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Δ^9-tetrahydrocannabinol treatment improved endothelium-dependent relaxation on streptozotocin/nicotinamide-induced diabetic rat aorta

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Objective: In this study, we investigated the possible effect of Δ^9-tetrahydrocannabinol (THC), a peroxisome proliferator-activated receptor gamma (PPARγ) agonist, on metabolic control and vascular complications of diabetes in streptozotocin/nicotinamide (STZ/NIC) induced type 2 diabetes mellitus. *Material and methods:* Type 2 diabetes was induced with 65 mg/kg STZ, 15 minute later 85 mg/kg NIC was given intraperitoneally (i.p.) to rats. Three days after diabetes induction, THC (3 mg/kg/day, i.p.) was given for 7 days to diabetic rats. Body weight and plasma glucose levels of rats were measured in all groups before and at the end of 3 weeks after diabetes induction. Acetylcholine (Ach) and sodium nitroprusside (SNP) potency and maximum relaxant effects were calculated on aortic rings pre-contracted with noradrenaline (NA). *Results:* At the end of 3 weeks, blood glucose levels of diabetic group significantly increased in comparison with the control group. Increased plasma glucose levels were significantly decreased by the treatment of THC. Ach induced relaxation was impaired whereas endothelium-independent relaxation to SNP was unaffected on isolated diabetic rat aorta. THC treatment enhanced Ach induced relaxation on diabetic rat aortas. *Discussion:* These results suggested that THC improved endothelium-dependent relaxation in STZ/NIC induced diabetic rat aorta and that these effects were mediated at least in part, by control of hyperglycemia and enhanced endothelial nitric oxide bioavailability.

Keywords: rat, aorta, Δ^9-tetrahydrocannabinol, type 2 diabetes mellitus, STZ/NIC induced diabetes, endothelial dysfunction

Type 2 diabetes mellitus comprises an array of dysfunctions resulting from the combination of resistance to insulin and inadequate insulin secretion. These disorders are characterized by hyperglycemia and associated with microvascular and macrovascular complications. In both diabetic patients and animal models, endothelial dysfunction is asserted to have a key role in the progression of macro- and microangiopathy (5, 33).

The most psychoactive substance in cannabis is Δ^9-tetrahydrocannabinol (THC) and its derivatives have recently taken interest for researchers because of the possible therapeutic use in diabetes. Although the evidence regarding the effects of cannabis on diabetes is complex, ranging from anecdotal reports on benefits and harms to experimental research on

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cannabinoids (7). *In vitro* studies have shown that THC can be useful in diabetes treatment via increasing insulin secretion (20), insulin sensitivity (10) and its antioxidant effects (4).

THC might have beneficial effects on diabetes, as it activates the peroxisome proliferator-activated receptor-γ (PPARγ) (25). PPARγ is a ligand-activated transcription factor, belonging to the nuclear receptor superfamily and is a regulator of lipid and glucose metabolism (29). PPARγ agonists improve insulin sensitivity, cause vasorelaxation and increase nitric oxide bioavailability; besides these, it also has anti-inflammatory effects. Because of these properties, it is one of the options for treating diabetes and avoiding possible complications (6, 15).

It is understood that no animal model is identical to any human disease; none of the available animal models of type 2 diabetes mellitus exactly simulate the human type 2 diabetes mellitus. However, streptozotocin/nicotinamide (STZ/NIC) rat models have several advantages over the other models and are considered to be one of the suitable experimental animal models for type 2 diabetes mellitus. For that reason, we used STZ/NIC combination in adult rats to generate type 2 diabetes modeling (19, 27).

In previous studies, it was shown that administration of THC affected the vascular activity in rat aorta (25). However, there is no published data regarding the effect of *in vivo* administration of THC on vascular activity of diabetic rat aorta.

The aim of this study was to investigate the effect of THC treatment on the vascular activity of STZ/NIC induced diabetic rat aorta.

