Original Article

Chronological Changes of α -Adrenoceptor-Mediated Vascular Constriction in Otsuka-Long-Evans-Tokushima Fatty Rats

Yasumasa YOSHIDA, Mitsumasa OHYANAGI, and Tadaaki IWASAKI

In recent years, it has been suggested that many factors are involved in the development of hypertension accompanying insulin resistance. Because changes in vascular reactivity could be one of these factors, we here investigated chronological changes of α -adrenoceptor (AR)-mediated peripheral arteriolar vasoconstriction in a rat model of type II diabetes. Otsuka-Long-Evans-Tokushima fatty (OLETF) rats that naturally develop insulin resistance at the age of 16 weeks and type II diabetes at the age of 30 weeks (DM group) and control rats (N group) were used. Arterioles with a diameter of approximately 100 μ m were removed from the cremaster muscle of 8-, 16- and 40-week-old rats and their diameters were measured in a tissue bath. The concentration–response curve (CRC) was determined for phenylephrine and UK14,304 both with and without *N*^G-monomethyl-L-arginine (LNMMA). Although there were no significant differences in the CRC for phenylephrine between the 8-week-old DM group and N group, a leftward shift was seen for the 16- and 40-week-old DM groups. There were no significant differences in the CRC for UK14,304 between the two groups at any age, but in the presence of LNMMA, a leftward shift was seen in the 8- and 16-week-old but not in the 40-week-old DM groups. One possible explanation for these results is that impaired endothelium-dependent dilatation may have offset the reduction in arteriolar smooth muscle contraction. In conclusion, in the OLETF rats, the sensitivity of α_1 -AR-mediated arteriolar vasoconstriction increased after the onset of insulin resistance. The sensitivity of α_2 -AR-mediated arteriolar smooth muscle contraction and endothelium-dependent vascular relaxation were both presumed to be impaired after the onset of type II diabetes. (*Hypertens Res* 2003; 26: 559–567)

Key Words: α -adrenoceptor, peripheral arteriolar vasoconstriction, insulin resistance

Introduction

In recent years, several studies have documented that hypertension often accompanies diseases such as obesity, glucose intolerance, and type II diabetes (1–3). Furthermore, it has been reported that insulin resistance deteriorates in non-obese hypertensive patients (4, 5). Therefore, insulin resistance could be a risk factor for hypertension and *vice versa*. The onset mechanism of hypertension accompanying increased insulin resistance is thought to involve many factors, and changes in vascular reactivity could be one of these fac-

tors. In a rat model of type II diabetes caused by increased insulin resistance, it has been reported that α_1 -adrenoceptor (AR)-mediated arterial vasoconstriction was elevated in large vessels (6), but few studies have investigated the effects of insulin resistance on α -AR-mediated vasoconstriction of arterioles, which function as resistant vessels and are deeply involved with tissue blood flow.

In this study, we hypothesized that changes in α -AR-mediated arteriolar vasoconstriction are one of the mechanisms involved in the onset of hypertension accompanying increased insulin resistance. Therefore, we investigated the chronological changes in α -AR-mediated arteriolar vasocon-

From the Department of Internal Medicine, Cardiovascular Division, Hyogo College of Medicine, Nishinomiya, Japan.

Address for Reprints: Mitsumasa Ohyanagi, M.D., Ph.D., the Department of Internal Medicine, Cardiovascular Division, Hyogo College of Medicine, 1–1, Mukogawa-cho, Nishinomiya 663–8501, Japan. E-mail: ohyanagi@hyo-med.ac.jp

Received December 20, 2002; Accepted in revised form February 28, 2003.

Fig. 1. Schematic diagram of the experimental set-up.

striction in a rat model that naturally develops type II diabetes due to increased insulin resistance to clarify the effects of insulin resistance on α -AR-mediated arteriolar vasoconstriction.

Methods

All procedures were conducted in accordance with the institutional guidelines for animal research of Hyogo College of Medicine. We studied male Otsuka-Long-Evans-Tokushima fatty (OLETF) rats (DM group; 24 at age 8 weeks, 22 at age 16 weeks, and 19 at age 40 weeks) and male Long-Evans Tokushima-Otsuka (LETO) rats (N group; 23 at age 8 weeks, 20 at age 16 weeks, and 18 at age 40 weeks). OLETF rats naturally develop type II diabetes due to increased insulin resistance, and LETO rats do not. Rats were obtained from Otsuka Pharmaceutical (Tokushima, Japan) and kept at a specific pathogen-free facility under controlled temperature ($22 \pm 2^\circ\text{C}$) and humidity ($55 \pm 5\%$) with a 12-h light and dark cycle. They were fed standard laboratory chow and given tap water *ad libitum*.

