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Restoration of endothelium-dependent relaxation in both hypercholesterolemia and diabetes by chronic taurine

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Abstract

We examined the effects of taurine on levels of low-density lipoprotein (LDL) cholesterol and glucose, and an endothelium-dependent relaxation in response to acetylcholine in cholesterol-fed or streptozotocin-induced diabetic mice. The acetylcholine-induced concentration-dependent relaxation was significantly attenuated in aortic rings from cholesterol-fed and streptozotocin-induced diabetic mice. The attenuated vasodilation in both cholesterol-fed and streptozotocin-induced diabetic mice was normalized by the chronic administration of taurine. The endothelium-independent relaxation of aortic rings induced by sodium nitroprusside was not significantly different between control, cholesterol-fed and streptozotocin-induced diabetic mice. The increased serum levels of LDL cholesterol in cholesterol-fed and diabetic mice were returned to normal by the chronic administration of taurine. The chronic administration of taurine had no effects on serum glucose levels. These results suggest that the impaired endothelium-dependent vasodilation seen in both cholesterol-fed and streptozotocin-diabetic mice can be normalized by the chronic administration of taurine and this effect may be, at least in part, due to lowering of serum LDL levels.

Keywords: Taurine; Hypercholesterolemia; Diabetes; Endothelium; LDL (low-density lipoprotein); Cholesterol

1. Introduction

Impaired endothelium-dependent relaxations in atherosclerosis have been reported in the rabbit aorta (Habib et al., 1986; Verbeuren et al., 1986; Bossaller et al., 1987; Jayakody et al., 1987; Simon et al., 1993), monkey iliac artery (Freiman et al., 1986), pig coronary artery (Yamamoto et al., 1987; Shimokawa and Vanhoutte, 1989) as well as human coronary artery in vitro (Bossaller et al., 1987; Forstermann et al., 1988) and in vivo (Lunder et al., 1986). In contrast, there is little information concerning the endothelium-dependent relaxation in response to acetylcholine in cholesterol-fed mice. The impairment of endothelium-dependent relaxation is thought to play an important role in the pathogenesis of coronary spasm. Oxidative modification of low-density lipoprotein (LDL) cholesterol by the endothelium is thought to be an important step in the initiation of atherosclerosis (Steinbrecher et al., 1984; Quinn et al., 1987; Berliner et al., 1990).

It is well-known that vascular disease is one of the complicating features of diabetes mellitus in humans (Christrieb, 1973). The reactivity of vascular smooth muscles and the endothelium to vasoactive agents in diabetic animals has been extensively studied (Agrawal and McNeill, 1987; Harris and MacLeod, 1988; Kamata et al., 1992). It has been shown that the relaxation response of aortic strips to endothelium-dependent agents is decreased in streptozotocin-induced diabetic rats (Oyama et al., 1986; Pieper and Gross, 1988; Kamata et al., 1989a,b; Abiru et al., 1993; Poston and Taylor, 1995) and alloxan-induced diabetic rabbits (Tesfamariam et al., 1989; Abiru et al., 1990a,b, 1991). However, there are few studies which have directly assessed the role of hyperlipidemia or oxidized LDL in the endothelial cell dysfunction in diabetes. To our knowledge, no studies have investigated the effect of cholesterol-lowering therapy on endothelial function in animal models of diabetes or in humans.

Taurine (2-aminoethanesulfonic acid) is a sulfur-containing amino acid that is widely distributed in various animal tissues. World-wide epidemiological studies (Nara et al., 1990) and investigations with animal models (Yamori et al., 1983; Yamauchi-Takihara et al., 1986; Sugiyama et

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al., 1989; Goodman and Shibabi, 1990; Petty et al., 1990; Bellentani et al., 1986; Murakami et al., 1992) have suggested beneficial effects of dietary taurine on cardiovascular disease. It has been reported that an increased cellular concentration of taurine is associated with an elevated rate of bile acid synthesis, a reduced free cellular cholesterol concentration and an enhanced expression of high-affinity LDL receptors (Stephan et al., 1987).

In the present study, we examine the effects of taurine on serum LDL levels and endothelium-dependent relaxation of isolated aortas from both cholesterol-fed and streptozotocin-induced diabetic mice.

