K 8644-induced contractions in aortae from diabetic (DIA) and insulin-treated (hyperinsulinaemic) diabetic (INS-T DIA) groups. * significance, $p < 0.05$, $n = 5$ each.

and Kamata (1999) reported that treatment of STZ-induced type 1 diabetic rats with high dose insulin for 4 weeks leads to insulin resistance. They also reported that the raised in blood pressure in STZ-diabetic rats treated with high insulin are secondary to the insulin resistance. Our results also support this phenomena. On the other hand, although the direct effects of PIO on vasculature have been studied so far, there is no data on the direct-acute effect of PIO on vascular reactivity in hyperinsulinaemic/diabetic state. In a recent study by Majithiya et al. (2005), it has been reported that PIO at higher concentrations (>10 μmol/l) depressed phenylephrine-induced contractions in endothelium-free aorta from STZ-diabetic rats. In the present study, we found that vasodepressor effect of the drug (10 μmol/l) did not differ in hyperinsulinaemic aorta. We also observed that there is no acute synergistic action between insulin and PIO on the reactivity of both diabetic and hyperinsulinaemic aorta in the concentrations used in this study. It is well known that insulin reduces the vasoconstrictor responses to various vasoactive substances such as noradrenaline (Zemel et al. 1991), angiotensin II (Verma et al. 1997), vasopressin (Standley et al. 1991), 5-HT (Kahn et al. 1993) and atrial vasoactive peptide (Wu et al. 1994) on vascular smooth muscle. Endothelium-dependent relaxant effect of insulin has also

been reported in a number of studies (Anderson and Mark 1993; Steinberg et al. 1994; Wu et al. 1994; Scherrer et al. 1995). An exact mechanism(s) of nitric oxide-independent (direct) vasodepressor effect of insulin on vasculature is not yet clear. Several reports suggest that insulin alters vascular tone *via* direct effects on intracellular Ca^{2+} concentrations in vascular smooth muscle cells (Standley et al. 1991, 1993; Kahn et al. 1993; Ram et al. 1993). Kahn et al. (1993) has reported that insulin attenuates 5HT-induced contractions on vascular smooth muscle cells by decreasing Ca^{2+} influx *via* voltage-operated channels. On the other hand, troglitazone and PIO directly inhibit the voltage-dependent Ca^{2+} channels in arterial cells (Song et al. 1997; Kawasaki et al. 1998; Nakamura et al. 1998.) and it is reported that inhibitory action of troglitazone on Ca^{2+} currents was not affected by 10 μmol/l insulin after 10 min incubation period, on guinea pig endothelium denuded mesenteric artery (Nakamura et al. 1998). The mechanism(s) of the inhibitory effect of TZDs derivatives on Ca^{2+} channels is not well known yet. It has been suggested that their mode of action differs from that of known organic Ca^{2+} channel antagonists in mesenteric arteries of guinea pig (Nakamura et al. 1998). On the other hand, it was reported that 1 h PIO incubation augments the vasodepressor effect of insulin in aorta to norepinephrine, but not to angiotensin (Kotchen et al. 1996). More recently, troglitazone has been shown to act synergistically with insulin in 1-h incubation period in endothelium-denuded rat aortic rings. In the mentioned study, although low concentration of insulin did not affect phenylephrine and KCl contractions, insulin plus troglitazone attenuated dramaticaly the maximum contractions to these agents. Additionally, EC_{50} values for phenylephrine and KCl were further increased after insulin plus PIO incubations (Goud et al. 1998). Goud and co-workers explained well this synergism between insulin and troglitazone by measuring intracellular Ca^{2+} concentrations thus they concluded that troglitazone inhibits Ca^{2+} influx. This is also supported by abolishment of contractions of Bay K 8644, a voltage-operated Ca^{2+} channel agonist, by troglitazone. Interestingly, in the last two *in vitro* studies, mentioned above, on rat aorta, insulin concentrations were choosen as nondepressive concentrations. In our study, besides low concentration, we tested vasodepressive concentration of insulin (high concentration) (Verma et al. 1997; Walker et al. 1997). Combined incubations of PIO with two different concentrations of insulin did not exert further depression on 5-HT-induced contractions in our study, as the same phenomena was observed by the others to angiotensin by PIO: while PIO plus insulin did not affect angiotensin responses, the drugs depressed phenylephrine contractions. The discrepencies of the results raise a question that whether interaction can be different depending on a vasoactive agent used. All of these agents use both receptor and voltage operated channels for Ca^{2+} mobilization,