Materials and Methods

Animals

Male Sprague-Dawley rats (8–10 weeks) were randomly divided into four groups. Each group consisted of 6 rats. THC was imported by the permission (permit no. 2010/80) of the Drugs Department of Turkey Republic, Ministry of Health, General Directorate of Pharmaceuticals and Pharmacy. The experimental procedures were approved (approval no: 2012/94) by Animal Ethical Committee of the Istanbul University. The first group (control group) received physiological saline solution, i.p. for 7 days. The second group comprised of STZ/NIC induced diabetic control rats. Diabetes was induced by a single i.p. dose of 65 mg/kg streptozotocin (STZ), 15 minute after the administration of 85 mg/kg, i.p. nicotinamide (NIC). STZ and NIC were freshly prepared in saline solution. Plasma glucose levels were determined by using tail vein blood samples (Accu-check, Roche Diagnostics), 72 h after the STZ/NIC injection. Rats with glucose concentration of 200 mg/dl or higher were considered as diabetic. The third group was the THC-treated group (3 mg/kg/day, i.p for 7 days), and the fourth group was the THC-treated diabetic group. Three days after the diabetes induction, THC (3 mg/kg/day, i.p.) was given for 7 days in the diabetic group. Body weight and plasma glucose of rats were measured in all groups before and at the end of 3 weeks after diabetes induction. All rats were kept under identical conditions for 3 weeks with free access to food and water.

Drugs

THC was provided by Lipomed THC-135-100LE. STZ used in the experiments was obtained from Sigma Chemical (St. Louis, MO, USA) and other chemicals were obtained from Merck (Darmstadt, Germany).

Experimental design

The rats were anestethetized with sodium thiopenthal, on day 15 following the last 9-THC injections. After sacrifice, the thoracic aorta was rapidly removed and placed into Krebs–Henseleit solution (KHS, nM: NaCL 119, KCL 4.70, $MgSO_4.7H_20$ 1.20, KH_2PO_4 1.20, $CaCl_2$ 2.50, $NaHCO_3$ 25, Glucose 11.1), cut into 3–4 mm aorta rings and was mounted with a resting tension of 1 g in an isolated organ bath (Ugo-basile, 4050, Italy) containing 25 ml KHS, aerated (95% O_2 and 5% CO_2) at 37 °C. The responses of isolated organs were recorded isometrically using a force-displacement transducer (BİOPAC MP36, USA) integrated Tissue Bath System (Commat, Ankara, Turkey). Following a one-hour incubation period, concentration-response relationships of the aorta were obtained with doses of noradrenaline (NA). NA (10^{-9}–10^{-4} mol/l) was added in a cumulative manner until a maximal response was achieved. After the addition of each dose, a plateau response was obtained before the addition of a subsequent dose. A cumulative relaxation curve to acetylcholine (Ach) (10^{-9}–10^{-4} mol/l) was obtained in each strip pre-contracted submaximally (EC90 10^{-5}) by addition of NA. A cumulative relaxation curve to sodium nitroprusside (SNP) (10^{-9}–10^{-4} mol/l) was obtained for each strip pre-contracted submaximally (approx. EC90 10^{-5} mol/l) by addition of NA. At the end of each experiment, the tissue was blotted dry, measured and weighed.

Statistical analysis

All isolated organ responses were expressed as apparent affinity constant (pD_2) and percentage of corresponding maximal responses to each agonist (E_{max} for NA and % $Inh._{max.}$ for Ach.). Contractile responses to noradrenaline were calculated as the increase in tension in milligrams, in response to agonist per milligram of aorta (mg tension / mg wet weight). The statistical analyses were performed by one-way ANOVA, followed by Tukey's multiple comparison tests for isolated-organ bath experiments. The statistical analyses were carried out using SPSS version 11.0 and Microsoft Office Excel. The values were expressed as the mean ± SEM to show variation in groups. $p < 0.05$ was considered statistically significant.

Results

General characteristics of animals

Body weight and blood glucose levels for four groups are shown in Table I. Body weight of rats did not change initially and even after three weeks in any of the groups. THC treatment did not affect the final weight of rats.

