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THE EFFECT OF SUCCINIC ACID MONOMETHYL ESTER (SAM) ON THE RESPONSES OF ISOLATED THORACIC AORTA IN STREPTOZOTOCIN-DIABETIC RATS

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Succinic acid monomethyl ester (SAM) was recently proposed as an insulinotropic tool in non-insulin-dependent diabetes mellitus. The present study was designed to define whether SAM has the vascular effect in thoracic aorta of streptozotocin (STZ)-diabetic rats. (1) Body weights of diabetic rats were significantly increased after SAM treatment ($P < 0.05$). (2) Ten-day SAM treatment did not significantly affect blood glucose levels in SAM-treated control and SAM-treated STZ-diabetic rats. (3) Maximum tension responses to noradrenaline and KCl (80 mmol l^{-1}) were not significantly different among all the experimental groups. (4) pD_2 ($-\log EC_{50}$) values for noradrenaline of untreated diabetic rats were significantly less than those of controls, SAM-treated control and SAM-treated diabetic rats ($P < 0.01$, $P < 0.001$ and $P < 0.05$, respectively). SAM treatment normalized the decreased sensitivity of noradrenaline response in diabetic rats. (5) Fast, slow and total components of responses to noradrenaline (10^{-5} mol $l^{-1} \cong EC_{90}$) were not significantly different among all the experimental groups. (6) There were no significant differences between aorta precontracted with noradrenaline from controls and STZ-diabetic (untreated and SAM-treated) rats in pD_2 values and the potency of maximum relaxation to acetylcholine or in pD_2 values to sodium nitroprusside. In conclusion, 10-day SAM treatment increases the sensitivity of diabetic–aortic rings to noradrenaline compared to untreated diabetic control rats.
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KEY WORDS: streptozotocin, succinic acid monomethyl ester, aorta, contraction.

INTRODUCTION

MacDonald *et al.* discovered that the methyl esters of succinic acid were new potent insulin secretagogues [1–3]. Then, several reports confirmed the insulinotropic action of these esters [4–9]. In addition, the monomethyl ester of succinic acid (SAM) was recently found to protect pancreatic islet B-cells against the impairment of glucose-stimulated insulin release caused by either glucopenia or starvation [10,11] and against streptozotocin (STZ)-induced diabetes mellitus [12].

Altered reactivity of vascular smooth muscle and impaired endothelium-dependent relaxation may be involved in the pathogenesis of diabetic vascular complications. Studies on vascular reactivity using the STZ-diabetic rat have reported inconsistent results with increased, decreased and unchanged responsiveness to noradrenaline, acetylcholine and sodium nitroprussiate [13–17].

The present study was designed to define whether SAM has the vascular effect in the thoracic aorta of STZ-diabetic rats.

MATERIALS AND METHODS

Animals

Adult male and female Wistar rats, weighing 200–300 g were used in this study. They received a standard pellet diet and had free access to tap water. The rats ($n = 36$) were divided into four groups:

I. Control group ($n = 11$);
II. SAM-treated control group ($n = 9$);

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III. STZ-induced diabetic control group ($n = 8$); and
IV. SAM-treated diabetic group ($n = 8$).

Two aorta rings were taken from all the animals and prepared as the following two subgroups:

A. E (+): aortic strips with intact endothelium.
B. E (−): aortic strips with denuded endothelium.

Succinic acid monomethyl ester (SAM) treatment

The rats in group II (SAM-treated control group) and group IV (SAM-treated STZ-diabetic group) were intraperitoneally injected with SAM (2×60 mg kg^{-1}) (Sigma, S-9259, Lot. 128F3660) in saline for 10 days.

Induction of experimental diabetes

Rats were injected intravenously (into the lateral tail vein) with streptozotocin (60 mg kg^{-1}) (Sigma, S-0130, Lot. 10H0657) in saline and then, the rats were maintained for 6 weeks with free access to food and water. Plasma glucose levels (with Dextrostix, Bayer Diagnostic, Lot. 2175053) and body weights were measured at the beginning of this study, 3 days after STZ injection and 6 weeks after the induction.

Aortic strips

Rats were killed by stunning followed by decapitation. A segment (3–5 cm) of thoracic aorta was removed and placed in an ice-cold Krebs–Ringer solution (KRS) of the following composition (mmol l^{-1}): NaCl (118), KCl (4.7), $CaCl_2$ (2.5), $MgSO_4.7H_2O$ (1.2), KH_2PO_4 (1.2), $NaHCO_3$ and glucose (11.1) and then trimmed free of adhering fat and connective tissue and cut into rings of 3 mm in width. The rings were opened by cutting the vessels longitudinally and in group E (−), the endothelium was then removed using a cotton thread. The removal of the endothelium was confirmed by the inability of arteries precontracted with noradrenaline to relax in response to acetylcholine. Subsequently, they were fixed with stainless steel clips at both ends and then placed in 15-ml organ baths containing KRS, gassed with carbogen (95% O_2 + 5% CO_2, pH 7.4 at 37°C). The preparation was connected to an isometric force displacement transducer (Grass Ins. FT03 isometric transducer) connected to a recorder (Grass Model 5) and were equilibrated for 90 min at optimal resting tension of 1 g. During this time, the KRS in the organ bath was replaced every 20 min.