We measured body weight and systolic blood pressure (SBP) by the tail-cuff method using an electro-sphygmomanometer (UR-5000, Ueda, Tokyo, Japan).

After anesthetizing each rat using pentobarbital (50 mg/kg), the right cremaster muscle was removed and placed in dissecting solution at a temperature of $0-4^\circ\text{C}$, and arterioles with an internal diameter of approximately $100\ \mu\text{m}$, which vascularized the center of the cremaster mus-

cle, were excised as approximately 2-mm sections under a stereoscopic microscope. The excised arterioles were transferred to a perfusion chamber filled with dissecting solution at a temperature of $0-4^\circ\text{C}$. Each arteriole was suspended in a chamber filled with Krebs's solution at pH 7.4 and 34°C , and a glass micropipette was inserted into both ends of the arteriole to perfuse the arteriole with the solution. Only arterioles exhibiting a normal myogenic response (those showing at least 30% spontaneous constriction when heated to 34°C) were used. After adjusting the internal pressure of the arteriole to 40 mmHg, which is the normal physiological pressure, the internal diameter of the arteriole was measured by photographing the arteriole using a CCD camera (C2400 Hamamatsu Photonix, Tokyo, Japan) mounted on an inverted microscope and analyzing captured images using an Argus 10 image processor (Hamamatsu Photonix). Changes in the diameter of the arterioles in response to addition of various agents were determined. At the end of each experiment, after confirming the absence of leakage and the presence of the myogenic response, the arteriole was maximally dilated using $100\ \mu\text{mol/l}$ nitroprusside in order to ensure that there was no marked difference between the nitroprusside-induced maximum diameter and the diameter prior to induction of spontaneous constriction. Arterioles that did not meet the above conditions were discarded (Fig. 1).

Blood was withdrawn from the aorta after separation of the cremaster muscle. Fasting and postcibal plasma glucose levels were determined by a standard glucose oxidase method and plasma insulin concentration by a specific radio-

Table 1. The Diameter of the Arterioles in Response to the Agents (Phenylephrine, UK 14,304 with (+) and without (-) LNMMA) for 8-, 16- and 40-Week-Old DM and N Group Rats

	Pre (μ m)	Post (μ m)	Max (%)	NP (μ m)
Phenylephrine				
DM group				
8W ($n = 6$)	118 $\pm$ 13	86 $\pm$ 11	33 $\pm$ 7	120 $\pm$ 14
16W ($n = 6$)	151 $\pm$ 17	109 $\pm$ 17	34 $\pm$ 7	151 $\pm$ 18
40W ($n = 6$)	144 $\pm$ 20	107 $\pm$ 13	27 $\pm$ 8	143 $\pm$ 20
N group				
8W ($n = 6$)	108 $\pm$ 18	87 $\pm$ 13	25 $\pm$ 11	122 $\pm$ 26
16W ($n = 6$)	151 $\pm$ 11	118 $\pm$ 19	27 $\pm$ 7	148 $\pm$ 14
40W ($n = 6$)	147 $\pm$ 23	108 $\pm$ 17	34 $\pm$ 9	146 $\pm$ 23
UK14,304 LNMMA -				
DM group				
8W ($n = 9$)	141 $\pm$ 24	103 $\pm$ 20	34 $\pm$ 8	138 $\pm$ 2
16W ($n = 8$)	142 $\pm$ 18	106 $\pm$ 15	29 $\pm$ 8	141 $\pm$ 15
40W ($n = 7$)	156 $\pm$ 54	110 $\pm$ 27	32 $\pm$ 6	155 $\pm$ 51
N group				
8W ($n = 9$)	122 $\pm$ 17	94 $\pm$ 14	33 $\pm$ 9	124 $\pm$ 16
16W ($n = 7$)	146 $\pm$ 25	109 $\pm$ 19	37 $\pm$ 10	145 $\pm$ 22
40W ($n = 6$)	171 $\pm$ 40	122 $\pm$ 30	40 $\pm$ 13	168 $\pm$ 38
UK14,304 LNMMA +				
DM group				
8W ($n = 9$)	141 $\pm$ 28	102 $\pm$ 23	34 $\pm$ 10	142 $\pm$ 27
16W ($n = 8$)	152 $\pm$ 24	110 $\pm$ 19	33 $\pm$ 7	152 $\pm$ 21
40W ($n = 6$)	158 $\pm$ 42	112 $\pm$ 27	34 $\pm$ 6	157 $\pm$ 40
N group				
8W ($n = 8$)	132 $\pm$ 19	96 $\pm$ 15	32 $\pm$ 7	133 $\pm$ 18
16W ($n = 7$)	141 $\pm$ 26	106 $\pm$ 19	39 $\pm$ 11	142 $\pm$ 23
40W ($n = 6$)	167 $\pm$ 30	117 $\pm$ 20	36 $\pm$ 10	166 $\pm$ 32