As indicated in Table I, plasma glucose levels were significantly elevated in diabetic rats (409.71 ± 29.12 mg/dl) in comparison with control rats (106.83 ± 2.75 mg/dl, $p < 0.05$). The increase in plasma glucose levels were significantly decreased by the treatment with THC (298.17 ± 40.18 mg/dl, $p < 0.05$).

Contractile response to noradrenaline

The contraction values, pD_2 and E_{max} of aortic strips as a response to NA of all experimental groups are shown in Table III. pD_2 values of NA were significantly decreased in diabetic rats (7.72 ± 0.10) compared with the control rats (8.15 ± 0.08, $p < 0.05$). THC treatment could not improve the decreased pD2 values of NA in diabetic rats (7.49 ± 0.05). E_{max} of NA did not change in aortic strips of all groups (Fig. 3).

Table I. Body weight and blood glucose levels of groups

After 3 weeks		
Blood glucose (mg/dl)		**Body weight (g)**
Control	106.83 ± 2.75	214.67 ± 7.48
Diabetic	409.71 ± 29.12[a]	193.33 ± 12.10
THC	124.67 ± 8.56	199.25 ± 11.68
Diabetic+THC	298.17 ± 40.18[a,b]	189.75 ± 33.17

Values are mean ± SEM, [a] $p < 0.05$ compared to the control and [b] $p < 0.05$ compared to the diabetic group. Plasma glucose levels were elevated in STZ/NIC-induced type 2 diabetes and decreased by the treatment of THC. THC treatment did not affect the body weight of STZ/NIC-induced type 2 diabetic rats

Table II. pD_2 and $Inh._{max.}$ % values for Ach and SNP-induced relaxations of aortic rings of groups

	Ach		**SNP**	
	$Inh._{max.}$	**% pD_2**	**$Inh._{max.}$**	**% pD_2**
Control	88.30 ± 6.06	7.73 ± 0.26	92.61 ± 3.04	8.93 ± 0.16
Diabetic	52.97 ± 6.32[a]	6.34 ± 0.18[a]	98.67 ± 2.30	8.40 ± 0.23
THC	80.78 ± 2.79	7.38 ± 0.17	97.25 ± 2.99	8.27 ± 0.20
Diabetic+THC	73,94 ± 3,18[ab]	7.48 ± 0.18[b]	101.31 ± 3.99	8.34 ± 0.10

Values are mean ± SEM, [a] $p < 0.05$ compared to the control and [b] $p < 0.05$ compared to the diabetic group. Sensivity (pD_2) and Maximum Relaxation ($Inh._{max.}$ %) of Ach were decreased on the STZ/NIC-induced type 2 diabetic group and improved with THC treatment

Table III. pD_2 and E_{max} values (mg / mg ww) for NA-induced contraction of aortic rings from groups

	NA-E_{max}	**NA-pD_2**
Control	258.58 ± 29.42	8.15 ± 0.08
Diabetic	256.58 ± 28.60	7.72 ± 0.10[a]
THC	284.86 ± 54.03	8.16 ± 0.15
Diabetic+THC	311.73 ± 31.29	7.49 ± 0.05[a]

Values are mean ± SEM, [a] $p < 0.05$ compared to the control and [b] $p < 0.05$ compared to the diabetic group. Sensivity (pD_2) of NA were decreased on the STZ/NIC-induced type 2 diabetic and THC-treated STZ/NIC-induced diabetic rat aorta

Vascular relaxation

Endothelium-dependent relaxation values as a response to Ach (pD_2, and $Inh._{max.}$ %) of aortic strips pre-contracted with NA (submaximally, approx. EC90), are summarized in Table II. pD_2 of Ach were decreased in the diabetic rats ($pD_2 = 6.34 \pm 0.18$, $p < 0.05$) compared with the control rats ($pD_2 = 7.73 \pm 0.26$) and were improved in THC-treated diabetic rats (pD2 = 7.48 ± 0.18, $p < 0.05$). $Inh._{max.}$ % of Ach ($Inh._{max.}\% = 52.97 \pm 6.32$, $p < 0.05$) were decreased in the diabetic rats compared to those values of control rats ($Inh._{max.}\% = 88.30 \pm 6.06$) and they were improved in THC-treated diabetic rats ($Inh._{max.}\% = 73.94 \pm 3.18$, $p < 0.05$) (Fig. 1).

pD_2 and $Inh._{max.}$ % of SNP did not change in aortic strips in any of the groups (Table II and Fig. 2).