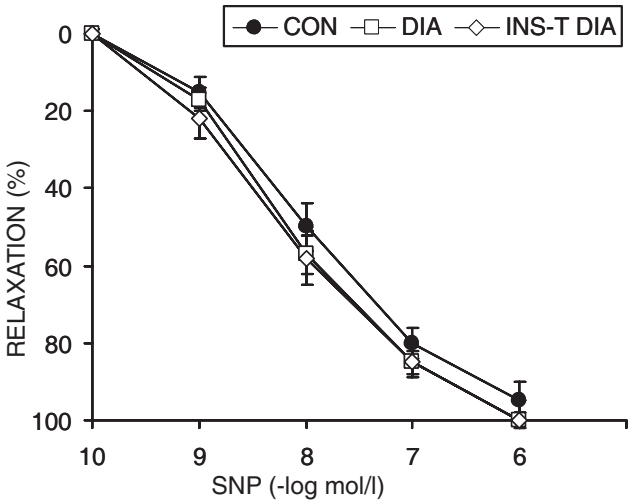


Figure 4. Concentration-response curves for sodium nitroprusside (SNP) in aortic groups. $n = 5$ each; CON, control group; DIA, diabetic group; INS-T DIA, insulin-treated (hyperinsulinaemic) diabetic group.

and accumulating data strongly suggest that TZDs inhibit only voltage-operated channels. On the other hand, in our study, responses to Bay K 8644 were not completely abolished by PIO indicating the drug may have different additional effect(s) on smooth muscle other than voltage-operated Ca^{2+} channels. Any possibility of the involvement of endothelium derived relaxing factors and, because of short exposure time, any involvement of activation of peroxisome proliferator activated receptors on the vasodepressor effect of PIO to 5-HT can be considered.

As a conclusion, we have found that the direct vasodepressor effect of insulin was blunted in hyperinsulinaemic rat aorta. Insulin sensitiser drug PIO, at therapeutic concentration, depresses dramatically contractions induced by 5-HT, partly by *via* Ca^{2+} channels. The drug does not modulate directly vasodepressor effect of insulin in hyperinsulinaemic/diabetic state. However, direct vasodepressor effect of PIO on vasculature may be beneficial in these states with hypertension.

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References

Alexander W. D., Oake R. J. (1987): The effect of insulin on vascular reactivity to norepinephrine.Diabetes **26**, 611–614

Anderson E. A., Mark A. L. (1993): The vasodilator action of insulin. Implications for the insulin hypothesis of hypertension. Hypertension **21**, 136–141

Bailey C. J., Day C. (2001): Thiazolidinediones today. Brit. J. Diab. Vasc. Dis. **1**, 7–1

Baron A. D. (1993): Cardiovascular actions of insulin in humans. Implications for insulin sensitivity and vascular tone. Baillieres Clin. Endocrinol. Metab. **7**, 961–987

Baron A. D. (1994): Hemodynamic actions of insulin. Am. J. Physiol. **267**, E187–202

Baron A. D. (1996): The coupling of glucose metabolism and perfusion in human skeletal muscle. Diabetes **45** (Suppl. 1), 105–109

Bhanot S., McNeill H. (1996): Insulin and hypertension: a causal relationship? Cardiovasc. Res. **31**, 212–221

Buchanan T. A., Meehan W. P., Jeng Y. Y., Yang D., Chan T. M., Nadler J. L., Scott S., Rude R. K., Hseuh W. A. (1995): Blood pressure lowering by pioglitazone: evidence for a direct vascular effect. J. Clin. Inves. **96**, 354–360

Goud C., Pitt B., Webb R. C., Richey J. M. (1998): Synergistic actions of insulin and troglitazone on contractility in endothelium-denuded rat aortic rings. Am. J. Physiol. **275**, E882–887

Kahn A. M., Seidel C. L., Allen J. C., O'Neil R. G., Shelat H., Song T. (1993): Insulin reduces contraction and intracellular calcium concentration in vascular smooth muscle. Hypertension **22,** 735–742

Kawasaki J., Hirano K., Nishimura J., Fujishima M., Kanaide H. (1998): Mechanisms of vasorelaxation induced by troglitazone, a novel antidiabetic drug, in the porcine coronary artery. Circulation **98**, 2446–2452

Kobayashi T., Kamata K. (1999): Effect of insulin treatment on smooth muscle contractility and endothelium-dependent relaxation in rat aortae from established STZ-induced diabetes. Br. J. Pharmacol. **27**, 835–842

Kotchen T. A., Zhang H. Y., Reddy S., Hoffmann R. G. (1996): Effects of pioglitazone on vascular reactivity *in vivo* and *in vitro*. Am. J. Pysiol. **270**, R660–666

Laakso M., Edelman S. V., Brechtel G., Baron A. D. (1990): Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man: a novel mechanisms for insulin resistance. J. Clin. Inves. **85**, 1844–1852

Laakso M., Edelman S. V., Brechtel G., Baron A. D. (1992): Impaired insulin mediated skeletal muscle blood flow in patients with NIDDM. Diabetes **41**, 1076–1083

Majithiya J. B., Paramar A. N., Balaraman R. (2005): Pioglitazone, a PPAR gamma agonist, restores endothelial function in aorta of STZ-induced diabetic rats. Cardiovasc. Res. **66**, 150–161