Concentration–response curves

After equilibration, the thoracic aortae strips were exposed to 10^{-5} mol l^{-1} noradrenaline until the contraction reached the plateau (approx. 15 min) in order to measure the fast and slow components of vascular response to noradrenaline. The fast component was measured from the baseline to the point at which the rate of contraction started to decrease abruptly. The slow component was measured from this point to the top of the contraction. The total response was the sum of these two components [18]. Concentration–response curves were obtained with noradrenaline. Noradrenaline ($10^{-9}/3 \cdot 10^{-9}$–$10^{-4}/3 \cdot 10^{-4}$ mol l^{-1}) 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. In addition, the aortic strips were also exposed to 80 mmol l^{-1} KCl until the contraction reached the plateau (approx. 15 min). Cumulative relaxation curves to acetylcholine (Ach) (10^{-8}–10^{-4} mol l^{-1}) and sodium nitroprusside (SNP) (10^{-8}–10^{-4} mol l^{-1}) were obtained in each strip precontracted submaximally (approx. EC_{90}) by addition of noradrenaline.

At the end of each experiment, tissue was blotted dry, measured and weighed.

Data analysis

Contractile responses to noradrenaline and KCl were calculated as the increase in tension (in milligrams) in response to the agonist per milligram of aorta. Agonist pD_2 value ($= -\log EC_{50} = -\log$ contractile concentration$_{50}$) was calculated from each agonist dose–response curve by linear regression analysis of the linear portion of the curve and taken as a measure of the sensitivity of the tissues to each agonist. All values are expressed as (mean ± SEM). Statistical analysis of the data was performed using one-way analysis of variance (ANOVA) followed by Tukey–Kramer Multiple comparisons test. $P < 0.05$ was considered as statistically significant.

RESULTS

The body weights of the rats in each group are given in Table I. The body weights of STZ-control rats

Table I
Effects of streptozotocin-induced diabetes, SAM treatment on plasma glucose and body weight

Groups	*Body weight (g)*	*Blood glucose (mg dl^{-1})*
Group I ($n = 11$)	298 ± 10*	$101 \pm 3^{1,2}$
Groul II ($n = 9$)	308 ± 4**	$111 \pm 2^{3,4}$
Group III ($n = 8$)	250 ± 6	488 ± 42
Group IV ($n = 8$)	286 ± 2***	432 ± 32

*Significant from STZ-control, $P < 0.001$; **significant from STZ-control, $P < 0.001$; ***significant from STZ-control, $P < 0.05$; [1]significant from STZ-control, $P < 0.001$; [2]significant from SAM-treated diabetic, $P < 0.001$; [3]significant from STZ-control, $P < 0.001$; [4]significant from SAM-treated diabetic, $P < 0.001$.

Table II
Reactivity of endothelium-intact and denuded rat aorta from SAM-treated and untreated control and diabetic rats to noradrenaline

Groups (NA)	E_{max}		pD_2	
	E(+)	*E(−)*	*E(+)*	*E(−)*
Group I ($n = 11$)	235 ± 9	209 ± 22	7.1 ± 0.2[1]	6.4 ± 0.2[4]
Group II ($n = 9$)	239 ± 20	230 ± 18	7.0 ± 0.3[2]	6.4 ± 0.1[5]
Group III ($n = 8$)	211 ± 19	222 ± 17	5.4 ± 0.2	5.0 ± 0.2
Group IV ($n = 8$)	230 ± 22	223 ± 24	6.4 ± 0.2[3]	6.0 ± 0.2[6]

[1]Significant from diabetic, $P < 0.001$; [2]significant from diabetic, $P < 0.001$; [3]significant from SAM-treated diabetic, $P < 0.05$; [4]significant from diabetic, $P < 0.05$; [5]significant from diabetic, $P < 0.001$; [6]significant from SAM-treated diabetic, $P < 0.01$.

(untreated — group III) was significantly less than untreated controls (group I), SAM-treated controls (group II) and SAM-treated STZ-diabetic rats (group IV), respectively ($P < 0.001$, $P < 0.001$, $P < 0.05$).