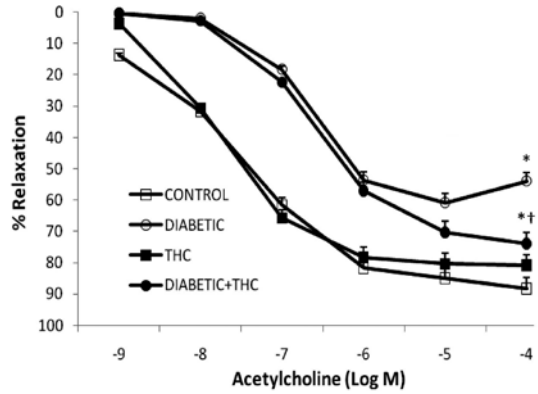

Fig. 1. Acetylcholine (Ach) concentration-response curves in noradrenaline (NA, 10^{-6}M)-precontracted rat aorta rings. Diabetes decreased relaxation responses to Ach and treatment with THC improved it. *$p < 0.05$ compared to the control, *†$p < 0.05$ compared to the diabetic group. Each point is the mean ± SEM of experiments

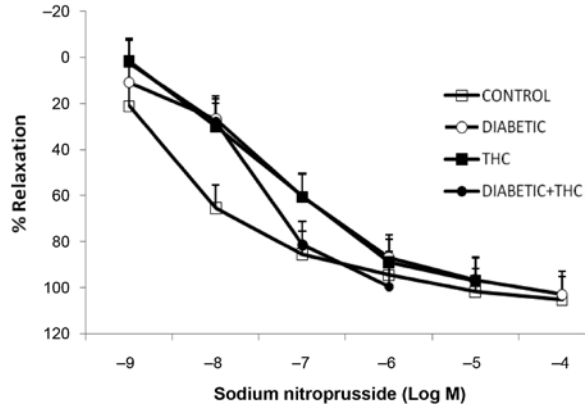

Fig. 2. Sodium nitroprusside (SNP) concentration-response curves in noradrenaline (NA, 10^{-6}M)-precontracted rat aorta rings. Neither diabetes nor treatment with THC affected relaxation responses to SNP. Each point is the mean ± SEM of experiments

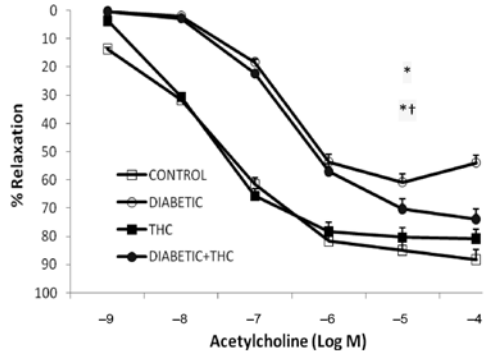

Fig. 3. Contractile response curves of noradrenaline (NA) in rat aorta rings. Neither diabetes nor treatment with THC affected contraction responses to NA. Each point is the mean ± SEM of experiments

Discussion

Type 2 diabetes mellitus is one of the most prevalent metabolic disorders worldwide. Medications in use have intended controlling blood glucose levels, hence the complications of the type 2 diabetes mellitus. It has been claimed that cannabis and its derivative compounds can be used in diabetes mellitus treatment. Therefore, major active components of cannabis THC have been tested for the treatment of type 2 diabetes mellitus and its vascular complications.

THC appears principally to exert its pharmacological action by stimulating the endocannabinoid system, via the cannabinoid cell-surface receptors CB1 and CB2. This system appears to have a role in the regulation of body weight and food intake (22).