Pre, diameter prior to warming under the physiological internal pressure. Post, diameter after warming to 34°C under internal physiological pressure. Max (%), diameter in presence of maximum concentration of test agent / diameter after warming to 34°C under internal physiological pressure $\times$ 100; no significant differences were observed between DM and N group in Max (%). NP, diameter in presence of nitroprusside after the concentration-response curve was obtained. W, week; LNMMA, N^G -monomethyl-L-arginine; DM group, diabetic rats; N group, non-diabetic rats.

immunoassay.

Experiment 1: To investigate α_1 -AR-mediated vasoconstriction, in the presence of rauwolscine (10^{-6} mol/l), propranolol (10^{-6} mol/l), and indomethacin (10^{-5} mol/l), phenylephrine (an α_1 -adrenoceptor agonist) concentration was increased in steps from 10^{-10} mol/l to 10^{-5} mol/l, the degree of vasoconstriction relative to the maximum constriction (100%) was measured, and a concentration-response curve (CRC) was drawn.

Experiment 2: To investigate α_2 -AR-mediated vasoconstriction, in the presence of prazosin (10^{-6} mol/l), propranolol (10^{-6} mol/l), and indomethacin (10^{-5} mol/l), UK14,304 (α_2 -AR agonist) concentration was increased in steps from 10^{-10} mol/l to 10^{-5} mol/l and a CRC was drawn.

Experiment 3: To investigate changes in the sensitivity of α_2 -AR-mediated vasoconstriction without NO-mediated endothelium-dependent dilatation, in the presence of prazosin

(10^{-6} mol/l), propranolol (10^{-6} mol/l), indomethacin (10^{-5} mol/l), and N^G -monomethyl-L-arginine (LNMMA) (10^{-4} mol/l), the concentration of UK14,304 was increased in steps from 10^{-10} mol/l to 10^{-5} mol/l and a CRC was drawn.

The results of Experiments 2 and 3 were compared in order to ascertain the effects of LNMMA.

Experiment 4: To investigate the amount of α -AR mRNA in the aorta, the aortas were isolated from 8- and 16-week-old DM and N group rats ($n = 4$ per group). The adventitia was dissected away from the aorta and the endothelium was scraped off with a cell scraper. The aortas were stored at - 80°C for up to 14 days until used for measurement. Total RNAs were extracted using ISOGEN (Nippon Gene, Tokyo, Japan) according to the product manual, followed by treatment with RQ1 RNase-free DNase for 45 min at 37°C to prevent genomic DNA contamination. RNA concentration was determined spectrophotometrically at 260 nm. An

Fig. 2. Characteristic features of the DM and N group rats. (A) Fasting-plasma glucose; (B) postcibial-plasma glucose; (C) fasting-plasma insulin; (D) postcibial-plasma insulin; (E) body weight; (F) systolic blood pressure. * $p < 0.01$, § $p < 0.001$.