2. Materials and methods

2.1. Animals

Male ICR mice aged 5 weeks and weighing 26.7 ± 1.3 g were housed under constant climatic conditions (temperature 21–22°C, relative air humidity 50 ± 5%). The diets and water were given ad libitum to all animals. This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals adopted by the Committee on the Care and Use of Laboratory Animals of Hoshi University, which is accredited by the Ministry of Education.

2.2. Experimental design

Mice were randomly divided into two groups. Control mice received a standard mouse diet and cholesterol-fed mice received a diet supplemented with 2% cholesterol (w/w) and 0.5% cholic acid (w/w). This feeding program was adhered to for 10 weeks and then the experiments were performed.

8–10-week-old male ICR mice received a single injection of streptozotocin (200 mg/kg) in the tail vein in order to induce diabetes. Age-matched controls were injected with a similar volume of citrate buffer. Streptozotocin-induced diabetic mice were fed a normal diet. Food and water were given ad libitum to all animals. The experiments were performed 10 weeks after the injection.

Cholesterol-fed and streptozotocin-induced diabetic mice received saline, taurine (50 or 100 mg/kg p.o. daily for 10 weeks). We administered these drugs at the start of cholesterol-feeding or streptozotocin injection.

2.3. Measurement of isometric force

After 10 weeks of dietary intervention or streptozotocin injection, the age-matched control, cholesterol-fed mice, streptozotocin-induced diabetic mice and hypercholesterolemic and diabetic mice that had been administered drugs were anesthetized with ether. A midline incision was made and blood was obtained from the abdominal aorta to be used to estimate serum cholesterol and serum glucose levels. The blood was centrifuged at 3000 rpm for 10 min at 4°C and the serum was isolated and stored at −80°C. After the rats were killed by bleeding, the aorta was rapidly dissected and placed in ice-cold modified Krebs-Henseleit solution (KHS, composition in mM: NaCl, 118.0; KCl, 4.7; $NaHCO_3$, 25.0; $CaCl_2$, 1.8; NaH_2PO_4, 1.2; $MgSO_4$, 1.2; dextrose, 11.0). Each aorta was separated from surrounding connective tissue and cut into rings (3 mm long). Special care was taken not to damage the endothelium. The rings were then suspended in organ bath chambers, between a clip and a force-displacement transducer (TB-611T, Nihon Kohden, Japan) by means of two stainless steel wires inserted into the lumen, under a resting tension of 1.5 g (preliminary determined to be optimum), to measure isometric force. The organ chamber was filled with 10 ml of KHS at 37°C and gassed with 95% O_2–5% CO_2. Following a 1-h equilibration period, prostaglandin $F_{2\alpha}$ was added to the organ bath at a concentration high enough (10^{-6}–3×10^{-6} M) to induce ring contraction. After the prostaglandin $F_{2\alpha}$-induced contraction reached a plateau, 10^{-5} M acetylcholine was added to the organ bath to confirm the integrity of the endothelium. The aortic rings relaxed completely at this concentration of acetylcholine. The removal of endothelial cells by rubbing was confirmed by the fact that the tonic contraction of the aortic ring induced by prostaglandin $F_{2\alpha}$ was not affected by acetylcholine. The effects of drugs were then tested. The tissue was allowed to relax and equilibrate for 40 min before the next application of drugs. Because the maximal contraction of aortic rings in response to prostaglandin $F_{2\alpha}$ was enhanced significantly in cholesterol-fed mice, for the relaxation studies aortic rings were precontracted with an equieffective concentration of 10^{-6} to 3×10^{-6} M prostaglandin $F_{2\alpha}$ so that the rings would develop a tension of approximately 860 mg. When the prostaglandin $F_{2\alpha}$-induced contraction reached a plateau, the relaxing agents were cumulatively added. The relaxation in response to each agent was expressed as a percentage of the decreased contractile force induced by 10^{-6} to 3×10^{-6} M prostaglandin $F_{2\alpha}$.

2.4. Measurement of serum cholesterol and glucose

Serum cholesterol levels were determined using a commercially available enzyme kit (Wako Chemical Company, Osaka, Japan). The concentration of glucose in serum was determined by the *o*-toluidine method (Dubowski, 1962).