In human studies, stimulation of CB1 receptors by THC has been shown to cause increased food intake, blockade of these receptors, such as by treatment with the selective CB1 receptor antagonist rimonabant, has been shown to have beneficial effects on diabetes, such as weight loss (14). However, the present study indicated that THC treatment did not cause weight gain in normal and diabetic rats. And this is thought to be the cause of administration dose and/or duration of THC.

STZ/NIC rat models have several advantages and are considered to be one of the most suitable experimental animal models of type 2 diabetes mellitus. For that reason, STZ/NIC combination was used to generate type 2 diabetes models (19).

Several studies have indicated that THC interferes in both the release of insulin and its action. It has been found that THC increases insulin secretion via CB1 and CB2 receptors (16, 20), whereas, in contrast to the above findings some studies have shown that THC decreases insulin secretion via CB1 receptors in pancreas and causes beta cell apoptosis (19, 25).

Besides, literature has also shown that THC enhances insulin action by decreasing TNF-α level (35) and increasing gene expression of IRS-1, IRS-2 and GLUT4 (10). PPARγ agonists are now widely used in the management of type 2 diabetes to improve insulin sensitivity (31) by increasing IRS-1, IRS-2, PKB/Akt, and GLUT4 (37). These effects on insulin sensitivity can be thought as THC being a PPAR gamma receptor agonist (17).

The data of the present study indicated that plasma glucose levels significantly decreased in THC-treated STZ/NIC-induced type 2 diabetic rats. Most of the studies about the effect of THC on glucose metabolism are *in vitro* studies done in cell culture. Our study is an *in vivo* study that shows the systemic net effect of THC on glucose metabolism, so the results can be extrapolated to diabetes associated with an increased risk for vascular deterioration. Endothelial cells regulate basal vascular tone and vascular reactivity with the release of a variety of contracting and relaxing factors (9). It has been shown that hyperglycemia, which is one of the most important symptoms of diabetes, can cause endothelial cell damage by several mechanisms, including apoptosis and increased reactive oxygen species formation (11, 30, 32).

The effects of diabetes on vascular responsiveness of the rat aorta have been widely studied but there are conflicting results. Although, some researchers demonstrated that the maximum relaxation response of Ach decreased but not the sensitivity in diabetes (28, 34). The results of the present study, in accordance with others (12, 15) revealed that diabetes decreased the maximum relaxation and the sensitivity of Ach.

In our study THC treatment enhanced the maximum relaxation ($Inh._{max}$) responses and the sensitivity (pD_2) of Ach in diabetic rat aortas.

Hyperglycemia is the major causal factor in the development of endothelial dysfunction in patients with diabetes mellitus. Controlled hyperglycemia remains the best way to improve endothelial function and to prevent atherosclerosis and other cardiovascular complications of diabetes. Because of that, THC treatment shows an improving effect on endothelial dysfunction in diabetic rats and is basically linked with blood glucose regulation.

In type 2 diabetes, hyperglycemia causes the production of reactive oxygen species (ROS) that makes oxidative injury. Also, oxidative stress plays critical roles in the development of diabetes complications. Recent studies have shown that the endocannabinoid system may significantly influence reactive oxygen species production, and subsequent tissue injury (14). Some studies have shown that THC acts as potent antioxidant without any cannabinoid receptor activation (12).

One of the PPAR gamma agonist, rosiglitazone has antioxidant effects and it increases NO bioavailability and catalase activity independently from blood glucose regulation (2); besides THC does the same effect via increasing superoxide dismutase activity (25). Therefore, these results show that THC might contribute to decrease the vascular complications of diabetes.

On the other hand, insulinotropic effect of THC (16, 20) can indirectly affect its relaxant response, because insulin itself may relax smooth muscle by releasing NO.

So these findings indicate that THC improves vascular responsiveness in diabetic rat aortas, and these effects are mediated, at least in part, by control of hyperglycemia and enhanced endothelial nitric oxide bioavailability by PPAR gamma agonism (1, 18).