oligonucleotide primer (1A-sense, 5'-CGAGTCTACGTAG TAGCCAAGAGA-3'; 1A-antisense, 5'-GTCTTGGCAGCT TTCTTCTCTC-3'; 1B-sense, 5'-ATCGTGGCCAAGAGGA CC-3'; 1B-antisense, 5'-TTTGGCTGCTTTCTTTTC-3'; 1D-sense, 5'-GTGTACGTGGTCGCACG-3'; 1D-antisense, 5'-C TTGGCAGCCTTTTCTCG-3'; 2A-sense, 5'-AGAAACG CTTACGTTTCGTG-3'; 2A-antisense, 5'-TGTAAGCAGC ACAGCCCG-3'; 2B-sense, 5'-GAGTACAACCTCAAGCG CACT-3'; 2B-antisense, 5'-ATTCTTGCCAGAAAGCACC T-3'; 2C-sense, 5'-CGGAAGCGGTAGAGTACAACC-3'; 2C-antisense, 5'-AGAACTCGACGGAGCGC-3') (7) was synthesized corresponding to a position in the amplified target mRNA sequence that was inserted at a position n base pairs (bp) upstream from either the 5' or 3' end. This primer was then used in combination with the appropriate forward or reverse primer from the original pair and conditions were optimized to result in amplification of a polymerase chain reaction (PCR) product of the predicted size visible as a single band on ethidium gel (n bp shorter than the original PCR product). If this primer was a forward primer, a new forward primer was synthesized that linked a T7 recognition sequence to the original forward-primer sequence, which was

in turn linked to this primer sequence. A new reverse-primer sequence was also synthesized linking the reverse primer to an 18 poly-T tail. These primers were used to synthesize a PCR product that formed a DNA template for transcription of RNA by use of a T7 RNA polymerase (Toyobo, Tokyo, Japan). The RNA formed was then extracted, treated with RNase I, and reextracted. This synthetic RNA was used as competitive RNA. Serial dilutions of the competitive RNA were made and added to equal quantities of total RNA extracted from DM and control rats in a series of reverse transcription (RT)-PCR reaction mixtures. Electrophoresis of the amplified products was performed on 1.5% agarose gel containing Tris acetate/EDTA and ethidium bromide. An Hae III digest of 174 DNA was used as a molecular size standard. Gels were visualized with UV irradiation and photographed. When the target mRNA exceeded the competitive RNA, the product was the longer target product, but when the quantity of synthetic competitive RNA in the tube equaled the quantity of target mRNA present, amplification of bands of both size was equal. This allowed us to compare the concentration the target mRNA among DM and N group rat samples in relation to the dilution sequence (8).

Fig. 3. Concentration–response curve of phenylephrine (10^{-10} to 10^{-5} mol/l) in 8-, 16- and 40-week-old DM group and N group rats. $p < 0.01$ (Bonferroni/Dunn variance analysis).

Table 2. Bonferroni/Dunn Variance Analysis of CRC for Phenylephrine and UK14,304 either with (+) or without (–) LNMMA in 8-, 16- and 40-Week-Old DM-Group and N-Group Rats

		Bonferroni/Dunn variance analysis of CRC
Phenylephrine	8W	n.s.
	N group vs. DM group	
	16W	leftward shift for DM group ($p < 0.01$)
UK14,304	40W	leftward shift for DM group ($p < 0.01$)
	8W	n.s.
	N group vs. DM group	
UK14,304	16W	n.s.
	40W	n.s.
	8W	leftward shift for LNMMA + ($p < 0.05$)
UK14,304	16W	leftward shift for LNMMA + ($p < 0.05$)
	40W	leftward shift for LNMMA + ($p < 0.001$)
	8W	leftward shift for LNMMA + ($p < 0.01$)
UK14,304	16W	leftward shift for LNMMA + ($p < 0.05$)
	40W	n.s.
UK14,304 LNMMA +	8W	n.s.
	DM group 8W vs. DM group 16W	
	16W	n.s.
UK14,304 LNMMA +	40W	rightward shift for 40W DM rats ($p < 0.05$)
	DM group 16W vs. DM group 40W	
	16W	n.s.
UK14,304 LNMMA +	40W	n.s.
	N group 16W vs. N group 40W	
	16W	n.s.
UK14,304 LNMMA +	40W	rightward shift for 40W DM group rats ($p < 0.01$)
	N group vs. DM group	
	40W	

CRC, concentration–response curve; W, week; LNMMA, N^G -monomethyl-L-arginine; DM group, diabetic rats; N group, non-diabetic rats.

Statistical Analysis

All numerical results were expressed as the mean $\pm$ SD. An unpaired Student's t -test was used to compare the % maximum vasoconstriction values (Table 1), and differences in characteristic features between rats of different ages (Fig. 2) were determined by ANOVA, followed by Tukey's test for individual data. Bonferroni/Dunn variance analysis was used to compare the CRCs for each agonist. In all analyses, differences were considered statistically significant at a value of $p < 0.05$.