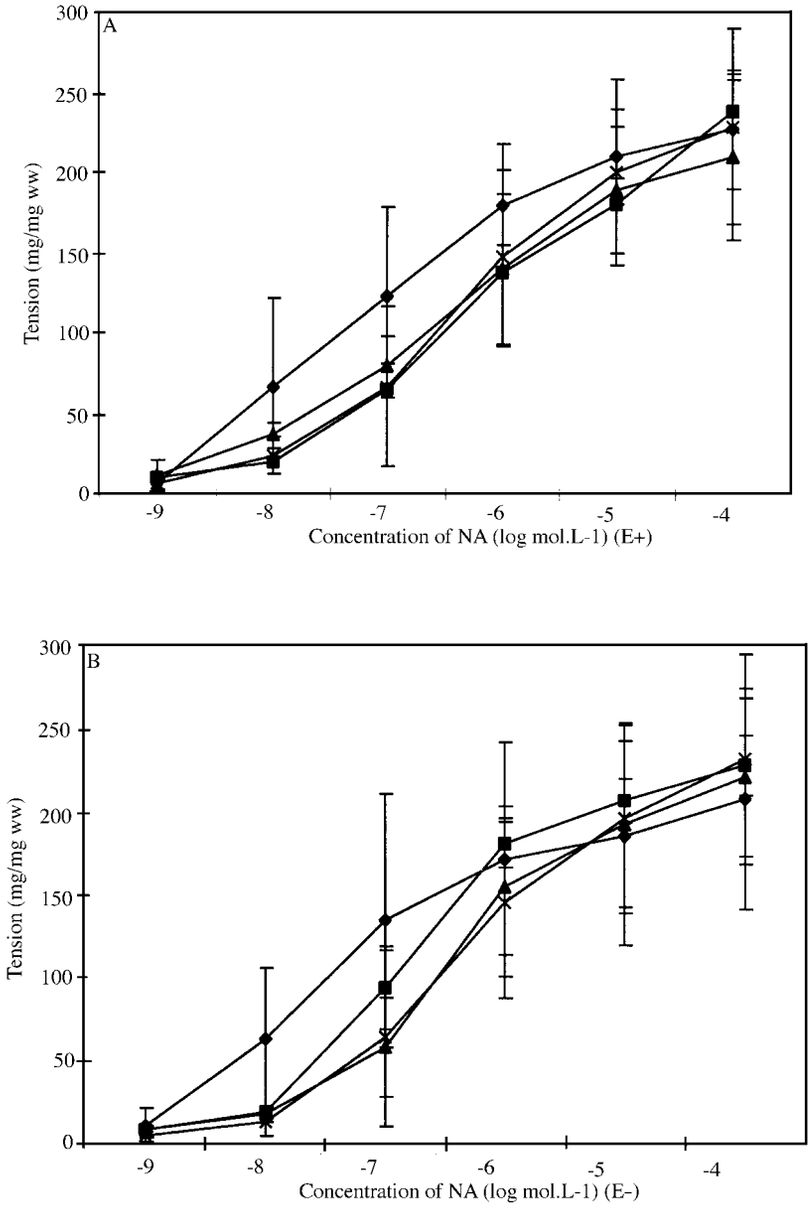


Fig. 1. The concentration−response curves (♦, control; ■, SAM-control; ▲, STZ-control and X, STZ + SAM) to noradrenaline in (A) endothelium-intact and (B) endothelium-denuded aortas from SAM-treated and untreated controls and diabetic rats.

Table III
E_{max} values for KCl in endothelium-intact and denuded aorta from SAM-treated and untreated control and diabetic rats

Groups (KCl)	E_{max}	
	E(+)	*E(−)*
Group I ($n = 11$)	184 ± 13	209 ± 19
Group II ($n = 9$)	216 ± 13	200 ± 11
Group III ($n = 11$)	158 ± 21	163 ± 16
Group IV ($n = 8$)	159 ± 10	168 ± 18

The body weights of diabetic rats were significantly increased after SAM treatment ($P < 0.05$).

Blood glucose levels of rats in each group are also shown in Table I. Blood glucose levels of untreated diabetic rats (group III) and SAM-treated diabetic rats (group IV) were significantly greater than those of untreated control (group I) and SAM-treated controls (group II) ($P < 0.001$, $P < 0.001$, respectively). SAM treatment (nevertheless, in treatment time used in this study) did not significantly affect blood glucose levels in SAM-treated control rats (group II) and SAM-treated diabetic rats (group IV).

Agonist-induced contractions

Table II shows the pD_2 ($= -\log EC_{50}$) values of noradrenaline and maximum responses (absolute tension in milligrams) obtained to noradrenaline in aortic strips from control (untreated and SAM-treated) and diabetic (untreated and treated) rats. Concentration–response curves of the aortae to noradrenaline (10^{-9}–10^{-4} mol l^{-1}) are shown in Fig. 1. When maximum contractions were expressed as milligram per milligram tissue wet weight, maximum tension responses to noradrenaline were not significantly different among all the experimental groups. However, pD_2 values of aortas with endothelium for noradrenaline of untreated diabetic rats (group III) were significantly less than those of the control (group I), SAM-treated control (group II) and SAM-treated diabetic (group IV) rat ($P < 0.001$, $P < 0.001$ and $P < 0.05$, respectively). pD_2 values of aortas without endothelium (subgroup E −) were similar ($P < 0.01$, $P < 0.001$ and $P < 0.01$, respectively) to those of with intact endothelium. Similarly, the maximum contractions (absolute tension in milligrams per milligram of wet tissue weight) to KCl (80 mmol l^{-1}) were not significantly different among all the experimental groups (including E + and E − subgroups) (Table III).

