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$^{-1}$ $\approx EC_{50}$) 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.

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 ($n = 9$);
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.

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. 10F0657) 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), CaCl₂ (2.5), MgSO₄·7H₂O (1.2), KH₂PO₄ (1.2), NaHCO₃ 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% O₂ + 5% CO₂), 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 10⁻³ 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 (10⁻⁵/3·10⁻⁵/10⁻⁴/3·10⁻⁴ 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) (10⁻⁵−10⁻⁴ mol l⁻¹) and sodium nitroprusside (SNP) (10⁻³−10⁻⁴ mol l⁻¹) were obtained in each strip precontracted submaximally (approx. EC₅₀) 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 pD₂ value (=-log EC₅₀ = -log contractile concentration₉₀) 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 were measured at the beginning of this study, 3 days after STZ injection and 6 weeks after the induction.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Blood glucose (mg dl⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (n = 11)</td>
<td>298 ± 10°</td>
<td>101 ± 3°</td>
</tr>
<tr>
<td>Groul II (n = 9)</td>
<td>308 ± 4°</td>
<td>111 ± 2°</td>
</tr>
<tr>
<td>Group III (n = 8)</td>
<td>250 ± 6</td>
<td>488 ± 42</td>
</tr>
<tr>
<td>Group IV (n = 8)</td>
<td>286 ± 3°</td>
<td>432 ± 32</td>
</tr>
</tbody>
</table>

° Significant from STZ-control, P < 0.001; ** significant from STZ-control, P < 0.001; *** significant from STZ-control, P < 0.05; significant from STZ-control, P < 0.001; significant from SAM-treated diabetic, P < 0.001; 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

<table>
<thead>
<tr>
<th>Groups (NA)</th>
<th>$E_{\text{max}}$</th>
<th>$pD_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E(+)</td>
<td>E(−)</td>
</tr>
<tr>
<td>Group I (n = 11)</td>
<td>235 ± 9</td>
<td>209 ± 22</td>
</tr>
<tr>
<td>Group II (n = 9)</td>
<td>239 ± 20</td>
<td>230 ± 18</td>
</tr>
<tr>
<td>Group III (n = 8)</td>
<td>211 ± 19</td>
<td>222 ± 17</td>
</tr>
<tr>
<td>Group IV (n = 8)</td>
<td>230 ± 22</td>
<td>223 ± 24</td>
</tr>
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</table>

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

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.

(un)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 ($P < 0.001$, $P < 0.001$, $P < 0.05$).
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 (Table III).

Figure 2 illustrates the fast, slow and total components of contraction induced by noradrenaline ($10^{-9}$ 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. $EC_{50}$), 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 \(\alpha\)-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 non-selective alpha agonist, has comparable affinity for \(\alpha_1\) and \(\alpha_2\)-adrenoceptors. Fast component of the contraction-induced noradrenaline is a consequence of activation of \(\alpha_1\)-adrenoceptors whereas the slow component reflects activation of \(\alpha_2\)-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>
<thead>
<tr>
<th>Groups (SNP)</th>
<th>(E(+))</th>
<th>(E(–))</th>
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<tbody>
<tr>
<td>Group I ((n = 11))</td>
<td>7.9 ± 0.2</td>
<td>8.1 ± 0.1</td>
</tr>
<tr>
<td>Group II ((n = 9))</td>
<td>7.7 ± 0.2</td>
<td>7.9 ± 0.2</td>
</tr>
<tr>
<td>Group III ((n = 8))</td>
<td>7.5 ± 0.2</td>
<td>7.2 ± 0.2</td>
</tr>
<tr>
<td>Group IV ((n = 8))</td>
<td>7.4 ± 0.1</td>
<td>7.4 ± 0.1</td>
</tr>
</tbody>
</table>

Table V pD\(_2\) values for SNP in endothelium-intact and denuded from SAM-treated and untreated control and diabetic rats
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]—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