It is known that SNP acts through direct stimulation of vascular smooth muscle cells independently from an intact endothelium. Some of the previous studies claimed that SNP-induced endothelium-independent relaxation decreased in diabetes (23). However many others have shown that diabetes had no effect on SNP responses (13). Our results show that SNP responses were not different between control and other groups. THC improved the endothelium-dependent response to Ach but did not change the endothelium-independent response to SNP.

In vitro studies have indicated that the cannabinoids have affinities to other receptors besides cannabinoid receptors, when applied directly into the organ bath (8, 25, 36). Among them is PPAR gamma receptors. It has been found that THC is a PPAR gamma ligand and causes vasorelaxation in isolated aorta via PPAR gamma receptors and independently from CB1 receptors (38).

However in present the study, the administration of THC for 3 mg/kg/day for 7 days did not change the maximum relaxation responses and sensitivity of Ach in non-diabetic rat thoracic aorta.

Previous studies showed no vasodilating effect of THC (21). It can be speculated that THC was administered systemically not directly into organ bath.

Many studies reported inconsistent results with increased (3, 24) or unchanged (36) responsiveness to NA in aortic rings of diabetic rats. The present study demonstrates that NA-induced maximum contraction did not change in aortic rings of diabetic rats but pD_2 values of NA were significantly decreased in diabetic rats and THC-treated diabetic rats compared with the control rats. Some of the previous studies (26) have claimed that administration of THC causes acute vasoconstriction in different arteries. But we did not see any vasoconstrictive effect of *in vivo* used THC. As discussed above, the reason of this difference can be that THC was not administered directly into the organ bath, but given *in vivo* to the rat. THC treatment improved the control of hyperglycemia in diabetic rats and

Ach-induced relaxation but did not change SNP-induced relaxation and NA-induced maximum contraction in diabetic rat aortic ring responses. These findings indicate that THC improved vascular responsiveness in diabetic rat aortas, and that these effects were mediated by improving hyperglycemia and at least in part, by enhanced endothelial nitric oxide bioavailability by PPAR gamma agonism.

In our study, we showed that THC given 3 mg/kg/day for 7 days decreased hyperglycemia without any weight gain in diabetic rats, and as linked to that ameliorated Ach-induced endothelium-dependent relaxation responses. But we also need further studies that will show the effects of long term and different dose usage of THC. As, we think that, in long-term administration, THC may increase food intake and so may result in weight gain and might worsen the prognosis of diabetes.

These effects suggest the possible beneficial effects of THC in *in vivo* experimental diabetes and its vascular complications.