2.5. Drugs

6S-[6α(2S*,3S*),8β(R*),9β,11α]-5-(Methylamino)-2-[[3,9,11-trimethyl-8-[1-methyl-2-oxo-2-(1H-pyrrol-2-yl)-ethyl]-1,7-dioxaspiro[5,5]undec-2-yl]methyl]-4-benzoxazolecarboxylic acid (A23187), streptozotocin and sodium nitroprusside were purchased from Sigma (St. Louis, MO,

USA). Acetylcholine was purchased from Daiichi Pharmaceutical (Tokyo, Japan). Taurine was purchased from Taisho Pharmaceutical (Tokyo, Japan). Prostaglandin $F_{2\alpha}$ was purchased from Ono Pharmaceutical (Osaka, Japan). Prostaglandin $F_{2\alpha}$, sodium nitroprusside and acetylcholine were dissolved in 0.9% saline immediately before each experiment. Concentrations are expressed as the final concentration of each drug in the organ bath.

2.6. Statistics

Data are expressed as the means ± S.E. Statistical differences were measured using the Student's *t*-test for unpaired observations, following a one-way analysis of variance.

3. Results

3.1. Relaxation in response to acetylcholine, A23187 or sodium nitroprusside in age-matched control cholesterol-fed and streptozotocin-induced diabetic mice aortas

When the prostaglandin $F_{2\alpha}$ (10^{-6} to 3×10^{-6} M)-induced contraction reached a plateau, acetylcholine (10^{-9} – 10^{-5} M) or A23187 (10^{-8}–10^{-6} M) was cumulatively added. These data are summarized in Figs. 1–4. In aortic rings from age-matched control mice, acetylcholine (10^{-9}–10^{-5} M) caused concentration-dependent relaxation, with a maximum response at 10^{-5} M (Figs. 1 and 2). The relaxation caused by acetylcholine was significantly decreased in rings from cholesterol-fed and streptozotocin-induced diabetic mice (Figs. 1 and 2). After chronic administration of taurine (50 or 100 mg/kg p.o. daily for 10 weeks), aortic rings from cholesterol-fed and streptozotocin-induced diabetic mice relaxed in a normal way to acetylcholine (Figs. 1 and 2). As shown in Figs. 3 and 4, the decrease in A23187-induced relaxations in both cholesterol-fed and streptozotocin-induced diabetic mice was also preserved by the chronic administration of taurine (100 mg/kg p.o. daily for 10 weeks). Treating the control mice with taurine had no significant effect on the relaxation caused by acetylcholine (data not shown). The relaxation caused by sodium nitroprusside (10^{-9}–10^{-5} M) was not significantly different in aortic rings from the different groups (data not shown). Treating the control mice with taurine had no significant effect on the relaxation caused by sodium nitroprusside (data not shown).

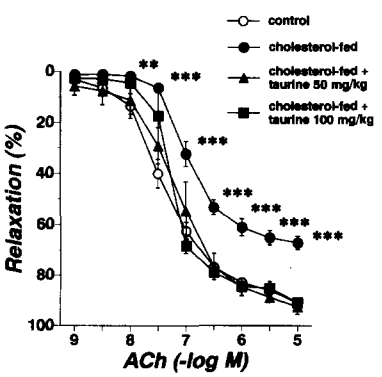

Fig. 1. Concentration–response curves for acetylcholine (ACh)-induced relaxation of aortic rings obtained from age-matched control mice, cholesterol-fed mice and cholesterol-fed mice given taurine. The aortic rings were initially contracted with prostaglandin $F_{2\alpha}$ (10^{-6}–3×10^{-6} M). The ordinate scale represents the relaxation of aortic rings as a percentage of the contraction induced by prostaglandin $F_{2\alpha}$ (10^{-6}–3×10^{-6} M). Each data point on the graph represents the mean S.E. of six experiments; the vertical lines indicate the S.E. and are only included when they exceeded the dimension of the symbols used. ** $P < 0.01$, *** $P < 0.001$.