Table IV
pD_2 and percent Inh_{max} values for acetylcholine in endothelium-intact aorta from SAM-treated and untreated control and diabetic rats

Groups (Ach)	*% Inh.$_{max}$*	*pD_2*
Group I ($n = 11$)	61 ± 9	6.0 ± 0.2
Group II ($n = 9$)	64 ± 7	5.6 ± 0.4
Group III ($n = 8$)	72 ± 7	5.1 ± 0.4
Group IV ($n = 8$)	60 ± 4	5.4 ± 0.2

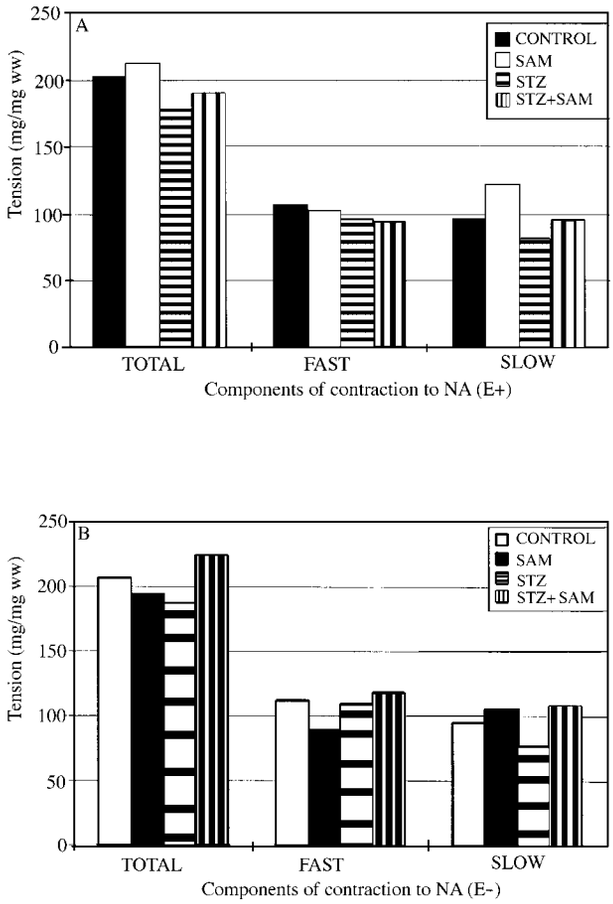


Fig. 2. Fast, slow and total components of noradrealine-induced contraction in (A) endothelium-intact and (B) endothelium-denuded aortas from SAM-treated and untreated control and diabetic rats.

Figure 2 illustrates the fast, slow and total components of contraction induced by noradrenaline (10^{-5} mol l^{-1}) in all the experimental groups (including E + and E − subgroups). As shown in Fig. 2, either fast and slow or total components of responses to noradrenaline were not significantly different among all the experimental groups.

Agonist-induced relaxations

In the experiments examining relaxations to the agonists in aorta strips precontracted with noradrenaline (submaximally, approx. EC_{90}), there were no significant differences among aorta from control (untreated and SAM-treated) and STZ diabetic (untreated and SAM-treated) animals in pD_2 ($= -\log EC_{50} = -\log$ relaxant concentration$_{50}$) values and in the potency of maximum relaxation to acetylcholine (Table IV) or in pD_2 values to sodium nitroprusside (Table V).

DISCUSSION

The methyl esters of succinic acid were introduced by MacDonald *et al.* as new potent insulin secretagogues [1–3]. Then several reports confirmed the insulinotropic action of these esters [4–7] and succinic acid monomethyl ester (SAM) was found to protect pancreatic islet B-cells against streptozotocin (STZ)-induced diabetes mellitus [12]. Based primarily on observations made with these compounds, it has been inferred that the mitochondrial oxidation of succinate is responsible for this insulinotropic effect [6,19].

The investigations of the pathogenesis of the vascular complications of diabetes have not yet yielded definitive knowledge. Although the effects of diabetes on the vascular responsiveness of the rat aorta and the other vascular beds have been widely investigated, with disputable results, there are several variables which should perhaps be considered when comparing results: strain of rat employed, drug employed to induce diabetes, duration of diabetes, age of animals, presence or absence of endothelium and the method used to calculate maximum response.