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REFERENCES

1. Ajay M, Achike FI, Mustafa AM, Mustafa MR: Effect of quercetin on altered vascular reactivity in aortas isolated from streptozotocin-induced diabetic rats. Diabetes Res. Clin. Pract. 73, 1–7 (2006)
2. Bagi Z, Koller A, Kaley G: PPAR gamma activation, by reducing oxidative stress, increases NO bioavailability in coronary arterioles of mice with type 2 diabetes. Am. J. Physiol. 286, 742–748 (2004)
3. Bisogno T, Melck D, Bobrov MYu, Gretskaya NM, Bezuglov VV, De Petrocellis L, Di Marzo V: N-acyl-dopamines: novel synthetics CB(1) cannabinoid-receptor ligands and inhibitors of anandamide inactivation with cannabimimetic activity in vitro and in vivo. Biochem. 351, 817–824 (2000)
4. Carroll CB, Zeisler ML, Hanemann CO, Zajicek JP: Δ9-THC exerts a direct neuroprotective effect in a human cell culture model of Parkinson's disease Neuropathol. Appl. Neurobiol. 38, 535–547 (2012)
5. De Vriese AS, Verbeuren TJ, Van de Voorde J, Lameire NH, Vanhoutte PM: Endothelial dysfunction in diabetes. Br. J. Pharmacol. 130, 963–974 (2000)
6. Ferré P: The biology of peroxisome proliferator-activated receptors: relationship with lipid metabolism and insulin sensitivity. Diabetes 53, 43–50 (2004)
7. Frisher M, White S, Varbiro G, Voisey C, Perumal D, Crome I, Khideja N, Bashford J: The role of cannabis and cannabinoids in diabetes. The British Journal of Diabetes and Vascular Disease 10, 267–273 (2010)
8. Fortes ZB, Garcia LJ, Scivoletto R: Vascular reactivity in diabetes mellitus: Role of the endothelial cell. Br. J. Pharmacol. 79, 771–781 (1983)
9. Furchgott RF, Vanhoutte PM: Endothelium-derived relaxing and contracting factors. FASEB J. 3, 2007–2018 (1989)
10. Gallant M, Odei-Addo F, Frost CL, Levendal RA: Biological effects of THC and a lipophilic cannabis extract on normal and insulin resistant 3T3-L1 adipocytes Phytomedicine 16, 942–949 (2009)
11. Hammes HP, Federoff HJ, Brownlee M: Nerve growth factor prevents both neuroretinal programmed cell death and capillary pathology in experimental diabetes. Mol. Med. 1, 527–534 (1995)
12. Hampson AJ, Grimaldi M, Axelrod J, Wink D: Cannabidiol and (–)Delta9-tetrahydrocannabinol are neuroprotective antioxidants. Proc. Natl. Acad. 95, 8268–8273 (1998)
13. Ho WS, Hiley CR: Vasodilator actions of abnormal-cannabidiol in rat isolated small mesenteric artery. Br. J. Pharmacol. 138, 1320–1332 (2003)
14. Horváth B, Mukhopadhyay P, Haskó G, Pacher P: The endocannabinoid system and plant-derived cannabinoids in diabetes and diabetic complications. Am. J. Pathol. 180, 432–442 (2012)
15. Hsueh WA, Bruemmer D: Peroxisome proliferator-activated receptor gamma: implications for cardiovascular disease. Hypertension 43, 297–305 (2004)