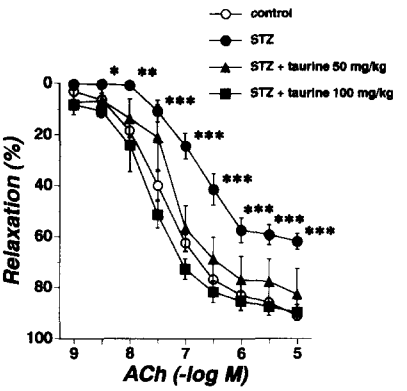

Fig. 2. Concentration–response curves for acetylcholine (ACh)-induced relaxation of aortic rings obtained from age-matched control mice, streptozotocin (STZ)-induced mice and STZ-induced mice that had been treated with taurine. The aortic rings were initially contracted with prostaglandin $F_{2\alpha}$ (10^{-6}–3×10^{-6} M). The ordinate scale represents the relaxation of aortic rings as a percentage of the contraction induced by prostaglandin $F_{2\alpha}$ (10^{-6}–3×10^{-6} M). Each data point on the graph represents the mean S.E. of six experiments; the vertical lines indicate the S.E. and are only included when they exceeded the dimension of the symbols used. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

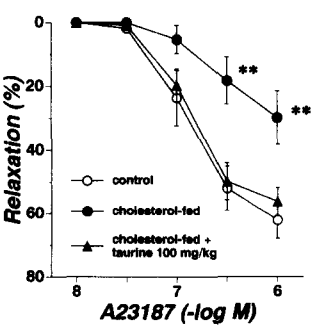

Fig. 3. Concentration–response curves for A23187-induced relaxation of aortic rings obtained from age-matched control mice, cholesterol-fed mice and cholesterol-fed mice given taurine. The aortic rings were initially contracted with prostaglandin $F_{2\alpha}$ (10^{-6}–3×10^{-6} M). The ordinate scale represents the relaxation of aortic rings as a percentage of the contraction induced by prostaglandin $F_{2\alpha}$ (10^{-6}–3×10^{-6} M). Each data point on the graph represents the mean S.E. of six experiments; the vertical lines indicate the S.E. and are only included when they exceeded the dimension of the symbols used. ** $P < 0.01$.

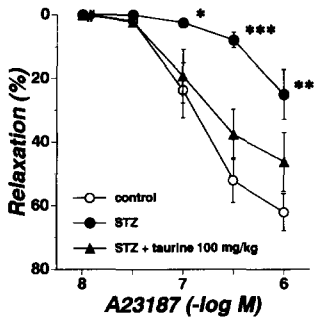

Fig. 4. Concentration–response curves for A23187-induced relaxation of aortic rings obtained from age-matched control mice, streptozotocin (STZ)-induced mice and streptozotocin-induced mice that had been treated with taurine. The aortic rings were initially contracted with prostaglandin $F_{2\alpha}$ (10^{-6}–3×10^{-6} M). The ordinate scale represents the relaxation of aortic rings as a percentage of the contraction induced by prostaglandin $F_{2\alpha}$ (10^{-6}–3×10^{-6} M). Each data point on the graph represents the mean S.E. of six experiments; the vertical lines indicate the S.E. and are only included when they exceeded the dimension of the symbols used. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

3.2. *Effects of chronic administration of taurine on serum cholesterol and glucose levels in cholesterol-fed and streptozotocin-induced diabetic mice*

In cholesterol-fed and streptozotocin-induced diabetic mice, serum total cholesterol and LDL cholesterol levels were significantly increased (Figs. 5 and 6). Chronic administration of taurine (50 or 100 mg/kg p.o. daily for 10 weeks) significantly reduced the total cholesterol and LDL cholesterol levels in cholesterol-fed and streptozotocin-induced diabetic mice as shown in Figs. 5 and 6. Serum glucose levels were not different between age-matched control mice, cholesterol-fed mice and cholesterol-fed mice that received taurine (Fig. 5). The increased levels of serum glucose in streptozotocin-induced diabetic mice were not affected by the chronic administration of taurine (Fig. 6).