The present results demonstrate that aorta with or without endothelium from 6-week STZ-diabetic rats are significantly less sensitive than those of controls, SAM-treated controls and SAM-treated diabetic rats while maximum contractions to noradrenaline were not significantly different among all of the groups. The unchanged maximal contraction and decreased sensitivity (pD_2) to noradrenaline of STZ-diabetic rats observed in the present study is in agreement with some of the previous reports [20–26]. There was also no significant differences between diabetic and control aorta with or without endothelium in the maximum contraction to KCl. These data are also in agreement with previous studies by Fiolde Cuneo *et al.* [23] and Mulhern and Docherty [24]. In contrast to the observations above, an increased or decreased maximal response to noradrenaline and KCl in aortic preparations from diabetic rats has also been found [27–33]. Furthermore, another research group has shown an increased sensitivity to agonists in diabetic aorta [34–36] and, on the other hand, MacLeod [31], Head [32], Agrawal [13,37] and Kamata [38] have found no change in EC_{50} of noradrenaline and KCl in thoracic aortic rings from diabetic and control rats. It was demonstrated that SAM significantly increased phosphoinositide (PI) hydrolysis and the effects of SAM might be mediated, at least in part, by increases in PI-derived second messengers, particularly diacylglycerol (DAG) [39–41]. Phosphoinositide hydrolysis (PI) results in the generation of the second messengers, inositol 1,4,5-tri-phosphate (IP_3) and 1,2 diacylglycerol (DAG). IP_3 has been shown to be involved in the release of Ca^{2+} from intracellular stores while DAG activates protein kinase C (PKC), resulting in protein phosphorylation and the generation of physiological responses. It has been suggested that the increment in IP_3 production and the activation of PKC could be responsible, at least in part, for the enhanced contractile response of aorta from normal and diabetic rats to noradrenaline [42–48]. In addition, the deficiency of insulin [22], change in calcium homeostasis [49], impairment of the adrenergic nerve fibers [20] and alteration in free radical tissue–defense mechanisms [50] have been proposed to be responsible for the altered contractile responsiveness of diabetic vascular smooth muscle to α-adrenergic stimulation. In this study, we demonstrated that SAM treatment increases the sensitivity to noradrenaline (pD_2) of aortic strips obtained STZ-diabetic rats compared to STZ untreated diabetic control rats. The mechanism(s) responsible for increasing effect of SAM treatment on vascular sensitivity of STZ-diabetic rats might be, at least in part, the restoration of the above altered vascular functions, especially phosphoinositide hydrolysis [39–41], cytoprotective [12] and antioxidant effects of SAM (unpublished data, [51]).

The contractile response of the rat aorta to noradrenaline is biphasic, consisting of a fast and a slow component [52]. The fast component of the contraction has been demonstrated to be due to mobilization of intracellular calcium whereas the slow component is directly dependent upon an influx of extracellular calcium [49,53–55]. Noradrenaline, a nonselective alpha agonist, has comparable affinity for α_1 and α_2-adrenoceptors. Fast component of the contraction-induced noradrenaline is a consequence of activation of α_1-adrenoceptors whereas the slow component reflects activation of α_2-adrenoceptors [54]. The total response and the fast and the slow components to noradrenaline in the present study were found to be not significantly different in all groups whereas Özçelikay *et al.* [18] has shown an enhanced total response and the increased fast and the decreased slow components to noradrenaline in 10-week-STZ-diabetic rat aortas. On the other hand, Rinaldi and Cingolani [56] found an unchanged total response and increased fast and decreased slow components to noradrenaline in spontaneously diabetic rats. Furthermore, in Scarborough and Carrier's study [57], the slow component of contraction in

Table V
pD_2 values for SNP in endothelium-intact and denuded from SAM-treated and untreated control and diabetic rats

Groups (SNP)	*pD_2*	
	E(+)	*E(−)*
Group I ($n = 11$)	7.9 ± 0.2	8.1 ± 0.1
Group II ($n = 9$)	7.7 ± 0.2	7.9 ± 0.2
Group III ($n = 8$)	7.5 ± 0.2	7.2 ± 0.2
Group IV ($n = 8$)	7.4 ± 0.1	7.4 ± 0.1

response to noradrenaline in aortas from diabetic rats was increased significantly compared to the control tissues. The differences between our results and the previous studies [18,56] could be at least due to the species of diabetic rats or the duration of diabetes.

In the present study, there were no significant differences between the diabetic tissues and control tissues in the responsiveness to the vasodilators sodium nitroprusside and acetylcholine. The sensitivity of diabetic aortas to relaxant substances were also not changed when compared with controls. Our results are in agreement with some of the previous studies [22,24,32,58–61] although the others have shown the impaired endothelium-dependent relaxation to acetylcholine in diabetic rats [62,63]. The discrepancy between our results and those of other groups may be due to an alteration in the regulation of post-receptor excitation–contraction coupling and an impairment of endothelium function.