Masiello P., Broca C., Gross R., Roye M., Manteghetti M., Hillaire-Buys D., Novelli M., Ribes G. (1998): Experimental NIDDM: development of a new model in adult rats administered streptozotosin and nicotinamide. Diabetes **47**, 224–229

McNally P. G., Lawrence I. G., Watt P. A., Hillier C., Burden A. C., Thurston H. (1995): The effect of insulin on the vascular reactivity of isolated resistance arteries taken from healthy volunteers. Diabetologia **38**, 467–473 (in German)

Nakamura Y., Ohya Y., Onaka U., Fujii K., Abe I., Fujishima M. (1998): Inhibitory action of insulin-sensitizing agents on calcium channels in smooth muscle cells from resistance arteries of guinea-pig. Brit. J. Pharmacol. **123**, 675–682

Ozturk Y., Altan V. M. Yildizoglu-Ari N. (1996): Effects of experimental diabetes and insulin on smooth muscle functions. Pharmacol. Rev. **48**, 69–112

Passa P. (1993): Insulin resistance and hypertension. Clin. Exp. Hypertens. **15**, 1047-1059

Pieper G. M. (1999): Enhanced, unaltered and impaired nitric oxide-mediated endothelium-dependent relaxation in experimental diabetes mellitus: importance of disease duration. Diabetologia **42**, 204–213 (in German)

Ram J. L., Fares M. A., Standley P. R., Therell L. L., Thyagaranjan R. V., Sowers J. R. (1993): Insulin inhibits vasopressin elicited contraction of vascular smooth muscle cells. J. Vasc. Med. Biol. **4**, 250–255

Sahilli M., Yöntem Ö., Altan V. M., Ari N. (1999): Effects of insulin and troglitazone on serotonin-induced contractions in rat aorta. Fund. Clin. Pharmacol. **13** (Suppl. 1), 277

Scherrer U., Randin D., Vollenweider P., Vollenweider L., Nicod P. (1995): Nitric oxide release accounts for insulin's effects in humans. J. Clin. Invest. **94**, 2511–2515

Song J., Walsh M. F., Igwe R., Ram J. L., Barazi M., Dominguez L. J., Sowers J. R. (1997): Troglitazone reduces contraction by inhibition of vascular smooth muscle cell Ca^{2+} currents and not endothelial nitric oxide production. Diabetes **46**, 659–664

Standley P. R., Zhang F., Ram J. L, Zemel M. B., Sowers J. R. (1991): Insulin attenuates vasopressin-induced calcium transients and a voltage-dependent calcium response in rat vascular smooth muscle cells. J. Clin. Invest. **88**, 1230–1236

Standley P. R., Ram J. L., Sowers J. R. (1993): Insulin attenuation of vasopressin induced calcium responses in arterial smooth muscle from Zucker rats. Endocrinology **13**, 1693–1699

Steinberg H. O., Brechtel G., Johnson A., Fineberg N., Baron A. D. (1994): Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent: a novel action of insulin to increase nitric oxide release. J. Clin. Invest. **94**, 1172–1179

Tack C. J. Smits P., Willemsen J. J., Lenders J. W., Thien T., Lutterman J. A. (1996): Effects of insulin on vascular tone and sympathetic nervous system in NIDDM. Diabetes **45**, 15–22

Vasudevan A. R., Balasubramanyam A. (2004): Thiazolidinediones: a review of their mechanisms of insulin sensitization,thera peutic potential, clinical efficacy, and tolerability. Diabetes Technol. Ther. **6**, 850–863

Verma S., Bhanot S., Yao L., McNeill J. H. (1997): Vascular insulin resistance in fructose-hypertensive rats. Eur. J. Pharmacol. **322**, R1–2

Walker A. B., Dores J., Buckingham R. E., Savage M. W., Williams G. (1997): Impaired insulin-induced attenuation of noradrenaline-mediated vasoconstriction in insulin-resistant obese Zucker rats. Clin. Sci. **93**, 235–241

Wu H., Jeng Y. Y., Yue C., Chyu K., Hsueh W., Chan T. M. (1994): Endothelial-dependent vascular effects of insulin and insulin-like growth factor I in the perfused rat mesenteric artery and aortic ring. Diabetes **43**, 1027–1032

Yagi S., Takata S., Kiyokawa H., Yamamoto M., Noto Y., Ikeda T., Hattori N. (1988): Effects of insulin on vasoconstrictive responses to norepinephrine and angiotensin II in rabbit femoral artery and vein. Diabetes **37**, 1064–1067

Zemel M. B., Reddy S., Sowers J. R. (1991): Insulin attenuation of vasoconstrictor responses to phenylephrine in Zucker lean and obese rats. Am. J. Hypertens. **4**, 537-539

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

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