16. Klein TW, Lane B, Newton CA, Friedman H: The cannabinoid system and cytokine network. Proc. Soc. Exp. Biol. Med. 225, 1–8 (2000)
17. Liu J, Li H, Burstein SH, Zurier RB, Chen JD: Activation and binding of peroxisome proliferator-activated receptor gamma by synthetic cannabinoid ajulemic acid. Mol. Pharmacol. 63, 983–992 (2003)
18. Majithiya JB, Paramar AN, Balaraman R: Pioglitazone, a PPAR gamma agonist, restores endothelial function in aorta of streptozotocin-induced diabetic rats. Cardiovasc. Res. 66, 150–161 (2005)
19. Masiello P, Broca C, Gross R, Roye M, Manteghetti M, Hillaire-Buys D, Novelli M, Ribes G: Experimental NIDDM: development of a new model in adult rats administered streptozotocin and nicotinamide. Diabetes 47, 224–229 (1998)
20. Matias I, Gonthier MP, Orlando P, Martiadis V, De Petrocellis L, Cervino C, Petrosino S, Hoareau L, Festy F, Pasquali R, Roche R, Maj M, Pagotto U, Monteleone P, Di Marzo V: Regulation, function and dysregulation of endocannabinoids in models of adopise and β-pancreatic cells in obesity and hyperglycaemia. J. Clin. Endocrinol. Metab. 91, 3171–3180 (2006)
21. Mulhern M, Docherty JR: Effect of experimental diabetes on the responsiveness of rat aorta. Br. J. Pharmacol. 97, 1007–1012 (1989)
22. Nogueiras R, Veyrat-Durebex C, Suchanek PM, Klein M, Tschöp J, Caldwell C, Woods SC, Wittmann G, Watanabe M, Liposits Z, Fekete C, Reizes O, Rohner-Jeanrenaud F, Tschöp MH: Peripheral, but not central, CB1 antagonism provides food intake-independent benefits in diet-induced obese rats. Diabetes 57, 2977–2991 (2008)
23. Offertáler L, Mo FM, Bátkai S, Liu J, Begg M, Razdan RK, Martin BR, Bukoski RD, Kunos G: Selective ligands and cellular effectors of a G protein-coupled endothelial cannabinoid receptor. Mol. Pharmacol. 63, 699–705 (2003)
24. O'Sullivan SE, Kendall DA, Randall MD: The vascular effects of Δ^9-tetrahydrocannabinol (THC) and its interactions with the endocannabinoid anandamide. Br. J. Pharmacol. 145, 514–526 (2005)
25. O'Sullivan SE, Tarling EJ, Bennett AJ, Kendall DA, Randall MD: Novel time-dependent vascular actions of Delta9-tetrahydrocannabinol mediated by peroxisome proliferator-activated receptor gamma. Biochem. Biophys. Res. Commun. 25, 824–831 (2005)
26. O'Sullivan SE, Kendall DA, Randall MD: Vascular effects of D9-tetrahydrocannabinol (THC), anandamide and N-arachidonoyldopamine (NADA) in the rat isolated aorta. Eur. J. Pharmacol. 507, 211–221 (2005)
27. Ozyazgan S, Kutluata N, Afşar S, Ozdaş SB, Akkan AG: Effect of glucagon-like peptide-1(7-36) and exendin-4 on the vascular reactivity in streptozotocin/nicotinamide-induced diabetic rats. Pharmacology 74, 119–126 (2005)
28. Özçelikay AT, Tay A, Dinçer T, Meral S, Yildizoğlu-Ari N, Altan VM: The effects of chronic L-arginine treatment on vascular responsiveness of streptozotocin-diabetic rats. Gen. Pharmacol. 33, 299–306 (1999)
29. Picard F, Auwerx J: PPAR(γ) and glucose homeostasis. Annu. Rev. Nutr. 22, 167–197 (2002)
30. Pieper GM, Gross GJ: Selective impairment of endothelium-dependent relaxation by oxygen-derived free radicals: distinction between receptor versus non-receptor mediator. Blood Vessels 26, 44–47 (1989)
31. Rangwala SM, Lazar MA: Peroxisome proliferator-activated receptor gamma in diabetes and metabolism. Trends Pharmacol. 25, 331–336 (2004)
32. Sakuma I, Stuehr DJ, Gross SS, Nathan C, Levi R: Identification of arginine as a precursor of endothelium-derived relaxing factor. Proc. Natl. Acad. 85, 8664–8667 (1988)
33. Shinozaki K, Ayajiki K, Kashiwagi A, Masada M, Okamura T: Malfunction of vascular control in lifestyle-related diseases: mechanisms underlying endothelial dysfunction in the insulin-resistant state. J. Pharmacol. Sci. 96, 401–405 (2004)
34. Silan C, Uzun O, Comunoğlu NU, Gokçen S, Bedirhan S, Cengiz M: Gentamicin-induced nephrotoxicity in rats ameliorated and healing effects of resveratrol: Biol. Pharm. Bull. 30, 79–83 (2007)
35. Stephens JM, Lee J, Pilch PF: Tumor necrosis factor-alpha-induced insulin resistance in 3T3-L1 adipocytes is accompanied by a loss of insulin receptor substrate-1 and GLUT4 expression without a loss of insulin receptor-mediated signal transduction: J. Biol. Chem. 272, 971–976 (1997)
36. Wang SB, Yang XY, Tian S, Yang HG, Du GH: Effect of salvianolic acid A on vascular reactivity of streptozotocin-induced diabetic rats. Life Sci. 85, 499–504 (2009)
37. Wu Z, Xie Y, Morrison RF, Bucher NL, Farmer SR: PPARγ induces the insulin-dependent glucose transporter GLUT4 in the absence of C/EBPα during the conversion of 3T3 fibroblasts into adipocytes. J. Clin. Invest. 101, 22–32 (1998)
38. Zobalı F, Çakici I, Karasu C: Effects of peroxynitrite on the reactivity of diabetic rat aorta. Pharmacology 63, 58–64 (2001)