In conclusion, our data demonstrate that 10-day succinic acid monomethyl ester (SAM) treatment increases the sensitivity of diabetic–aortic rings to noradrenaline compared to untreated-diabetic control rats. However, its exact mechanism(s) of action on vascular reactivity remain unclear and require further investigation.

REFERENCES

1. Fahien LA, Mac Donald MJ, Kmiotek EH, Mertz RJ, Fahien CM. Regulation of insulin release by factors that also modify glutamate dehydrogenase. *J Biol Chem* 1988; **263**: 13610–14.
2. Mac Donald MJ, Fahien LA. Glyceraldehyde phosphate and methyl esters of succinic acid. Two 'new' potent insulin secretagogues. *Diabetes* 1988; **37**: 997–9.
3. MacDonald MJ, Fahien LA. Insulin release in pancreatic islets by a glycolytic and Krebs cycle intermediate: contrasting patterns of glyceraldehyde phosphate and succinate. *Arch Biochem Biophys* 1990; **279**: 104–8.
4. Akkan AG, Malaisse WJ. Iterative pulse administration of succinic acid monomethyl ester to streptozotocin diabetic rats. *Diabetes Res* 1993; **23**: 55–63.
5. Malaisse WJ, Rasschaert J, Villanueva-Penacarrillo ML, Valverde I. Respiratory, ionic and functional effects of succinate esters in pancreatic islets. *Am J Physiol* 1993; **264(Endocrinol Metab 27)**: E428–33.
6. Malaisse WJ, Sener A. Metabolic effects and fate of succinate esters in pancreatic islets. *Am J Physiol* 1993; **264(Endocrinol Metab 27)**: E434–40.
7. Vincent D, Villenueva-Penacarrillo ML, Malaisse-Lagae F, Leclercq-Meyer V, Valverde I, Malaisse WJ. In vivo stimulation of insulin release by succinic acid methyl esters. *Arch Int Pharmacodyn Ther* 1994; **327(2)**: 246–50.
8. Malaisse-Lagae F, Bakkali-Nadi A, Malaisse WJ. Insulinotropic response to enterally administered succinic acid and glutamic acid methyl esters. *Arch Int Pharmacodyn Ther* 1994; **328(2)**: 235–42.
9. Garcia-Martinez JA, Villanueva-Penacarrillo ML, Valverde I, Malaisse WJ. Stimulation of insulin release caused by intraduodenal administration of succinic acid methyl esters. *Arch Int Pharmacodyn Ther* 1995; **330(1)**: 116–24.
10. Eizirik DL, Welsh N, Pener A, Malaisse WJ. Protective action of succinic acid monomethyl ester against the impairment of glucose-stimulated insulin release caused by glucopenia or starvation: metabolic determinants. *Biochem Med Metab Biol* 1994; **53(1)**: 34–45.
11. Conget I, Zhang TM, Eizirik DL, Malaisse WJ. SAM prevents impairment of glucose-stimulated insulin secretion caused by hexose deprivation or starvation. *Am J Physiol* 1995; **268(4 Pt 1)**: E580–7.
12. Akkan AG, Malaisse WJ. Protective effect of succinic acid monomethyl ester against streptozotocin-induced diabetes mellitus. *Med Sci Res* 1993; **21**: 467.
13. Agrawal DK, McNeill JH. Vascular responses to agonist in rat mesenteric artery from diabetic rats. *Can J Physiol Pharmacol* 1987a; **65**: 1484–90.
14. Takiguchi Y, Satoh N, Hashimoto H, Nakashima M. Reversal effect of thyroxine on altered vascular reactivity in diabetic rats. *J Cardiovasc Pharmacol* 1989; **13**: 520–4.
15. White RF, Carrier GO. Vascular contraction induced by activation of membrane calcium ion channels is enhanced in streptozotocin-diabetes. *J Pharmacol Exp Ther* 1990; **253**: 1057–62.
16. Cameron NE, Cotter MA. lmpaired contraction and relaxation in aorta from streptozotocin-diabetic rats: role of polyol pathway. *Diabetologia* 1992: **35**: 1011–19.
17. Taylor PD, Mc Carthy AL, Thomas CR, Poston L. Endothelium-dependent relaxation and noradrenaline sensitivity in mesenteric resistance arteries of streptozotocin-induced diabetic rats. *Br J Pharmacol* 1992; **107**: 393–9.
18. Özçelikay AT, Pekiner C, Arý N, Öztürk Y, Özüarý A, Altan VM. The effect of vanadyl treatment on vascular responsiveness of streptozotocin-diabetic rats. *Diabetologia* 1994; **37**: 572–8.
19. Zawalich WS, Zawalich KC. Biochemical mechanisms involved in monomethyl succinate-induced insulin secretion. *Endocrinology* 1992; **131**: 649–54.
20. Sullivan S, Spark HV. Diminished contractile response of aortas from diabetic rabbits. *Am J Physiol* 1979; **236**: 301–6.
21. Turlapaty PD, Lum G, Altura BM. Vascular responsiveness and serum biochemical parameters in alloxan diabetes mellitus. *Am J Physiol* 1980; **239(6)**: E412–21.
22. Fortes ZB, Leme JG, Scivoletto R. Vascular reactivity in diabetes mellitus: role of the endothelial cell. *Br J Pharmacol* 1983; **79**: 771–81.
23. Fiol-de Cuneo M, Ruiz RD, Lacuara JL, Santillan-de Torres R. Contractility and pharmacological reactivity of isolated vascular smooth muscle from diabetes rats. *Pharmacology* 1988; **36(4)**: 228–37.
24. Mulhern M, Docherty RJ. Effect of experimental diabetes on the responsiveness of rat aorta. *Br J Pharmacol* 1989; **97**: 1007–12.
25. Sarioglu Y, Utkan T, Utkan NZ, Bozkurt A. Effect of chronic experimental diabetes on vascular smooth muscle function in dog femoral artery. *Meth Find Exp Clin Pharmacol* 1993; **15(3)**: 161–7.
26. Nielsen H, Bonnema SJ, Flyrbjerg A. Effect of diabetes, insulin treatment and osmolality on contractility of isolated rat resistance arteries. *Pharmacol Toxicol* 1995; **77**: 209–15.
27. Owen MP, Carrier GO. Alteration in vascular smooth muscle sensitivity to vasoconstrictor agents by streptozotocin induced diabetes. *Proc West Pharmacol Soc* 1979; **22**: 363–6.