Results

No significant differences were observed in fasting plasma insulin or fasting plasma glucose between the 8-week-old DM group and the 8-week-old N group or between the 16-week-old DM group and the 16-week-old N group, but both parameters were significantly higher in the 40-week-old DM group than in the 40-week-old N group. The levels of post-cibal plasma insulin and postcibal plasma glucose (1 h after eating) in the 16- and 40-week-old DM groups were significantly higher than those in the 16- and 40-week-old N groups. Furthermore, the body weight of the 16- and 40-week-old DM groups was significantly higher than that of

Fig. 4. Concentration–response curve of UK14,304 (10^{-10} to 10^{-5} mol/l) in the absence and presence of 10^{-5} mol/l of LNMMA in 8-, 16- and 40-week-old (W) DM and N group rats. N group vs. DM group LNMMA + : # $p < 0.01$; N group LNMMA + vs. - : * $p < 0.05$, $p < 0.001$; DM group LNMMA + vs. - : § $p < 0.05$, $p < 0.01$; DM group 16W vs. 40W LNMMA + : † $p < 0.05$, Bonferroni/Dunn variance analysis.

Fig. 5. α -AR mRNA in the aorta. Upper panel: 8-week-old DM and N group rats. Lower panel: 16-week-old DM and N group rats. Data were determined by two group t-test. * $p < 0.05$ vs. the N group rats.

the 16- and 40-week-old N groups. SBP in the 16- and 40-week-old DM groups was significantly higher than that in the 16- and 40-week-old N groups (Fig. 2).

The average diameter of arterioles at the state of spontaneous constriction achieved by applying 40 mmHg of physiological internal pressure and warming to 34°C in the observation chamber was $101 \pm 27 \mu\text{m}$. In all experiments, there was no significant difference in maximum vasoconstriction between the DM and N groups (Table 1).

Experiment 1: No marked changes in the CRC for α_1 -AR-mediated arteriolar vasoconstriction were seen between the 8-week-old DM rats and the 8-week-old N rats, but a significant leftward shift was observed in the 16- and 40-week-old

DM rats compared to the 16- and 40-week-old N rats (Fig. 3; Table 2).

Experiment 2: There were no significant differences in the CRC for α_2 -AR-mediated arteriolar vasoconstriction between the DM and N groups at any age (Fig. 4; Table 2).

Experiment 3: In the presence of LNMMA, there was a significant leftward shift in the CRC for α_2 -AR-mediated arteriolar vasoconstriction in the 8- and 16-week-old DM rats and in the 8-, 16- and 40-week-old N rats, but not in the 40-week-old DM rats. In addition, there was a significant rightward shift in the CRC for α_2 -AR-mediated arteriolar vasoconstriction in the presence of LNMMA for the 40-week-old DM rats when compared to that for the 8- or 16-week-old

DM rats or the 40-week-old N rats (Fig. 4; Table 2).

Experiment 4: The rat aorta vascular smooth muscle expressed α 1B, 1D and 2A. In contrast, no α 1A, 2B or 2C expression was detected. The competitive RT-PCR revealed no significant differences in expression of any of the mRNAs (1B, 1D and 2A) between 8-week-old DM and age-matched control rats. The RT-PCR showed a significant increase in the expression of 1B in 16-week-old DM rats compared with age-matched control rats. There were no significant differences in the expression of 1D or 2A mRNA between 16-week-old DM rats and age-matched control rats (Fig. 5).

Discussion

In type II diabetes caused by increased insulin resistance, enhanced vasoconstriction has been reported, and this augmentation may be due to impaired insulin-dependent vasodilatation (9, 10), changes in the reactivity of vasoconstriction to vasopressors (11, 12), and/or impaired endothelium-dependent vasodilatation mediated by oxidative stress due to expression of NAD(P)H oxidase accompanying the insulin-induced activation of endothelin (13–15).

α -Adrenoceptors are known to be involved with vasoconstriction in vascular smooth muscle. There are two subtypes of α -AR: α_1 -AR and α_2 -AR. While α_1 -ARs are distributed in the aorta and muscular arteries and α_2 -ARs are distributed near capillaries, both α_1 -ARs and α_2 -ARs are distributed in arterioles. Furthermore, α_2 -ARs are also distributed in the vascular endothelium and are believed to be involved in endothelium-dependent vasodilatation.