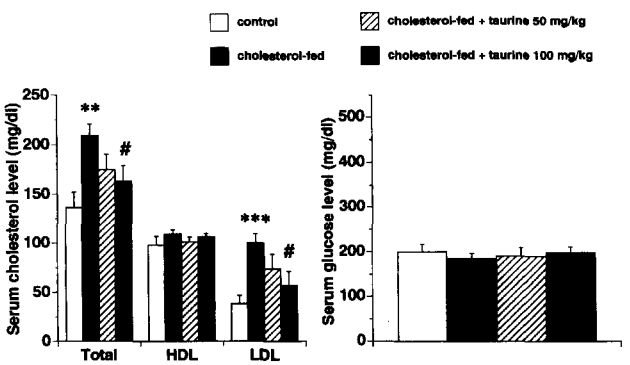

Fig. 5. Effects of taurine on levels of cholesterol and glucose in cholesterol-fed mice. Cholesterol-fed mice received taurine (50 or 100 mg/kg p.o. daily for 10 weeks). ** $P < 0.01$, *** $P < 0.001$, cholesterol vs. control; # $P < 0.05$, cholesterol-fed group vs. cholesterol-fed mice receiving drug.

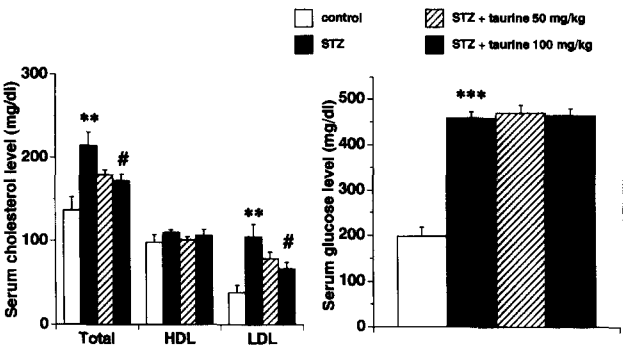

Fig. 6. Effects of taurine on levels of cholesterol and glucose in streptozotocin (STZ)-induced diabetic mice. Streptozotocin-induced mice received taurine (50 or 100 mg/kg p.o. daily for 10 weeks). ** $P < 0.01$, *** $P < 0.001$, diabetic vs. control; # $P < 0.01$, diabetic group vs. streptozotocin-diabetic mice receiving drugs.

4. Discussion

In the present study, we found that chronic administration of taurine normalizes the endothelium-dependent relaxation of isolated aorta from cholesterol-fed and streptozotocin-induced diabetic mice and significantly reduces the total cholesterol and LDL cholesterol levels in cholesterol-fed and streptozotocin-induced diabetic mice.

A reduction in the release of endothelium-derived relaxing factor (EDRF) from the vascular endothelium and a decrease in endothelium-dependent relaxation have been demonstrated in vascular tissue obtained from cholesterol-fed rabbits and in human atherosclerotic coronary arteries (Freiman et al., 1986; Habib et al., 1986; Verbeuren et al., 1986; Bossaller et al., 1987; Jayakody et al., 1987; Yamamoto et al., 1987; Forstermann et al., 1988; Shimokawa and Vanhoutte, 1989; Simon et al., 1993). Consistent with these findings, we found that acetylcholine-induced endothelium-dependent relaxation was significantly attenuated in cholesterol-fed mice. Since the endothelium-independent relaxation of mouse aortic rings by sodium nitroprusside was not changed in cholesterol-fed mice, the activity of guanylate cyclase in the smooth muscle of the aorta was not altered. These results suggest that the mouse is also useful as an animal model of hypercholesterolemia.

Impaired endothelium-dependent relaxation has been observed in blood vessels of genetically diabetic rats (Durante et al., 1988; Kappagoda et al., 1989; Miyata et al., 1992, 1993), streptozotocin-induced diabetic rats (Oyama et al., 1986; Pieper and Gross, 1988; Kamata et al., 1989a,b, 1992; Abiru et al., 1993; Poston and Taylor, 1995) and alloxan-induced diabetic rabbits (Tesfamariam et al., 1989; Abiru et al., 1990a,b, 1991). In the present study, we used mice as an animal model of diabetes and have shown that acetylcholine-induced endothelium-depen-

dent relaxation was reduced in streptozotocin-induced diabetic mice, suggesting that the mouse is also useful as an animal model of diabetes.