28. Owen MP, Carrier GO. Calcium dependence of norepinephrine-induced vascularcontraction in experimental diabetes. *J Pharmacol Exp Ther* 1980; **212**: 253–8.
29. Scarborough NL, Carrier GO. Increased alpha2-adrenoceptor mediated vascular contraction in diabetic rats. *J Autonom Pharmacol* 1983; **3**: 177–83.
30. Ramanadham S, Lyness WH, Tenner TE Jr. Alteration in aortic and tail artery reactivity to agonists after streptozocin treatment. *Can J Physiol Pharmacol* 1984; **62(4)**: 418–23.
31. MacLeod KM. The effect of insulin treatment on changes in vascular reactivity in chronic, experimental diabetes. *Diabetes* 1985; **34(11)**: 1160–7.
32. Head RJ, Longhurst PA, Panek RL, Stitzel RE. A contrasting effect of the diabetic state upon the contractile responses of aortic preparations from the rat and rabbit. *Br J Pharmacol* 1987; **91(2)**: 275–86.
33. White RE, Carrier GO. Enhanced vascular alpha-adrenergic neuroeffector system in diabetes importance of calcium. *Am J Physiol* 1988; **255(5Pt2)**: H1036–42.
34. Harris KH, MacLeod KM. Influence of the endothelium on contractile responses of arteries from diabetic rats. *Eur J Pharmacol* 1988; **153(1)**: 55–64.
35. MacLeod KM, McNeill JH. Alpha adrenoceptor mediated responses in aorta from three mpnth streptozocin diabetic rats. *Proc West Pharmacol Soc* 1982; **25**: 245–7.
36. Martin W, Furchgott RF, Villani GM, Jothianandan D. Depression of contractile responses in rat aorta by spontaneously released endothelium-derived relaxing factor. *J Pharmacol Exp Ther* 1986; **237(2)**: 529–38.
37. Agrawal DK, Bhimji S, Mac Neill JH. Effect of chronic experimental diabetes on vascular smooth muscle function in function in rabbit carotid artery. *J Cardiovasc Pharmacol* 1987b; **9(5)**: 584–93.
38. Kamata K, Miyata N, Kasuya Y. Mechanisms of increased responses of the aorta to alpha-adrenoceptor agonists in streptozocin-induced diabetic rats. *J Pharmacobiodyn* 1988; **11(10)**: 707–13.
39. MacDonald MJ, Fahien LA, Mertz RJ, Rana RS. Effect of esters of succinic acid and other citric acid cycle intermediates on insulin release and inositol phosphate formation by pancreatic islets. *Arch Biochem Biophys* 1989; **269(2)**: 400–6.
40. Zawalich WS, Zawalich KC. Biochemical mechanisms involved monomethyl succinate-induced insulin secretion. *Endocrinology* 1992; **131**: 649–54.
41. Rasmussen H, Zawalich KC, Zawalich WS. Metabolic activation of Calcium(2 +)independent phosphoinositide hydrolysis in beta-cells and its role in the control of insulin secretion. *Endocrinology* 1995; **136(2)**: 577–84.
42. Berridge MJ. Phosphatidyl inositol hydrolysis: a multi-functional transducing mechanism. *Mol Cell Endocrinol* 1981; **24**: 115–40.
43. Cotecchia S, Leeb-Lundberg LMF, Hagen PO, Lefkawitz RJ, Caron MG. Phorbol esters effects on alpha-1-adrenoceptor binding and phosphoinositol metabolism in cultured vascular-smooth muscle cells. *Life Sci* 1985; **37**: 2389–98.
44. Hashimoto T, Mirata M, Itoh T, Kanmura Y, Kuriyama H. Inositol 1,4,5-triphosphate activates pharmacomechanical coupling in smooth muscle of the rabbit mesenteric artery. *J Physiol* 1986; **370**: 605–18.
45. Heagarty AM, Ollerenshaw JD. The phosphoinositide signalling system and hypertension. *J Hypertens* 1987; **5**: 515–24.