In OLETF rats, systemic glucose uptake, as measured by the glucose clamp method, is decreased at the age of 16 weeks, and glucose administration causes excessive insulin secretion. After the age of 18 weeks, these rats develop diabetes (16, 17). For this reason, they are widely used as an animal model for type II diabetes. In the present study, post-cibal increases in blood glucose and immunoreactive insulin (IRI) were observed in the 16-week-old DM rats, and increases in fasting blood glucose were observed in the 40-week-old DM rats. Our data are consistent with the above-mentioned reports.

Taylor *et al.* reported that α_1 -AR-mediated arteriolar vasoconstriction remained unchanged in streptozotocin-induced insulin-dependent diabetic (IDDM) rats (18). However, Ohuchi *et al.* found that α_1 -AR-mediated aortic constriction was augmented in 20-week-old Zucker fatty insulin-resistant rats (19).

In the present study, the α_1 -AR-mediated constriction of arterioles was augmented in 16- and 40-week-old DM rats. Our data suggest that in rats genetically disposed to developing type II diabetes with age, sensitivity of α_1 -AR-mediated arteriolar vasoconstriction is normal during the absence of insulin resistance, but is augmented when insulin resistance increases.

On the other hand, Taylor *et al.* reported that endothelium-

dependent dilatation was impaired in the arterioles of IDDM rats (18), while Wakabayashi *et al.* documented that the endothelium-dependent dilatation mediated by α_2 -AR was weakened in the aorta of IDDM rats (20). Bohlen *et al.* found that endothelium-dependent dilatation was maintained in the arterioles of non-insulin-dependent diabetic (NIDDM) rats (9- to 10-week-old Zucker fatty rats) (21). In a study by Sakamoto *et al.*, however, although endothelium-dependent dilatation in the aorta of OLETF rats was normal at the age of 16 weeks, it was impaired at the age of 25 weeks, and the authors concluded that the difference between their results and those of Bohlen *et al.* (21) was due to the difference in severity of NIDDM between the two studies (22). Furthermore, Takagawa *et al.* reported impaired endothelial α_2 -AR-mediated arteriolar relaxation in NIDDM rats (fructose-fed rats) (23).

In the present study, when NO-mediated endothelium-dependent vascular relaxation was blocked by LNMMA in 40-week-old rats, the sensitivity of α_2 -AR-mediated arteriolar vasoconstriction was significantly enhanced in the N group rats, but remained unchanged in the DM group rats. These results suggest that because endothelium-dependent arteriolar relaxation was already impaired in the 40-week-old DM rats, there was no change in sensitivity of α_2 -AR-mediated arteriolar vasoconstriction even when NO-mediated endothelium-dependent arteriolar relaxation was blocked. In the presence of endothelium-dependent arteriolar relaxation, the sensitivity of α_2 -AR-mediated constriction was not significantly different between the DM and N groups at any age, but in the absence of endothelium-dependent arteriolar relaxation, it was lower in the 40-week-old DM group rats than in the 8- and 16-week-old DM or the 40-week-old N group rats. If we consider that impaired endothelium-dependent dilatation offsets reductions in arteriolar smooth muscle contraction, the above results are reasonable. We therefore believe that our data suggests that impaired endothelium-dependent arteriolar relaxation and decreased sensitivity of α_2 -AR-mediated smooth muscle contraction were present in advanced diabetes stages.

The following factors are currently thought to be involved in the onset of hypertension accompanying hyperinsulinemia caused by increased insulin resistance: 1) antidiuretic activity; 2) elevated sympathetic nerve activity; 3) changes in electrolyte metabolism of cell membranes; 4) vascular endothelial injury; 5) changes in vascular reactivity; 6) genetic abnormality of myofibrils; and 7) cellular proliferation. The results of the present study confirmed the increase in α_1 -AR-mediated vasoconstriction and the lack of change in α_2 -AR-mediated arteriolar vasoconstriction due to impairment of both endothelium-dependent arteriolar relaxation and smooth muscle contraction. Our data are consistent with mechanisms 4) and 5) above.

Irving *et al.* reported that while impairment of acetylcholine-induced dilatation is not correlated with severity of insulin resistance, it is correlated to fasting plasma glucose

levels (24). In other words, impaired endothelium-dependent dilatation may correlate more closely with increased plasma glucose than with insulin resistance. Likewise, in the present study, the endothelium-dependent dilatation mediated by α_2 -AR was not impaired in 16-week-old DM rats with insulin resistance but no significant increases in fasting plasma glucose, but it was impaired in 40-week-old DM rats with significantly increased fasting plasma glucose.