Dietary supplementation with taurine increases the activity of 7α-hydroxylase, which is the rate-limiting enzyme in bile acid formation (Kallis and Wikvall, 1981). These findings suggest that taurine, by stimulating 7α-hydroxylase activity and resultant bile acid synthesis and by enhancing LDL receptor binding, may affect the development of atherosclerosis (Petty et al., 1990). Although cholesterol-lowering drugs have a beneficial effect on lipids, there are few reports concerning the positive effects of these agents on endothelial dysfunction in cholesterolemia or diabetic states after the chronic administration with these agents in animals. In cholesterol-fed and streptozotocin-induced diabetic mice, serum total cholesterol and LDL cholesterol levels were significantly increased and the increased cholesterol levels were normalized by the chronic administration of taurine. At least two explanations are possible with respect to the cholesterol-lowering effects of taurine: an increase in the conversion of cholesterol to bile acids due to the enhanced activity of hepatic cholesterol 7α-hydroxylase, the rate-limiting enzyme for bile acid synthesis, and an increase in bile acid conjugation due to the increased taurine concentration in the liver, with a subsequent increase in biliary bile acid secretion. It is, furthermore, likely that an enhanced LDL receptor binding in the liver produced by chronic administration of taurine may also be responsible for the cholesterol-lowering effects of taurine. The endothelium-dependent relaxation of aortic rings in response to acetylcholine was significantly attenuated in cholesterol-fed and streptozotocin-induced diabetic mice and the impaired endothelium-dependent relaxation was restored by the chronic administration of taurine. These results suggest that the endothelial dysfunction in cholesterol-fed and streptozotocin-induced diabetic mice is due to the increased LDL and that endothelium-dependent relaxation may be preserved by the chronic administration of taurine, at least in part, through lowering the serum LDL levels.

Endothelium dysfunction is intimately involved in the pathogenesis of atherosclerosis (Ross, 1986; DiCorleto and Chisolm, 1986; Bossaller et al., 1987; Steinberg et al., 1989; Yasue et al., 1990). The oxidative modification of LDL cholesterol by the endothelium is thought to be an important step in the alteration of various endothelial functions (Kugiyama et al., 1990; Jacob et al., 1990; Rajavashisth et al., 1990; Simon et al., 1990; Witztum and Steinberg, 1991; Flavahan, 1992) and the initiation of atherosclerosis (Steinberg et al., 1984). Lysophosphatidylcholine, which is transferred from oxidized LDL to the endothelial surface membrane, is involved in the mechanisms of attenuation of the endothelial function caused by oxidized LDL (Kugiyama et al., 1990, 1992; Yokoyama et al., 1990; Flavahan, 1993; Sugiyama et al., 1994). Indeed, we confirmed that endothelium-dependent relaxation of aortic rings in response to acetylcholine was significantly attenuated by pretreatment with lysophosphatidylcholine (Kamata et al., 1996). Therefore, the release of lysophosphatidylcholine from oxidized LDL must play an important role in endothelial function. If this is the case, the following sequence of events occurs in cholesterol-fed and streptozotocin-induced diabetic mice: serum LDL levels are increased in cholesterol-fed and streptozotocin-induced diabetic mice; increased LDL is oxidized on the endothelium; lysophosphatidylcholine is transferred from oxidized LDL; lysophosphatidylcholine may inhibit the endothelium-dependent vasodilation induced by agonists, thereby resulting in endothelial dysfunction in cholesterol-fed and streptozotocin-induced diabetic mice. In the present study, the chronic administration of taurine significantly lowered serum LDL levels. This cholesterol-lowering effect of taurine may improve the endothelium-dependent relaxation.

Oxygen radicals, which inactivate EDRF, have been implicated in the impaired endothelium-dependent relaxation of the blood vessels from streptozotocin-induced diabetic rats (Hattori et al., 1991; Langenstroer and Pieper, 1992; Pieper et al., 1993). Indeed, Pieper et al. (1992) have demonstrated, using a bioassay technique for EDRF, that free radicals mediate the destruction of nitric oxide (NO) in diabetic rat aorta. It is unclear at present whether increased LDL or oxidized LDL in the diabetic state can produce superoxide anions in the rat aorta. This requires further investigation.

Since A23187-induced relaxation was also reduced in both cholesterol-fed and streptozotocin-induced diabetic mice, the impairment of endothelium-dependent relaxation by high levels of cholesterol is not mediated by a muscarinic receptor antagonistic action.

In conclusion, we demonstrated that endothelium-dependent relaxation is significantly attenuated in aortic rings from cholesterol-fed and streptozotocin-induced diabetic mice. Chronic administration of taurine reduces serum LDL cholesterol levels and normalizes the endothelium-dependent relaxation.

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