46. Abebe W, MacLeod KM. Protein kinase C-mediated contractile responses of arteries from diabetic rats. *Br J Pharmacol* 1990; **101**: 465–71.
47. Abebe W, MacLeod KM. Influence of diabetes on norepinephrine-induced inositol 1,4,5-triphosphate levels in rat aorta. *Life Sci* 1991a; **49(13)**: PL85–90.
48. Abebe W, MacLeod KM. Enhanced arterial contractility to noradrenaline in diabetic rats is associated with increased phosphoinositide metabolism. *Can J Physiol Pharmacol* 1991b; **69(3)**: 355–61.
49. Scarborough NL, Carrier GO. Nifedipine and alpha adrenoceptors in rat aorta: I. Role of extracellular calcium in alpha-1 and alpha-2 adrenoceptor-mediated contraction. *J Pharmacol Exp Ther* 1984; **231**: 597–602.
50. Wohaieb SA, Godin DV. Alterations in free radical tissue-defence mechanisms in streptozotocin-induced diabetes in rat: effect of insulin treatment. *Diabetes* 1987; **36**: 1014–18.
51. Akkan AG, Sultuybek G, Ince E, Ozyazgan S, Senses V. Antioxidant effect of succinic acid monomethyl ester (SAM) in diabetic rats. Unpublished Data, 1997.
52. Bohr DF. Vascular smooth muscle: dual effects of calcium. *Science* 1963; **139**: 597–9.
53. Deth R, Van Breemen C. Relative contribution of Ca^{2+} release during drug induced activation of the rabbit aorta. *Pflügers Arch* 1974; **348**: 13–22.
54. Van Meel JCA, De Jonge A, Kalkman HO, Wilffert B, Timmermans PBMNM, Van Zwieten PA. Vascular smooth muscle contraction initiated by postsynaptic alpha2-adrenoceptor activation is induced by an influx of extracellular calcium. *Eur J Pharmacol* 1981; **69**: 205–8.
55. Janis RA, Triggle DJ. New developments in Ca^{2+} channel antagonists. *J Med Chem* 1983; **26**: 775–85.
56. Rinaldi GJ, Cingolani HE. The effect of diabetes on fast response to noradrenaline in rat aorta. *Diabetes* 1992; **41:** 30–4.
57. Scarborough NL, Carrier GO. Nifedipine and alpha adrenoceptors in rat aorta. II. Role of extracellular calcium in enhanced alpha-2 adrenoceptor-mediated contraction in diabetes. *J Pharmacol Exp Ther* 1984; **231(3)**: 603–9.
58. Wakabayashi K, Hatake K, Kimura N, Kakishita E, Nagai K. Modulation of vascular tonus by the endothelium in experimental diabetes. *Life Sci* 1987; **40**: 643–8.
59. Gebremedhin D, Koltai MZ, Pogatsa G, Magyar K, Hadhazy P. Differential contractile responsiveness of femoral arteries from healthy and diabetic dogs: role of endothelium. *Arch lnt Pharmacodyn Ther* 1987; **288(1)**: 100–8.
60. Kappagoda T, Jayakody L, Rajotte R, Thomson ABR, Senaretne MPJ. Endothelium-dependent relaxation to acetylcholine in the aorta of streptozocin-induced diabetic rat and the BB-diabetic rat. *Clin Invest Med* 1989; **12**: 187–93.
61. Karasu C, Altan VM. The role of endothelial cells on the alterations in vascular reactivity induced by insulin-dependent diabetes mellitus: effects of insulin treatment. *Gen Pharmacol* 1993; **24(3)**: 743–55.
62. Kamata K, Miyata N, Kasuya Y. lmpairment of endothelium-dependent relaxation and changes in levels of cyclic GMP in aorta from streptozocin-induced diabetic rats. *Br J Pharmacol* 1989; **97(2)**: 614–18.
63. Heygate KM, Lawrence IG, Bennett MA, Thurston H. Impaired endothelium-dependent relaxation in isolated resistance arteries of spontaneously diabetic rats. *Br J Pharmacol* 1995; **116**: 3251–9.