It has also been reported that exercise can improve impaired vasodilatation in OLETF rats (22) and that administration of metformin normalized the blood pressure of OLETF rats (25), thus documenting the reversibility of insulin resistance and hypertension. These findings suggest that factors other than increased vascular resistance caused by endothelial injury attributable to irreversible organic changes are important in the onset of hypertension.

The increased α_1 -AR-mediated vasoconstriction may act in a feedback mechanism for muscular relaxation due to hyperinsulinemia, and reduced sensitivity of α_2 -AR-mediated smooth muscle contraction may also act as a feedback mechanism for impaired endothelium-dependent arteriolar relaxation caused by glucose toxicity or oxidative stress.

The smooth muscle cells in the media of the rat aorta have previously been shown to express 1A-, 1B-, 1D- and 2D-ARs (26). However, the expression of 1A was not confirmed by the present method, and further investigation will be needed to examine this adrenoceptor. In the aorta of rats in the 8-week-old groups, no significant changes were recognized in any of the receptor mRNAs. These results agreed with the results on vascular responsiveness. In the 16-week-old DM rats, only the expression of 1B mRNA was increased and the expressions of the other subtypes remained the same. It has been suggested that activation of 1B-ARs causes mobilization of Ca^{2+} from intracellular inositol trisphosphate (IP₃)-sensitive stores (27). The increase of the expression of mRNA may induce the increase in the protein of 1B-AR. Such an increase of the receptor protein would in turn play a role in the enhancement of α_1 -AR-mediated vascular responsiveness.

Limitations

A previous work described a more marginally lower rate of suppression of norepinephrine release by UK14,304 in the medulla oblongata of hypertensive rats (28). The greater suppression in the present study may have been due to the fact that, although the cremaster arterioles were dissected out and denervated to the greatest extent possible, the nerves around the arterioles could not be removed completely. It was thus possible that α_2 -AR in the end of the remaining sympathetic nerves may have played a role in the responsiveness of UK14304 in arterioles. Further studies will be needed to investigate this possibility.

In conclusion, In type II diabetes caused by increased insulin resistance, the sensitivity of α_1 -AR-mediated arteriolar

vasoconstriction increased after the onset of insulin resistance. Furthermore, after the onset of type II diabetes, the sensitivity of the α_2 -AR-mediated arteriolar smooth muscle contraction and endothelium-dependent vascular relaxation were both presumed to be impaired. Enhanced α_1 -AR-mediated arteriolar vasoconstriction may therefore be a factor in the onset of hypertension.

References

1. Modan M, Halkin H, Almog S, *et al*: Hyperinsulinemia: a link between hypertension obesity and glucose intolerance. *J Clin Invest* 1985; **75**: 809–817.
2. Hypertension in Diabetes Study (HDS): I. Prevalence of hypertension in newly presenting type 2 diabetic patients and the association with risk factors for cardiovascular and diabetic complications. *J Hypertens* 1993; **11**: 309–317.
3. Salomaa VV, Strandberg TE, Vanhanen H, *et al*: Glucose tolerance and blood pressure: long term follow up in middle aged men. *BMJ* 1991; **302**: 493–496.
4. Ferrannini E, Buzzigoli G, Bonadonna R, *et al*: Insulin resistance in essential hypertension. *N Engl J Med* 1987; **317**: 350–357.
5. Gress TW, Nieto FJ, Shahar E, *et al*: Hypertension and antihypertensive therapy as risk factors for type 2 diabetes mellitus: Atherosclerosis Risk in Communities Study. *N Engl J Med* 2000; **342**: 905–912.
6. Tuck ML: Obesity, the sympathetic nervous system, and essential hypertension. *Hypertension* 1992; **19**(Suppl 1): 167–177.
7. Ping P, Faber JE: Characterization of alpha-adrenoceptor gene expression in arterial and venous smooth muscle. *Am J Physiol* 1993; **265**: H1501–H1509.
8. Haywood GA, Gullestad L, Katsuya T, *et al*: AT1 and AT2 angiotensin receptor gene expression in human heart failure. *Circulation* 1997; **95**: 1201–1206.
9. Feldman RD, Bierbrier GS: Insulin-mediated vasodilation: impairment with increased blood pressure and body mass. *Lancet* 1993; **342**: 707–709.
10. Baron AD: Insulin resistance and vascular function. *J Diabet Complications* 2002; **16**: 92–102.
11. Zemel MB: Insulin resistance vs. hyperinsulinemia in hypertension: insulin regulation of Ca^{2+} transport and Ca^{2+} -regulation of insulin sensitivity. *J Nutr* 1995; **125**: 1738S–1743S.
12. He Y, MacLeod KM: Modulation of noradrenaline-induced vasoconstriction in isolated perfused mesenteric arterial beds from obese Zucker rats in the presence and absence of insulin. *Can J Physiol Pharmacol* 2002; **80**: 171–179.
13. Hsueh WA, Anderson PW: Hypertension, the endothelial cell, and the vascular complications of diabetes mellitus. *Hypertension* 1992; **20**: 253–263.
14. Tesfamariam B, Brown ML, Cohen RA: Elevated glucose impairs endothelium-dependent relaxation by activating protein kinase C. *J Clin Invest* 1991; **87**: 1643–1648.
15. Arcaro G, Cretti A, Balzano S, *et al*: Insulin causes endothelial dysfunction in humans. *Circulation* 2002; **105**: 576–582.
16. Kawano K, Hirashima T, Mori S, *et al*: Spontaneous long-

- term hyperglycemic rat with diabetic complications, Otsuka Long-Evans-Tokushima Fatty (OLETF) strains. *Diabetes* 1992; **41**: 1422–1428.
17. Ishida K, Mizuno A, Min Z, et al: Which is the primary etiologic event in Otsuka Long-Evans-Tokushima Fatty rats, a model of spontaneous non-insulin-dependent diabetes mellitus, insulin resistance, or impaired insulin secretion? *Metabolism* 1995; **44**: 940–945.
 18. Taylor PD, Graves JE, Poston L: Selective impairment of acetylcholine-mediated endothelium-dependent relaxation in isolated resistance arteries of the streptozotocin-induced diabetic rat. *Clin Sci (Colch)* 1995; **88**: 519–524.
 19. Ohuchi Y, Han S, Kim S, et al: Augmented contractile function and abnormal Ca^{2+} handling in the aorta of Zucker obese rats with insulin resistance. *Diabetes* 1996; **45** (Suppl): S55–S58.
 20. Wakabayashi I, Hatake K, Kimura N, et al: Modulation of vascular tonus by the endothelium in experimental diabetes. *Life Sci* 1987; **40**: 643–648.
 21. Bohlen HG, Lash JM: Endothelial-dependent vasodilation is preserved in non-insulin-dependent Zucker fatty diabetic rats. *Am J Physiol* 1995; **268**: H2366–H2374.
 22. Sakamoto S, Minami K, Niwa Y, et al: Effect of exercise training and food restriction on endothelium-dependent relaxation in the Otsuka Long-Evans Tokushima Fatty rat, a model of spontaneous NIDDM. *Diabetes* 1998; **47**: 82–86.
 23. Takagawa Y, Berger ME, Tuck ML, et al: Impaired endothelial alpha-2 adrenergic receptor-mediated vascular relaxation in the fructose-fed rat. *Hypertens Res* 2002; **25**: 197–202.
 24. Irving RJ, Walker BR, Noon JP, et al: Microvascular correlates of blood pressure, plasma glucose, and insulin resistance in health. *Cardiovasc Res* 2002; **53**: 271–276.
 25. Kosegawa I, Katayama S, Kikuchi C, et al: Metformin decreases blood pressure and obesity in OLETF rats via improvement of insulin resistance. *Hypertens Res* 1996; **19**: 37–41.
 26. Faber JE, Yang N, Xin X: Expression of α -adrenoceptor subtypes by smooth muscle cells and adventitial fibroblast in rat aorta and in cell culture. *J Pharmacol Exp Ther* 2001; **298**: 441–452.
 27. Han C, Li J, Minneman KP: Subtypes of α 1-adrenoceptors in rat blood vessels. *Eur J Pharmacol* 1990; **190**: 97–104.
 28. Tuda K, Tuda S, Masuyama Y, Goldstein M: Norepinephrine release and neuropeptide Y in medulla oblongata of spontaneously hypertensive rats. *Hypertension* 1990; **15**: 784–790.